

4th International Symposium of Medicinal and Aromatic Plants

2-4 October 2018
Çeşme- İZMİR



4th INTERNATIONAL SYMPOSIUM
OF MEDICINAL AND AROMATIC
PLANTS ÇEŞME TURKEY 2018

SYMPOSIUM BOOK



tibbiaromatik2018.org

Dear Guests

We welcome you to the Forth International Symposium of Medicinal and Aromatic Plants and greet you with respect. We are highly honored and happy to see you in Çeşme-İzmir.

“Medicinal and aromatic plants” involves a large area in terms of their active ingredients as well as their consumption. Besides, their trading volume in the world is increasing every day. It is stated that trading volume of the medicinal plants market has increased from 60 billion dollars in 2000’s to 107 billion dollars in the year 2017.

It is known that Turkey has a huge economical potential in terms of medicinal and aromatic plants which are cultivated or collected from the nature, because of having various climatic and ecological conditions, different plant types of the flora, and being the center of origin of several plants. It is seen that the scientific research studies on medicinal plants are limited compared to many other cultivated ones. In this regard, it is clear that the scientific studies on medicinal and aromatic plants will have a valuable contribution to this field and they will provide a resource for the national economy. Besides that, transferring the outputs of these researches to the practice and providing a common use for the sector is also needed.

The fourth Symposium of Medicinal and Aromatic Plants which is carried out biennially is organized internationally by the Ege University Faculty of Agriculture Department of Field Crops, with the support of the Aegean Agricultural Research Institute of Ministry of Agriculture and Forestry, during 2-4 October 2018 in Çeşme, İzmir. This symposium has a special importance for us since the first scientific meeting about medicinal plants in Turkey was organized by our department in İzmir. I would like to commemorate with great gratitude Professor Vamık Tayşi who organized the first international symposium and also we express our thanks to Honorable Professor Ayhan Ceylan. Professor Ceylan attributed a special importance to this subject and initiated the first studies about growing and breeding techniques with the seeds from Germany in 1971. The researches about these plants have been continued in our department for 47 years, sometimes more intense and sometimes less but permanently.

The subject of medicinal and aromatic plants is related to different working disciplines. Therefore, during compiling the scientific committee, we particularly pay attention to this issue. We were attentive to include scientists from different fields in the scientific committee. If the attendants of the symposium are taken into account, it is seen that there are researchers from different disciplines, nationally and internationally.

There has been a great interest to the symposium and the total number of the national and international scientific submissions was 460. However, only 103 oral presentations and 255 poster presentations could be accommodated due to the limited symposium duration. On the behalf of the organizing committee we thank to all delegates who shared their research results with us. We also thank to honorable Prof. Dr. Menşure Özgüven, honorable Prof. Dr. Bernd Honermeier, honorable Prof. Dr. K. Hüsnü Başer and honorable Prof. Dr. Tayfur Akın for their contributions with invited presentations. Especially, private sector had great contributions to the organization of this symposium, thus we present our thanks to all of them individually.

We gratefully thank to Ege University Faculty of Agriculture Department of Field Crops, who organized the symposium, to the Aegean Agricultural Research Institute of Ministry of Agriculture and Forestry for their support, to the members of secretary and organizing committee of the symposium who have an important role in carrying out the symposium by their efforts, to the members of the scientific committee, to all delegates who shared their research results with us, and to all institutions and foundations that supported us during every stage of the symposium, to our guests and students.

Prof. Dr. Emine BAYRAM
Symposium Chair

KEYNOTE SPEECH

FROM BIOLOGICAL DIVERSITY TO VALUE ADDED PRODUCTS - MEDICINAL AND AROMATIC PLANTS

Menşure ÖZGÜVEN

Konya Food and Agriculture University, Turkey

Hippocrates, known as the father of medicine, said "Medicus curat, natura sanat" -"The physician treats, but nature heal". Hippocrates established today's modern medicine with this approach and most of the active ingredients in the modern-day medicines are derived from plants as origin.

Since ancient times, the use of medicinal plants against diseases has played an important role for humans. Medicinal plant residues were found in the excavations of 60,000 years old Neanderthal tombs, in Iraq - Shanidar. In the first half of the 20th century, the importance and uses of medical plants decreased for a while as the result of the reflection of the industrialization on the pharmaceutical sector and synthetic pharmaceutical raw materials. However, starting from 1950s, the medical plants have regained great importance and a new era has begun.

Depending on growing demand for natural products, the volume of medicinal and aromatic plants in the world market shows a rapid increase. Along with the increase in demand for these plants, which have been collected from the nature, the priority has been given to the studies on the agriculture of medicinal and aromatic plants. Today, medicinal and aromatic plants are cultivated in many countries and new varieties are developed from many plant species.

According to a report by the Federal Association of the Pharmaceutical Industry, in 2010, the share of biopharmaceuticals, phytopharmaceuticals and homeopathic medicines accounted for approximately 20% of total sales in the German pharmaceutical market. In addition, medicinal and aromatic plants are widely used in dietary supplements, food additives, functional foods, cosmetics and perfume industry, paint, herbal teas, agrochemicals, feed additives and veterinary medicine fields. Biodiversity is constantly explored and evaluated to discover new plants or new secondary metabolites and their effects, these findings are transformed into value-added products.

According to Vavilov's "Gene Centers Theory", Anatolia is defined as one of the most important gene pool centers of the world and this area has a great potential in terms of natural and cultivated medicinal and aromatic plants with its rich plant diversity, wide surface area and different climates with 11.466 number of plant species. However, this potential in Turkey is not adequately assessed to ensure more effective utilization of medicinal and aromatic plants and to increase the economic returns.

Today, the Developed Countries are trying to construct an economy based on the production of biological products with information content and high added value. The area that exploits the overall economic activity resulting from the sustainable production of food, feed, energy, chemical and industrial and medicinal products from biological sources is briefly defined as Knowledge Based BioEconomy. Medicinal and aromatic plants are important sources of bioeconomic inputs.

In the scope of this study, the main focus will be medicinal and aromatic plants which can be cultivated in Turkey and can provide added-value, some examples of potential value-added products will be given.

KEYNOTE SPEECH

CULTIVATION AND RESEARCH WITH MEDICINAL AND AROMATIC PLANTS IN GERMANY CURRENT IN SITUATION AND FUTURE REQUIREMENTS

Bernd HONERMEIER

Justus Liebig University Giessen (Germany), Institute of Agronomy and Plant Breeding, Chair of Agronomy, Biomedical Research Centre Seltersberg (BFS), Schubert-Str. 81, D-35392 Gießen (Germany)
e-mail: Bernd.Honermeier@agrar.uni-giessen.de

Medicinal and Aromatic Plants (MAPs) are used in Germany to produce herbal and homeopathic medicinal products as well as nutraceuticals, herbal spices, natural pigments and cosmetic products. The demand for plant-based materials for these products is far greater than the domestic production. Around 85 to 90% of the national demand for MAPs plant raw material is imported from many countries. Only a small area of around 12.000 ha arable land distributed among 750 farms and companies is presently used for cultivation of MAPs.

The most important medicinal and aromatic plants (MAPs) cultivated in Germany are essential oil producing plants like chamomile (*Matricaria chamomilla* L.), peppermint (*Mentha x piperita* L.), apple mint (*Mentha suaveolens* L.), garden parsley (*Petroselinum crispum* L.), fennel (*Foeniculum vulgare* L.), common oregano (*Origanum vulgare* L.), common sage (*Salvia officinalis* L.) and lemon balm (*Melissa officinalis* L.) that are characterized by different active compounds and therapeutic uses. In addition, several other crops containing different active compounds are used as medicinal plants in Germany. Relatively important MAPs are: flax seed (*Linum usitatissimum* L.) used as an oil and mucilaginous plant, St. John's wort (*Hypericum perforatum*) as a naphthodianthrone and phloroglucinols containing plant, foxglove (*Digitalis lanata*) as a cardenolide plant, valerian (*Valeriana officinalis*) used for its sedative effects, seabuckthorn/sallow thorn (*Hippophae rhamnoides*) used for vitamin-rich-diet as well as flavonoids and phenolic acids containing crops like marine thistle (*Silybum marianum*), buckwheat (*Fagopyrum esculentum*) and artichoke (*Cynara cardunculus* L.). Moreover, on small areas some more plants like echinacea (*Echinacea* sp.), great yellow gentian (*Gentiana lutea*), common wormwood (*Artemisia absinthium*), evening primrose (*Oenothera biennis*) and borage (*Borago officinalis*) are used for therapeutic applications.

Current research activities with MAPs are focussed particularly on breeding, cultivation, processing and quality management. Running breeding investigations that are concentrating on chamomile, lemon balm and valerian have been conducted for several years. New breeding activities have been started with caraway (*Carum carvi*), peppermint (*Mentha x piperita*) and St. John's wort (*Hypericum perforatum*). The aim is to provide hybrid or semi-hybrid cultivars with improved biomass yield and yield stability, better plant homogeneity, increased resistance against relevant fungus diseases and improved composition of chemical compounds. Based on government-funded projects progress has been made for example in breeding of valerian cultivars.

Cultivation research with MAPs is focussed on developing smart farm technologies e. g. GPS controlled soil tillage, sowing and weeding as well as the use of robots for mechanical weed control. Further work is carried out to improve seed germination of MAPs by coat technology and encrusting to protect seeds and juvenile plants against fungi, bacteria and insect pests. Within a further study charcoal will be investigated to improve degradation of herbicide residues after its application in MAPs.

Among well-known and more important plants there are many underutilized plants with not completely known botanical, chemical and agronomic properties. Therefore there is a need to examine plant characteristics of those plants to enable possible cultivation. Some examples for plants that are currently evaluated in pot or field experiments are e. g. grater celandine, foxglove, centaury, great yellow gentian, arnica, primula and garden ladies mantle.

A wide range of research investigations in laboratories and under on-farm conditions are currently conducted with pyrrolizidine alkaloids (PAs). These compounds are viewed negatively because of their high

liver toxicity in humans and in animals due to the main PA compounds called senecionine, seneciphylline and setosinine and the N oxides derived from them. To prevent a contamination of MAPs cultivated on fields we need to know the presence of these PA compounds in wild plants depending on soil, climate and growing conditions. Up to now we know that PAs are synthesized in several wild plants particularly in those that are belonging to Asteraceae e. g. *Senecio* species and *Tussilago farfara* as well as belonging to Boraginaceae like *Myositis arvensis*, *Lycopsis arvensis*, *Buglossoides arvensis* and *Borago officinalis*. Currently there are several studies in Germany in which we investigate how the PAs will be transferred from wild plant to the medicinal or aromatic plant and how we can control these PA containing plants or their residues by agronomic management on the field and by processing technology.

KEYNOTE SPEECH

CHEMISTRY AND BIOLOGICAL ACTIVITIES OF CARVACROL AND CARVACROL CONTAINING ESSENTIAL OILS

K. Husnu Can BASER

Near East University, Faculty of Pharmacy, Department of Pharmacognosy,
Turkish Republic of Northern Cyprus

Oregano essential oils obtained from the genera *Origanum*, *Thymus*, *Coridothymus*, *Thymbra*, *Satureja* and *Lippia* are rich in carvacrol, a monoterpenic phenol isomeric with thymol. Turkey is the biggest exporter of oregano herb and oil to the world markets. Oregano is mainly used in food, spice and pharmaceutical industries. Carvacrol is responsible for the biological activities of oregano. Many diverse activities of carvacrol such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, antiinflammatory, angiogenic, antiparasitic, antiplatelet, AChE inhibitory, antielastase, insecticidal, antihepatotoxic and hepatoprotective activities and uses such as feed additive, in honeybee breeding and in gastrointestinal ailments, etc. have been described. This paper highlights the biological activities of carvacrol and carvacrol bearing essential oils.

KEYNOTE SPEECH

ECONOMIC ADULTERATION IN HERBS: OREGANO EXAPLE

Tayfur AKIN

Kütaş Tarım Ürünleri Dış Tic. ve San A.Ş. & Kütaş Agro Ziraat ve Tic. A.Ş, Turkey

Adulteration of food ingredients is a major problem for Food Manufacturers who need to ensure that high quality correctly labelled food products are available to the final consumer. Food Manufacturers have the benefit of acquiring the final product, the product is no longer in its natural form; therefore, control of pureness is not easily observed.

Price competition especially has brought about the formulation of low-grade mixtures. These can be from pure blends of spent (exhausted) material or low cost non-characteristic ingredients (adulteration).

Most Commond adulterans are, Olive, Strawberry, Cistus, Mrytle, Sumac, Hazelnut Leaves. In 2015, The study, published by the consumer group Which?, tested 78 samples of dried oregano purchased in the UK and Ireland. It found that 19 of the samples, or around 25 per cent, contained other ingredients such as olive and myrtle leaves.

With the implementation of FSMA (Food Safety Modernization Act) in the US, there is growing awareness for adulteration Worldwide. Codex efforts, re-writing the rules! Kutas co-chairs World Food Codex Herbs Committee. Draft proposal includes specific section for purity and will state «Dried culinary herbs shall be free from any economic adulteration.» (Section 3.2.2 on Adulteration) Turkey produces app. 10-12,000 MT of oregano raw material. This equals app. 7,-8,000 MT of final product However, Turkey export app. 17-18,000 MT. Adulteration will likely happen, because even a good crop size does not meet the global demand.

Physical appearance of Oregano does not have one single characteristic and it can be easily adulterated.

There are two major analysis to identify adulterated product.

1. GC Analysis and volatile oil measurement
2. Microscopic Analysis (Easy way)

Most recent adulterans used is pellet.

TABLE OF CONTENTS

ESSENTIAL OIL COMPOSITION OF SOME MOUNTAIN TEA (<i>Sideritis</i> spp.) SPECIES COLLECTED FROM FLORA OF TURKEY	1
<i>Ünal KARİK, Fatih ÇİÇEK, Erdiç OĞUR, İlknur KÖSOĞLU, Orçun ÇINAR</i>	
NON-WOOD PRODUCT POTENTIAL IN TAURUS CEDAR (<i>Cedrus libani</i> A. Rich): ESSENTIAL OIL.....	7
<i>Ayşe Betül AVCI, Nebi BİLİR</i>	
KÜTAHYA’NIN BAZI BÖLGELERİNDE GELENEKSEL OLARAK KULLANILAN BİTKİLERİN ETNOBOTANİK ÖZELLİKLERİ	12
<i>Hasan Basri KARAYEL</i>	
FARKLI LOKASYONLARDA YETİŞTİRİLEN BAZI ADAÇAYI TÜRLERİNİN (<i>Salvia Officinalis</i> L., <i>Salvia aethiopis</i> L., <i>Salvia fruticosa</i> Mill.)’İN UÇUCU YAĞ BİLEŞENLERİNDEKİ DEĞİŞİMLERİN İNCELENMESİ	18
<i>Hasan Basri Karayel</i>	
DETERMINATION OF ESSENTIAL OIL COMPOSITON OF BLANKET-LEAF (<i>Stachys byzantina</i> C. Koch.) BY MICROWAVE ASSISTED EXTRACTION COUPLED TO HEADSPACE SINGLE-DROP MICROEXTRACTION	28
<i>Zahra KAFIL, Marzieh BABASHPOUR-ASL, Marzieh PIRYAEI</i>	
EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF AQUEOUS AND NONAQUEOUS EXTRACT OF <i>Vitis vinifera</i> L. “WHITE SEEDLESS”. ..	35
<i>Marzieh BABASHPOUR-ASL, Marzieh PIRYAEI</i>	
EFFECTS OF DIFFERENT DRYING METHODS ON VOLATILE ORGANIC COMPOUNDS OF KADIOĞLU-12 APRICOT CULTIVAR	43
<i>Mustafa KAPLAN, Okan LEVENT, Mehmet ALPASLAN</i>	
DETERMINATION OF ORGANIC VOLATILE COMPOUNDS OF MALATYA KADIOĞLU-12 APRICOT CULTIVAR.....	50
<i>Mustafa KAPLAN, Okan LEVENT, Mehmet ALPASLAN</i>	
EXTRACTION OF MEDICAL AND AROMATIC PLANTS: ULTRASOUND	56
<i>Seydi YIKMIŞ, Mustafa KAPLAN, Harun AKSU, Mehmet ALPASLAN</i>	
DETERMINATION OF YIELD AND QUALITY CHARACTERISTICS OF <i>Mentha piperita</i> L. CLON AND VARIETIES CULTIVATED IN ISPARTA CONDITIONS	61
<i>Kemal YILMAZ, İsa TELCİ, Hasan ASLANCAN, Rafet SARIBAŞ, Tanju TURGUT Muhammed BIYIKLI</i>	
EFFECT OF DIFFERENT POTASSIUM DOSES ON THE YIELD, QUALITY COMPONENTS AND NUTRIENT CONTENT OF ANISE (<i>Pimpinella anisum</i> L.)	66
<i>Bülent YAĞMUR, Bülent OKUR, Nur OKUR</i>	
THE INFLUENCE OF DIFFERENT NITROGEN DOSES AND NUMBER OF HARVEST ON CERTAIN AGRICUTURAL PROPERTIES OF TARRAGON (<i>Artemisia dracunculus</i> L)	74
<i>Kemalettin KARA, Şeyma Nur TARHAN</i>	
DETERMINATION OF FLOWER AND SCENT CHARACTERISTICS IN OIL-BEARING ROSE (<i>Rosa damascena</i> Mill.) GENOTYPES DERIVED FROM MUTATION BREEDING	82
<i>Hasan BAYDAR, Sabri ERBAŞ, Keziban SARI</i>	
DETERMINATION OF SOME MORPHOLOGICAL, AGRONOMIC AND QUALITY PROPERTIES OF A CLONES WHICH GENERATED FROM A SELECTED POPULATION OF ISTANBUL OREGANO (<i>Origanum vulgare</i> subsp. <i>hirtum</i> (Link) Iestwaart)*	90
<i>Olçay ARABACI, Emine BAYRAM, Uğur TAN, Çiğdem SÖNMEZ</i>	

DETERMINATION OF THE ESSENTIAL OIL COMPOSITION ON FLOWER AND SEED OF DILL (<i>Anethum graveolens</i> L.) UNDER THE ŞANLIURFA CONDITIONS	96
<i>Abdulhabip ÖZEL, Orçun ÇINAR, İslim KOŞAR</i>	
AN ALTERNATIVE THERAPY FROM GREEN TEA IN PERIODONTAL DISEASE	100
<i>Arzu BEKLEN</i>	
DETERMINATION OF THE TOTAL FLAVONOID SUBSTANCE CONTENT OF ROWANBERRY EXTRACTS	104
<i>Burhan CEYLAN, Yeşim YEŞİLOĞLU</i>	
DETERMINATION OF THE CHLOROPHYLL OF THE ROWANBERRY LEAF	109
<i>Burhan CEYLAN, Yeşim YEŞİLOĞLU</i>	
DEVELOPMENTS IN TURKEY'S BAY LAUREL LEAF PRODUCTION AND TRADE	112
<i>Mustafa ÖZTÜRK, Mükrem TEMEL, Ahmet Bircan TINMAZ, Yasin ÖZDEMİR</i>	
DEVELOPMENT OF LIQUID DOSAGE FORM CONTAINING CONJUGATED LINOLEIC ACID AND L-CARNITINE	118
<i>Veysel Umut ÇELENK¹, Ercüment KARASULU</i>	
LIQUID DOSAGE FORM DEVELOPMENT and CHARACTERIZATION WITH β-(1,3 - 1,6) GLUCAN.....	123
<i>Simge UFACIK ÇELENK, Ercüment KARASULU</i>	
THE EFFECTS Of <i>Pinus pinea</i> L. NEEDLES ESSENTIAL OIL FORTIFIED EGG WHITE PROTEIN POWDER BASED EDIBLE FILMS ON SOME PROPERTIES OF LOR CHEESE.....	130
<i>Nazan KAVAS, Gökhan KAVAS</i>	
THE INVESTIGATION OF GIBBERELIC ACID EFFECTS ON SEED GERMINATION OF PHACELIA (<i>Phacelia tanacetifolia</i> Benthmam) UNDER SALT STRESS.....	134
<i>Ruveyde TUNCTURK, Barış KOCAMAN, Mahdi GHIYASI, Murat TUNCTURK</i>	
EVALUATION THE PERFORMANCE OF SOME SAFFLOWER	141
(<i>Carthamus tinctorius</i> L.) GENOTYPES UNDER MEDITERRANEAN CLIMATE CONDITIONS BASED ON SEED YIELD AND OIL YIELD.	
141	
<i>Mouloud BOUHOUHOU, Samy Mostafa MOHAMED, Elsayed AbouElfotowh OMER and Mourad BENSARI</i>	
CONSUMER HABITS TOWARDS MEDICINAL AND AROMATIC PLANTS IN ERZURUM PROVINCE	145
<i>BANU KADIOĞLU, Sibel KADIOĞLU, Gökhan TAŞGIN</i>	
A TRADITIONAL HEALING “TIRMIS OR TERMIYE” LUPIN (<i>Lupinus albus</i> L.)	149
<i>Sibel KADIOĞLU, Banu KADIOĞLU</i>	
TIBBİ VE AROMATİK BİTKİLER ENDÜSTRİSİ'NDE TEKNOLOJİ TRANSFERİ VE İNOVASYONUN FİRMALARIN ÜRETİM SÜREÇLERİNDEKİ STANDARDİZASYON, AKREDİTASYON VE PATENT HAKLARINA ETKİLERİ	163
<i>Armando ALİU, Mehmet MELEMEN, Dorian ALİU</i>	
USE OF LINSEED AS A MEDICAL AND AROMATIC PLANT IN THE HEALTH SECTOR.....	171
<i>Orhan KURT, Merve GÖRE</i>	
ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS OF ETHANOL EXTRACT OF GRAPE SEED, ROSEMARY, GREEN TEA AND OLIVE LEAF	178
<i>Burcu AKTAŞ, Hatice BASMACIOĞLU-MALAYOĞLU</i>	
DETERMINATION OF THRESHING AND SEPARATING PARAMETERS OF THE POPPY PLANT.....	182
<i>Deniz YILMAZ, Mukadder KARAMAN</i>	

IN VITRO MICROPROPAGATION of MEDICINAL PLANTS via BIOREACTORS.....	190
<i>Meltem BAYRAKTAR, Begüm GÜLER, Merve SEZEN, Aynur GÜREL</i>	
SOME PROPERTIES OF SOILS IN WHERE DIFFERENT AUTUMN-FLOWERING COLCHICUM (<i>Colchicum</i> spp.) SPECIES NATURAL GROWN IN TURKEY	197
<i>Erdoğan UYSAL, Erdal KAYA</i>	
SOME SOIL PROPERTIES OF DIFFERENT ASPHODELINE (<i>Asphodeline</i> spp.) SPECIES NATURAL GROWN IN FLORA OF TURKEY.....	203
<i>Erdoğan UYSAL, Erdal KAYA</i>	
PROPAGATION OF MASTIC TREE: FROM SEED TO TISSUE CULTURE.....	209
<i>Murat İSFENDİYAROĞLU</i>	
A STUDY ON VOLATILES, IN VITRO ANTICHOLINESTERASE INHIBITOR AND ANTIMICROBIAL ACTIVITY OF ENDEMIC <i>Allium tuncelianum</i>.....	214
<i>Rezzan ALIYAZICIOĞLU, Ufuk OZGEN, Nuriye KORKMAZ, Seyda AKKAYA, Sila Ozlem SENER, Merve BADEM, Nevin ULAS COLAK, Sengul ALPAY KARAOĞLU</i>	
BY MEDICINAL PLANTS	219
<i>Esra ERSOY ÖMEROĞLU, Aslı BAYER</i>	
DETERMINATION of VOLATILES, ANTIMICROBIAL and ANTIOXIDANT ACTIVITY of EXTRACTS from FLOWERS of CALENDULA (<i>Calendula officinalis</i> L.).....	223
<i>Didem ŞAHİNGİL, Ali Adnan HAYALOĞLU</i>	
USE OF MINT OIL FOR PROTECTING AND CONTROL AGAINST PHYTOPATHOGENIC FUNGI ON FRUITS AND VEGETABLES DURING POSTHARVEST STORAGE	229
<i>Rezzan KASIM, M. Ufuk KASIM, Aysun ÇAVUŞOĞLU</i>	
ANALYSIS OF FACTORS AFFECTING CONSUMPTION OF MEDICAL AND AROMATIC PLANTS IN URBAN AREA OF IZMIR PROVINCE	236
<i>Hülya ARSLAN, Sait ENGİNDENİZ, Bahar AYDIN CAN, Gökhan ÇINAR</i>	
CHEMICAL COMPOSITION OF SUMMER SAVORY (<i>Satureja hortensis</i>) ESSENTIAL OILS FROM EASTERN ANATOLIA REGION, TURKEY.....	244
<i>Furkan ÇOBAN, Hakan ÖZER, Ufuk ATMACA</i>	
ORGANIC PRODUCTION OPPORTUNITIES WITH NEWLY DEVELOPED BIOLOGICAL FERTILIZERS IN TURKISH TEA CLONE PAZAR-20.....	249
<i>Ramazan ÇAKMAKÇI, Atefeh VARMAZYARI, Erdim ERDURAN</i>	
MORPHOLOGICAL AND YIELD EVALUATION OF FENNEL (<i>Foeniculum vulgare</i> L.) POPULATIONS OF DIFFERENT ORIGIN.....	255
<i>Mahmut ÇAMLICA, Gülsüm YALDIZ</i>	
TIP SHAPE OF LEAF AND BASE SHAPE OF LEAF OF TURKISH TOBACCO POPULATIONS AND AKHISAR 97 CULTIVAR IN BOLU ECOLOGICAL CONDITIONS	262
<i>Mahmut ÇAMLICA, Gülsüm YALDIZ</i>	
DETERMINATION OF DIURNAL VARIABILITY IN SUMMER SAVORY (<i>Satureja hortensis</i> L.) SPECIES IN ITS NATURAL GROWTH AREA.....	267
<i>Memet İNAN, Muzaffer KIRPIK, Durmuş Alpaslan KAYA</i>	
EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOTING OF <i>Laurus nobilis</i> L. STEM CUTTINGS.....	273
<i>Aysun ÇAVUŞOĞLU, M. Ufuk KASIM, Rezzan KASIM</i>	
DETERMINATION OF AGRONOMIC AND QUALITY CHARACTERISTICS OF BLACK CUMIN SPECIES (<i>Nigella sativa</i> L. and <i>N. damascena</i> L.) SOWED AT DIFFERENT TIMES.....	277
<i>Mehmet Necat İZGİ, İsa TELCİ</i>	

EVALUATION OF THE CURRENT STATUS OF IMPORTANT MEDICINAL AND AROMATIC PLANTS IN THE CONTEXT OF INCREASING THE EXPORT POTENTIAL	282
<i>Önder Volkan BAYRAKTAR, Görkem ÖZTÜRK, Doğan ARSLAN, Hüseyin ARSLAN, Sait ENGİNDENİZ</i>	
FORMULATION OF HAZELNUTS FUNCTIONAL PRODUCTS WITH HERBAL EXTRACTS	285
<i>Zuleyhan BARAN, Halil Ibrahim UGRAS, Mehmet Akif SEN, Dicle BARAN, Bora KARAGUL</i>	
USING PROPOLIS EXTRACT AS A FUNCTIONAL FOOD IN HAZELNUT PASTE ...	288
<i>Zuleyhan BARAN, Halil Ibrahim UGRAS, Mehmet Akif SEN, Dicle BARAN, Bora KARAGUL</i>	
THE HEAVY METAL, VOLATILE OIL PHYSICO-CHEMICAL and ANTIMICROBIAL PROPERTIES of SAGE PLANT COLLECTED FROM MUĞLA-BODRUM LOCATION....	292
<i>Erman DUMAN</i>	
INDIGO PLANT (<i>Isatis glauca</i> L.) SEED OIL AND SOME PHYSICO-CHEMICAL PROPERTIES.....	300
<i>Sabire DUMAN, Erman DUMAN</i>	
ELATERIDAE (INSECTA: COLEOPTERA) BIODIVERSITY OF TURKISH MEDICINAL PLANT FLORA.....	305
<i>Nilay GÜLPERÇİN</i>	
GENOTYPIC VARIATION IN SEEDS CHARACTERS IN SOME GENOTYPES OF <i>Citrullus colocynthis</i> UNDER WATER STRESSES	312
<i>EL MADIDI Said, BENMOUMOU Abedlhamid</i>	
EFFECT OF DIFFERENT TREATMENTS ON SEED GERMINATION OF <i>Dorystoechas hastata</i> (Lamiaceae), A MEDICINAL AND AROMATIC PLANT FROM ANTALYA	317
<i>Ceren SELİM, Songül SEVER MUTLU</i>	
VEGETATIVE PROPAGATION OF A RELICT ENDEMIC MEDICINAL AND AROMATIC PLANT OF ANTALYA-TURKEY, <i>Dorystoechas hastata</i> Boiss. & Heldr. EX BENTHAM (LAMIACEAE)	325
<i>Ceren SELİM, Songül SEVER MUTLU</i>	
EFFECTS OF SALICYLIC ACID APPLICATIONS ON ESSENTIAL OIL COMPOSITION AND SALT STRESS TOLERANCE IN SWEET BASIL (<i>Ocimum basilicum</i> L.).....	331
<i>Şengül Nacar KARAMAN, Emel Dıraz YILDIRIM, Halime KAHVECİ</i>	
THE REVIEW OF ARTICLES OF THE STUDYING ON MYRTLE IN THE WORLD..	335
<i>Ayşe EVEREST, Uysal YENİPINAR</i>	
PHARMACOGNOSTIC FEATURES OF THE AERIAL PARTS OF THE MEDICINAL plant <i>Echinops spinosus</i> L.....	349
<i>Amel BOUZABATA</i>	
EFFECTS OF FOLIAR ZINC AND HUMIC ACIDE APPLICATIONS ON THE ESSENTIAL OIL QUALITY AND SEED NUTRIENT CONTENTS OF BLACK CUMIN (<i>Nigella sativa</i> L.).....	355
<i>Zehra AYTAÇ, Nurdilek GÜLMEZOĞLU, Mine KÜRKÇÜOĞLU</i>	

ESSENTIAL OIL COMPOSITION OF SOME MOUNTAIN TEA (*Sideritis* spp.) SPECIES COLLECTED FROM FLORA OF TURKEY

Ünal KARİK¹, Fatih ÇİÇEK¹, Erdinç OĞUR¹, İlknur KÖSOĞLU¹, Orçun ÇINAR²

¹Aegean Agricultural Research Institute, İzmir/TURKEY

²West Mediterranean Research Institute, Antalya/TURKEY

Corresponding author email: unalkarik@gmail.com

Abstract

This study was carried out determining the essential oil content of some species of mountain tea (*Sideritis* spp.) spreading on the Flora of Turkey. *Sideritis* L. species are mostly known in our country as mountain, plateau or spike, and in some regions they are called sage. Despite being consumed only as herbal tea in our country, extracts are made to standard in *Sideritis* L. species in various countries in Europe. In the study, among the species of mountain tea found in our country flora; *Sideritis perfoliata* L., *Sideritis sipylea* Boiss., *Sideritis congesta* P.H.Davis & Hub.-Mor., *Sideritis lanata* L., *Sideritis tmolea* P. H. Davis, *Sideritis vulcanica* Hub.-Mor., *Sideritis stricta* Boiss. & Heldr. and *Sideritis condensate* Boiss. & Heldr. are used. Essential oil yield of the collected plant samples were determined by water distillation method with Clevenger aparate and essential oil components analysed by GC/MS. The yield of essential oils in the examined species were determined as 0,65%, 0,79%, 0,13%, 0,60%, 0,33%, 0,15%, 0,45% and 0,28% respectively. The main components and ratio of in the essential oil were α -pinene 30,16%, myrcene 28,76%, β -pinene 47,80%, germacrene 14,58%, caryophyllene oxide 21,11%, borneol 20,79%, β -pinene 40,61% and β -caryophyllene 34,70%.

Keywords: Mountain tea, *Sideritis* L., essential oil, composition

INTRODUCTION

The genus *Sideritis* L. belongs to the Labiateae Family, tribe Lamieae. This genus comprises more than 150 species which are distributed in temperate and tropical regions of the Northern Hemisphere, from Bahamas to Western China and from Germany to Morocco. Most species are mainly found in the Mediterranean area, from Canary Islands and Madeira to Caucasus; Spain and Turkey possess the highest number of different species. In Spain, most of them are established in the Southeast of Iberian Peninsula and Canary Islands whereas Marmara and Aegean regions predominate in Turkey (Guvenc, et al., 2005; Aslan et al., 2006; Logoglu et al., 2006). The genus name *Sideritis* L., derives from the Greek Word “sideros” (iron) in reference to the use given for these plants since ancient times to heal wounds caused by weapons made with this metal (Font Quer, 2000). In addition to this vulnerary property, *Sideritis* L. species have been popularly used for centuries for their anti-inflammatory, anti-ulcerogenic, digestive and antimicrobial properties (Gonzalez-Burgos et al., 2009).

The extensive and important use of *Sideritis* spp. in Mediterranean traditional medicine has been reported in many ethnopharmacological papers. Alongside its traditional use extensive mentioned, the importance of this genus lies in the type of disorders for which preparations of its aerial parts are used: gastritis, gastric ulcer, mucous membrane inflammation, burns and wounds, among others. For these purposes, infusions and decoctions are prepared from aerial parts and administered both orally and topically as compresses or baths. Moreover, some compounds that were isolated from *Sideritis* spp. have shown antiproliferative, anti-HIV or antifeedant activities (Font Quer, 2000; Gonzalez-Burgos et al., 2009).

Sideritis L. species grow as herbs or small shrubs, both annual or perennial and aromatic. Leaves are often narrow, opposite, entire or crenate–dentate, sessile to petiolate. The number of verticillaster ranges is from two to many and they have terminal spikes; bracteoles are absent. Calyx tubes are tubular–campanulate, 5–10 are veined and five more teeth that are equal or upper larger than the others. The corolla is mostly yellow, rarely white or red and shorter than the calyx. Nutlets are

ovoid, triquetrous, obtuse to round at the apex, smooth and glabrous. *Sideritis* L. species grow optimally in full sun and are well suited to drought conditions. They are found on rocky slopes and pastures, from a few meters above the sea level to more than 3000 m, and require moderately nutrient-rich soils and slightly alkaline (Davis, 1982; Davis et al., 1988). In addition, it is important to point out that the *Sideritis* L. genus contains a large number of endemic species: 46 species, 12 subspecies and two varieties grow in Turkey, 36 species, 10 subspecies and two varieties of them being endemic (77% endemism ratio) (Çarıkçı, 2007). Twenty-five *Sideritis* L. species grow in Morocco, 16 of which are endemic (Ghoumari et al., 2005). Iberian Peninsula and Balears Islands contain 49 *Sideritis* L. species, 36 of them being endemic; in the Canary Islands, this genus is represented by 19 endemic species (Fraga et al., 2009; Morales, 2000, 2010).

Several papers have been previously published about the ethnopharmacological uses of some *Sideritis* L. species. In general, the most frequently recorded medical uses of *Sideritis* spp. are related to their digestive, anti-inflammatory and vulnerary properties. Based on these properties, infusions and decoctions made from aerial parts have been employed for the treatment of gastrointestinal, respiratory and urogenital disorders as well as wound healing. Differences in uses have been observed among *Sideritis* spp. and the region where they grow. However, the uses in everywhere are based on plant properties. In Taurus Mountains of Turkey, a poultice prepared with boiled leaves of *Sideritis psidica*, barley flour, grated onion and pine tar and applied on the abdomen as a plaster is used to treat abdominal pain (Yeşilada et al., 1995). In K. Maras province of Turkey, Karaman and Kocabaş (2001) reported that *Sideritis syriaca* is used as diuretic agent and to relieve cough. An infusion prepared from leaves and flowers of *Sideritis leptoclada* is used because of its expectorant effect and to fight common cold in Inner-West of Anatolia (Turkey) (Kargıoğlu et al., 2008). The endemic specie *Sideritis trojana* is one of the most demanded and consumed medicinal plants in bazaars by Turkmens and villagers who live in the sacred mountain called Mount Ida, “the mountain of the Goodess” (Kazdağı, in modern Turkish) for the treatment of throat, peptic and chest illnesses (Çelik et al., 2008).

MATERIAL AND METHODS

Plants were harvested during the flowering period from Flora of Turkey. The plants belonging to the species used in the study were transformed into herbarium examples and after identification, stored in the herbarium of IZ. *Sideritis* L. species and collected areas were used in this study are listed below (Table 1.). Six of the *Sideritis* L. species examined in this study are endemic. These species are marked in the Table 1.

Table 1. *Sideritis* L. Species and locations.

Species	Location
<i>Sideritis perfoliata</i> L.	İzmir-Yunt Mountain
<i>Sideritis sipylea</i> Boiss.*	Manisa-Spil Mountain
<i>Sideritis congesta</i> P.H.Davis & Hub.-Mor.*	Antalya-Gazipaşa- Maha Mountain
<i>Sideritis lanata</i> L.	İzmir-Bozdağ Mountain
<i>Sideritis tmolea</i> P. H. Davis*	İzmir-Bozdağ Mountain
<i>Sideritis vulcanica</i> Hub.-Mor.*	Elazığ-Maden Mountain
<i>Sideritis stricta</i> Boiss. & Heldr.*	Antalya-Kemer-Ulupınar
<i>Sideritis condensate</i> Boiss. & Heldr.*	Antalya-Gazipaşa- Maha Mountain

*Endemic

Isolation of the Essential Oils

The essential oils from air-dried plant materials were isolated by hydrodistillation for 3 h, using a clevenger-type apparatus. The obtained oils were dried over anhydrous sodium sulphate and stored at +4°C in the dark until analysed and tested.

GC-MS analysis

The essential oil composition of samples was analyzed by gas chromatography (Agilent 5975C) coupled to flame ionization detector and mass spectrometry (Agilent 5975C) using capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm). Essential oils were diluted 1:50 ratio with hexane. GC-MS/FID analysis was carried out at split mode of 50:1. Injection volume and temperature were adjusted as 1 µl and 250 °C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 1 ml/ min. The oven temperature was programmed as follows: 60 °C for 10 minutes, increased at 20 °C/minute to 250 °C, and held at 250 °C for 8 minutes. MS spectra were monitored between 35 and 450 amu and the ionization mode used was electronic impact at 70 eV.

Identification of Compounds

The relative percentage of the components was calculated from GC-FID peak areas, and components were identified by Wiley 7n, Nist 05 and Flavour and Fragrance Natural and Synthetic Compounds (ver. 1.3) Libraries.

RESULTS AND DISCUSSION

The essential oil yield of eight *Sideritis* L. species were studied in this study is summarized in Table 2. The essential oil yield of *Sideritis perfoliata* L., *Sideritis sipylea* Boiss., *Sideritis congesta* P.H.Davis & Hub.-Mor., *Sideritis lanata* L., *Sideritis tmolea* P. H. Davis, *Sideritis vulcanica* Hub.-Mor., *Sideritis stricta* Boiss. & Heldr. and *Sideritis condensate* Boiss. & Heldr were obtained. The yield of essential oils in the examined species were determined as 0,65%, 0,79%, 0,13%, 0,60%, 0,33%, 0,15%, 0,45% and 0,28% respectively.

Many studies have been performed on the chemical composition of essential oil from *Sideritis* L. species. Some qualitative and quantitative differences between the components of the essential oil from the same species were observed. These differences both in the oil content and composition may be due to different reasons such as climatic and genetic factors, agronomical practices, or plant chemotype or nutritional status. Lamiaceae family is well-known because of its richness in essential oil. However, *Sideritis* species have been reported to be poor in essential oil (Kirimer et al., 2001).

The chemical composition of eight *Sideritis* L. essential oils are summarized in Table 2. We identified 18, 13, 13, 17, 15, 19, 30 and 30 components in essential oils belong to *Sideritis perfoliata* L., *Sideritis sipylea* Boiss., *Sideritis congesta* P.H.Davis & Hub.-Mor., *Sideritis lanata* L., *Sideritis tmolea* P. H. Davis, *Sideritis vulcanica* Hub.-Mor., *Sideritis stricta* Boiss. & Heldr. and *Sideritis condensate* Boiss. & Heldr. These components are representing 96,78%, 96,95%, 99,44% 92,92% 90,80%, 95,15%, 99,57% and 95,72% of the total composition of essential oils respectively. The chemical composition of *Sideritis perfoliata* L., *Sideritis sipylea* Boiss., *Sideritis congesta* P.H.Davis & Hub.-Mor., *Sideritis lanata* L., *Sideritis tmolea* P. H. Davis, *Sideritis vulcanica* Hub.-Mor., *Sideritis stricta* Boiss. & Heldr. and *Sideritis condensate* Boiss. & Heldr. were analyzed by GC/MS. The main components and ratio of in the essential oil were α-pinene 30,16%, myrcene 28,76%, β-pinene 47,80%, germacrene 14,58%, caryophyllene oxide 21,11%, borneol 20,79%, β-pinene 40,61% and β-caryophyllene 34,70% respectively. Other important components found in essential oils according to the species are limonene 17,92%, α-pinene 28,64%, α-pinene 35,38%, spathulenol 13,27%, β-caryophyllene 18,74%, terpinen -4-ol 13,03%, α-pinene 18,89% and germacrene-D 25,93%.

Başer (2002) and Kirimer et al. (2004) classified *Sideritis* L. species from Turkey into six groups depending on the main essential oil constituent; these groups were named “monoterpene hydrocarbon-rich”, “oxygenated monoterpene-rich”, “sesquiterpene hydrocarbon-rich”, “oxygenated sesquiterpene-rich”, “diterpene-rich” and “others”. 57% of Turkish *Sideritis* L. species contain monoterpene hydrocarbons as major constituents. α-pinene, β-pinene, α-phellandrene, sabinene and myrcene were found in high amounts.

The essential oil ratio of the natural *Sideritis congesta* species was 0,11%; and *Sideritis condensata* species was 0,18%. The main constituents of both natural and cultivated *Sideritis congesta* essential oil were β - pinene and α -pinene, in natural plants 41,97% and 30,93%; in cultivated plants 28,42% and 20,14%, respectively. The main constituents of *Sideritis condensata* essential oil were caryophyllene and germacrene. These constituents ratio were 21,47% and 19,69% in natural plants; 23,96% and 23,90% in cultivated plants (Gümüşçü et. al., 2011). Özcan et al. (2011) determined the oil yield of *S. congesta* 0,83% in their study, thirty-six components, representing 79,7% of the oil with high contents of muurol-5-en-4-a-ol (11,7%) and muurol-5-en-4-b-ol (33,0%). Muurol-5-en-4-b-ol and muurol-5-en-4-a-ol, linalool, bornyl acetate, α -cadinol, α -pinene and 1 epicubenol were the major constituents of *S. congesta* oil. Ezer et al. (1996) reported that in the essential oil of *S. congesta* α - and β -pinene were the main constituents, while β -caryophyllene and α - pinene were the major constituents of the oil of *S. condensata*. Kırimer et. al., (2003) studied on five endemic *Sideritis* L species and identified their essential oil composition. In mentioned study they found β -pinene 30% and α -pinene 13% as main components in the *S. stricta* essential oil.

Table 2. Essential oil composition of *Sideritis* L. species.

R.T.	Essential Oil (%)	S. pr.	S. sp.	S. cn.	S. ln.	S. tm.	S. vl.	S. st.	S. cd.
		0,65	0,79	0,13	0,60	0,33	0,15	0,45	0,28
	Components								
12,151	alpha-pinene	30,16	28,64	35,38			9,75	18,89	1,23
13,850	α -thujene							1,04	
15,757	beta-pinene	5,27	20,41	47,80			9,16	40,61	3,24
17,459	delta-3-carene			0,67					
17,575	delta-3-carene	4,78							
18,167	myrcene	2,43	28,76	0,66				1,55	
18,780	α -phellandrene							0,91	
19,174	heptanal						2,93		
19,372	beta-myrcene								0,29
19,520	α -terpinene							5,08	
19,842	limonene	17,92	1,30	1,08			3,75	2,36	
20,170	beta-phellandrene					2,24		0,69	
20,202	alpha-terpinene								0,37
20,210	γ -terpinene							1,94	
20,294	sabinene	1,01		0,92			2,08		
21,049	limonene								1,47
22,522	cis-beta-ocimene								0,25
23,432	alpha-terpinolene	5,30							
25,760	p-cymene							0,36	
27,444	3-octanol						1,79		
29,454	1-octen-3-ol					1,27	5,75		
30,070	alpha-cubebene			0,39				0,57	
30,961	bicycloelemene	1,48	1,14		3,06		1,75		0,65
31,345	alpha-copaene			0,53	1,65			0,38	0,76
31,840	Bornyl acetate							1,98	
32,839	linalool	8,15		1,60			1,84		1,06
33,275	linalyl acetate	2,09							
33,498	beta-Bourbonene								0,61
34,046	beta-copaene								0,22
34,854	terpinen-4-ol						13,03	0,43	
34,964	beta-caryophyllene	9,81			5,99	18,74		8,09	34,70
35,316	beta-ylangene								0,55
35,705	beta-elemene								1,70
35,898	beta-cubebene								0,36
36,487	trans-beta-farnesene					4,98	0,63		
37,449	neral		4,02		4,87	3,55			
37,663	alpha-terpineol	0,96			1,59		2,21		

37,849	borneol	1,02					20,79		
38,370	germacrene		1,25	0,61	14,58	7,68	2,90		
38,535	beta-bisabolene						10,96		
38,894	geranial		6,47		10,00	4,24			
39,018	bicyclogermacrene	2,52	1,97		5,36		1,22		7,94
39,153	geranyl acetate				1,44				
39,247	neryl acetate	0,73				1,42			
39,398	cis-muuroala-3,5-diene				2,88				0,34
39,410	trans-muuroala-3,5-diene							0,45	
39,430	delta-cadinene			1,38					1,31
39,495	germacrene D								25,93
39,567	beta-gurjunene					1,52			
39,650	13(16),14-labdien-8-ol							0,45	
39,783	alpha-curcumene					7,60			
39,785	α -humulene							1,00	
39,791	ar-curcumene				6,62				
40,087	alpha-farnesene								1,11
41,605	geraniol		0,97						
43,062	gamma-muurolene			6,49					
44,395	gamma-cadinene			1,94	1,08				1,31
45,927	methyl eugenol						1,16		
45,950	borneol							0,86	
45,973	caryophyllene oxide	0,94			11,90	21,11	1,86		2,58
46,240	muuroala-4(14),5-diene							0,90	
46,465	nerolidol				3,18				
47,275	humulene epoxide					1,32			
48,689	alpha-cis-bergamotene		0,66						
48,730	hexahydrofarnesyl acetone					2,30			
48,951	cis-Sesquisabinene hydrate								1,03
49,020	spathulenol	0,86	0,52		13,27	10,32	1,59		1,37
49,247	viridiflorol								0,26
49,942	tau-cadinol				2,14				
49,950	epibicyclosquiphellandrene					2,52		2,93	
49,960	trans-cadina-1,4-diene							0,73	
50,240	cis-muurool-5-en-4-alpha-ol							1,65	
50,750	cis-muurool-5-en-4-beta-ol							0,34	
50,860	caryophyllene oxide							0,59	
50,957	alpha-bisabolol	1,36							
50,980	1,10-diepi-cubenol							0,49	
51,000	1-epi-cubenol							1,48	
51,120	manool							0,58	
51,606	epi-alpha-muurolol								0,86
51,680	labd-7,13-dien-15-ol-acetate							1,05	
51,844	alpha-muurolol								1,45
52,003	beta-sesquiphellandrene								0,70
52,644	beta-Curcumen-12-ol								0,72
52,840	longifolol							1,19	
53,053	nuciferol		0,86		3,29				
57,555	2,6-trans,trans-farnesol								1,35
TOTAL (%)		96,78	96,95	99,44	92,92	90,80	95,15	99,57	95,72

S. pr.: *Sideritis perfoliata*, S. sp.: *Sideritis sipylea*, S. cn.: *Sideritis congesta*, S.ln.: *Sideritis lanata*, S. tm.: *Sideritis tmolea*, S. vl.: *Sideritis vulcanica*, S. st.: *Sideritis stricta*, S. cd.: *Sideritis condensate*

REFERENCES

Aslan, I., Kilic, T., Goren, A., Topçu, G., 2006. Toxicity of acetone extract of *Sideritis trojana* and 7-epicandiciol, 7-epicandiciol diacetate and 18-acetylsideroxol against stored pests *Acanthoscelides obtectus* (Say), *Sitophilus granarius* (L.) and *Ephestia kuehniella* (Zell). Industrial Crops and Products 23: 171–176.

- Başer, K.H.C., 2002. Aromatic biodiversity among the flowering plant taxa of Turkey. *Pure and Applied Chemistry* 74: 527–545.
- Çarıkçı, S., Col, C., Kilic, T., Azizoğlu, A., 2007. Diterpenoids from *Sideritis tmolea* P.H Davis. *Records of Natural Products* 1: 44–50.
- Çelik, S., Karabacak, E., Uysal, I., 2008. Plants have been collected from mythological Kazdağ (Mt. Ida) National Park, West Turkey by turkmens and their folk, cultural and social uses. *European Journal of Scientific Research* 19: 835–843.
- Davis, P.H., 1982. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, p. 7.
- Davis, P.H., Mill, R.R., Kit, Tan., 1988. *Flora of Turkey and the East Aegean Islands*. Edinburgh University Press, Edinburgh, p. 10.
- Dülgeroğlu C. 2013. Comparison of Wild and Cultivated Form of *Sideritis stricta* Boiss. & Heldr. (Lamiaceae), Regarding Their Ecological, Anatomical, Morphological and Volatile Oil Properties. Akdeniz University, Graduate School of Natural and Applied Sciences MSc Thesis in Biology, 133 pp.
- Ezer, N., R. Vila, S. Cañigual and T. Adzet, 1996. Essential oil composition of four Turkish species of *Sideritis*. *Phytochemistry*, 41(1): 203-205.
- Font Quer, P., 2000. *Plantas Medicinales. El Dioscorides Renovado*. Ediciones Peninsula, Barcelona.
- Fraga, B.M., Hernandez, M.G., Fernandez, C., Santana, J.M., 2009. A chemotaxonomic study of nine Canarian *Sideritis* species. *Phytochemistry* 70: 1038–1048.
- Ghoumari, H., Benajiba, M.-H., Azmani, A., Garcia-Granados, A., Martinez, A., Parra, A., Rivas, F., Socorro, O., 2005. Ent-kauranoid derivatives from *Sideritis moorei*. *Phytochemistry* 66, 1492–1498.
- Gonzalez-Burgos, E., Gomez-Serranillos, M.P., Palomino, O.M., Carretero, M.E., 2009. Aspectos botánicos y farmacológicos del género *Sideritis*. *Revista de Fitoterapia* 9: 133–145.
- Güvenç, A., Houghton, P.J., Duman, H., Coskun, M., Şahin, P., 2005. Antioxidant activity studies on selected *Sideritis* species native to Turkey. *Pharmaceutical Biology* 43: 173–177.
- Gümüşçü A. Osman Tugay Yüksel Kan 2011. Comparison of Essential Oil Compositions of Some Natural and Cultivated Endemic *Sideritis* Species *Advances in Environmental Biology*, 5(2): 222-226.
- Karaman, S., Kocabas, Y.Z., 2001. Traditional medicinal plants of K. Maras (Turkey). *The Sciences* 1, 125–128.
- Kargioğlu, M., Cenkcı, S., Serteser, A., Evliyaoğlu, N., Konuk, M., Şamil Kok, M., Bağcı, Y., 2008. An ethnobotanical survey of Inner-West Anatolia, Turkey. *Human Ecology* 36: 763–777.
- Kırimer, N., Baser, K.H.C., Demirci, B., Duman, H., 2004. Essential oils of *Sideritis* species of Turkey belonging to the section Empedoclia. *Chemistry of Natural Compounds* 40: 19–23.
- Kırimer, N., Tabanca, N., Demirci, B., Baser, K.H.C., Duman, H., Aytac, Z., 2001. The essential oil of a new *Sideritis* species: *Sideritis ozturkii* Aytac and Aksoy. *Chemistry of Natural Compounds* 37: 234–237.
- Kırimer, N., Tabanca, N., Ozek, T., Baser, K.H.C., Tumen, G., Duman, H., 2003, Composition of Essential Oils from Five Endemic *Sideritis* Species. *Journal of Essential Oil Research*, 4: 221-225.
- Loğoğlu, E., Arslan, S., Oktemer, A., Şakiyan, I., 2006. Biological activities of some natural compounds from *Sideritis sipylea* Boiss. *Phytotherapy Research* 20: 294–297.
- Morales, R., 2000. Diversidad en Labiadas Mediterraneas y Macaronesicas. *Portugaliae Acta Biologica* 19: 31–48.
- Morales, R., 2010. *Sideritis*. In: Castroviejo, S., Morales, R., Quintanar, A., Cabezas, S., Pujadas, A.J., Cirujanos, S. (Eds.), *Flora Iberica XII plantas vasculares de la Peninsula Iberica e Islas Baleares*. Real Jardin Botanico Consejo Superior de Investigaciones Cientificas, Madrid, pp. 1–56.
- Özcan, M., J.C. Chachat and A. Akgül, 2001. Essential oil composition of Turkish mountain tea (*Sideritis* spp.). *Food Chemistry*, 75(4): 459-463.
- Yeşilada, E., Honda, G., Sezik, E., Tabata, M., Fujita, T., Tanaka, T., Takeda, Y., Takaishi, Y., 1995. Traditional medicine in Turkey V. Folk medicine in the inner Taurus Mountains. *Journal of Ethnopharmacology* 46: 133–1

NON-WOOD PRODUCT POTENTIAL IN TAURUS CEDAR (*Cedrus libani* A. Rich): ESSENTIAL OIL

Ayşe Betül AVCI¹, Nebi BİLİR²

¹Ödemiş Vocational School, Ege University, 35760, Izmir, Turkey

²Forestry Faculty, Applied Sciences University of Isparta, 32260, Isparta, Turkey

Corresponding author email: nebibilir@sdu.edu.tr or nebilir@hotmail.com

Abstract

Taurus cedar or also called Lebanon cedar (Cedrus libani A. Rich.), is one of the most important tree species for Turkish forestry because of its valuable commercial wood and potential non-wood products (e.g, essential oil), occupies mainly in Taurus mountains of Turkey except of a few stands in Lebanon and Syria. It has the largest natural distribution in Turkey by 482391 ha of which 49% to be unproductive in whole the world. Utilization is very limited because of the limited occupation and productive forest of the species. In this context, wood production, which is an important utilization from the species, inhibited because of the limitation. In this situation, new products have to be investigated for an alternative utilization such as essential oil extraction from different part of trees.

We aimed to discussion alternative product based on limited damage to the forests, to give basic information for future studies such as quality and using of seeds for oil, and to contribute local and national economy based on early studies on the species in this paper.

Keywords: *Cedrus libani*, seed, essential oil, constituent, heritability

INTRODUCTION

Taurus cedar or also called Lebanon cedar (*Cedrus libani* A. Rich) has the largest natural distribution mainly on the Taurus Mountains in southern Turkey, and small populations are remained in Syria and Lebanon in whole the world (Boydak, 2003). It is also classified as one of the economically important species for Turkish forestry and the “National Tree Breeding and Seed Production Programme” (Koski and Antola, 1993) because of its valuable wood product and potential non-wood products (e.g, essential oil). Taurus cedar has the largest natural distribution in Turkey by 482391 ha of which 49% to be unproductive in whole the world (Anonymous, 2017). Utilization is very limited because of the limited occupation and productive forest of the species. In this context, wood production, which is an important utilization from the species, inhibited because of the limitation. However, cones of the species are collected to local people for plantations by Ministry of Forestry and Water Affairs (Fig.1). It could be said this is only an economical utilization on present forests of the species for local income. In this situation, new products have to be investigated for an alternative utilization such as essential oil extraction from different part of trees.

Some early researches were carried out on ratio and constituent of extraction of bark, wood, cones (i.e., Hafizoğlu, 1987a-b; Hafizoğlu and Holmbom, 1987a-b; Başer and Demirçakmak, 1995) and ratio of oil by Çetin *et al.*, (2009). They reported that the species had very rich for oil and extractives. These products are used for many purposes such as medicinal, cosmetics, insecticide, herbicide and fungicide. Çetin *et al.*, (2009) investigated essential oil, as a natural insecticide, obtained 1.72% from seeds of the species. Similar studies were also carried out by DıĖrak *et al.* (1999), Lozzio *et al.* (2008), and Esen and DıĖrak (2009).

Bilir and Avcı (2013) studied to determine potential of essential oil isolated from seeds of Taurus cedar sampled from 25 open pollinated families of each three natural populations to estimate rate and components of essential oil. They found average of essential oil rate was 1.82%, while there were large differences among populations and within population for the rate. Totally, 137 components were determined, while α -pinene (27.08%) and β -pinene (20.35%) were major components in the oil.

Heritability was low (0.27) for the oil rate, while it was very high (>0.78) for the rate of major components. Avcı and Bilir (2017) emphasized the importance of growth and geographic characters on content and main constituents of essential oil in the species.



Fig. 1. Cones of the species.

In this paper, we aimed to discuss alternative product based on limited damage to the forests, to give basic information for future studies such as quality and using of seeds for oil, and to contribute local and national economy based on early studies on the species.

MATERIAL AND METHODS

In this review paper, mainly the data collected based on a project (TOVAG-110O474) from following populations (Table 1) was used published by Bilir and Avcı (2013), and Avcı and Bilir (2017), and also related early publications (i.e., Hafizoğlu, 1987a-b; Hafizoğlu and Holmbom, 1987a-b; Çetin *et al.*, 2009).

Table 1. Some details of studied populations.

Population name and code	Latitude (N)	Longitude (E)	Altitude (m)
Egirdir (P1)	37°44'	30°49'	1565
Senirkent (P2)	38°05'	30°42'	1490
Sarkikaraagac (P3)	37°53'	31°17'	1620

Cedar seeds were separated from the cones collected from the 25 open pollinated families of three natural populations of the species. Essential oil was obtained by hydrodistillation for 3 h with using of Clevenger type apparatus from Taurus cedar seeds with four replications. The volatile oils were stored in dark glass bottles at 4 °C until analysis. The essential oil was analyzed by gas chromatography-mass spectrometry system in TUBITAK-MAM (Bilir and Avcı, 2013; Avcı and Bilir, 2017).

RESULTS AND DISCUSSION

Averages of essential oil content were 1.66%, 1.71% and 2.09% in the populations, while it was 1.82% in polled populations. It varied between 0.73% and 3.20% among families. Essential oil yield was 2.3 ml/tree ranged from 0.1 ml to 7.9 ml (Avcı and Bilir, 2017). Average of essential oil content was found 1.72% in seeds of six populations of the species (Çetin *et al.*, 2009). It was between 0.80% and 2.75% in the populations (Çetin *et al.*, 2009). High rate and rich components in essential oil were found in different parts of *Cedrus libani* (Hafizoglu, 1987a,b; Hafizoglu and Holmbom, 1987a,b; Çetin *et al.*, 2009). The rate was 1.82% in needles of *Cedrus atlantica* (Derwich *et al.*, 2010). The rate of essential oil could change according to part of the plants. For instance, rates

of essential oil were 0.21% in cones, 0.41% in cones, and 0.68% in wood of *Cedrus libani* (Lazzio, *et. al.*, 2007). It could be said that essential oil rate was higher in seeds than that of other parts of plants in *Cedrus libani*.

Table 1. The highest percentage and most abundant components in populations.

Components	RI*	Rates (%)			
		Population 1	Population 2	Population 3	Pooled populations
α -Pinene	932.29	28.74(19)**	27.40 (17)	33.47 (24)	29.3 (60)
1-Hexen-3-yne	974.89	7.89 (10)	4.267 (6)	5.184 (3)	5.78 (19)
β -Pinene	975.39	22.27 (15)	17.76 (15)	22.82 (23)	20.9 (53)
β -Myrcene	989.93	1.27 (3)	2.576 (1)	1.042 (12)	1.63 (16)
Cyclohexene	1027.7	1.08 (1)	8.41 (3)	1.407 (8)	3.63 (12)
D-Limonene	1028	2.81 (2)	2.382 (1)	3.1 (4)	2.76 (7)
β -Phellandrene	1028	-	19.97 (1)	3.72 (1)	11.84 (7)
Bicyclo[3,1,1]heptan-3-ol	1141	4.02 (7)	5.95 (8)	4.20 (7)	4.72 (22)
trans-Pinocarveol	1141	5.56 (4)	5.35 (2)	3.85 (7)	4.92 (13)
Isopinocarveol	1141.2	3.81 (2)	2.17 (6)	0.89 (3)	2.92 (11)
Bicyclo[3,1,1]hept-3-en-2-ol	1146.8	3.87 (8)	-	1.96 (12)	2.91 (22)
2(10)-Pinen-3-one	1163.8	5.78(17)	3.49 (22)	4.00 (14)	3.09 (53)
Borneol	1168.6	2.78 (3)	1.68 (6)	1.47 (6)	2.00 (15)
3-Cyclohexen-1-ol	1178.9	2.65 (4)	2.24 (7)	2.28 (15)	2.39 (26)
3-Cyclohexene-1-methanol	1193	-	3.57 (4)	5.54 (2)	4.55 (6)
p-menth-1-en-8-ol	1193	2.93 (10)	2.64 (9)	1.80 (16)	2.45 (35)
Bicyclo[3,1,1]hept-2-ene-2-carboxaldehyde	1197.1	4.38(11)	6.45 (6)	3.47 (9)	4.77 (26)
(1R)-(-)-Myrtenal	1197.6	3.34 (8)	2.94 (7)	5.67 (3)	3.98 (18)
3-Caren-10-al	1197.6	-	6.31 (3)	3.18 (4)	4.74 (7)
Bicyclo[3,1,1]hept-3-en-2-one	1211.4	5.77 (5)	-	2.34 (8)	4.05 (13)
3-Diazo-1,7,7-trimethylbicyclo[2,2,1]heptan-2-one	1211.9	1.56 (10)	2.07 (6)	2.32 (1)	1.99 (17)
trans-2-Caren-4-ol	1276.4	1.91 (5)	1.57 (3)	2.05 (3)	1.84 (11)
Bicyclo[2,2,1]heptan-2-ol	1285.5	7.69 (1)	6.68 (10)	3.88 (17)	6.08 (28)
Bornyl acetate	1286	-	6.36 (2)	4.93 (2)	5.65 (4)
Isobornyl propionate	1286	1.87 (11)	2.05 (13)	2.49 (5)	2.14 (29)

**, Retention Indice; **, Number of identified family of the components.

Totally, 137 components were determined, while α -pinene (27.08%) and β -pinene (20.35%) were major components in the oil. 137 components representing 99% of the total oil were identified in pooled populations. Only 25 of total components were in all the populations, while 28, 20 and 38 of total components were only in first, second and third population, respectively. Beside, 60 components were identified only in an each family. Major components of pooled populations were α -Pinene (29.5%) and β -pinene (21%). The other major components were 1-Hexen-3-yne (5.78%) and Bicyclo[2.2.1]heptan-2-ol (6.08%) based on rate and identified family numbers. The ten highest components were presented in together with rate and retention indices in Table 1 (Bilir and Avcı, 2013). Başer and Demirçakmak (1995) reported 37 components representing 90-93% of total essential oil in stem and root of *Cedrus libani*.

There were significant ($p \leq 0.05$) differences among populations for rate and components according to results of analysis of variance (Bilir and Avcı, 2013). Significant ($p \leq 0.05$) differences were also determined among the populations, age, diameter, and altitudinal classes for content of essential oil emphasized importance in terms of growth and geographic effects on oil content (Avcı and Bilir, 2017). It was known that there were many environmental and genetic effects on essential oil content and constituents (Gönüz and Özörgücü, 1999; Mori *et. al.*, 2009; Avcı, 2011).

Low heritable ($h^2 = 0.27$) was reported for oil content and high ($h^2 > 0.78$) for the content of major constituents (α -pinene and β -pinene) in the populations (Bilir and Avcı, 2013). It could be said that, while rate was under environmental effect, genetic factors was very effective on components based on estimated repeatability and coefficients of variations. Repeatability was 0.71 in rate of essential oil in *Eucalyptus camaldulensis* (Avcı and Bilir, 2009). It was 0.5 for 1,8-cineole of *Eucalyptus camaldulensis* (Doran, 1994). The genetic parameters were used in increasing of quality and quantity of essential oil (e.g., Burdon *et. al.*, 1992).

CONCLUSION

As emphasized by Bilir and Avcı (2013), seed sources should be selected/established to isolated essential oil in the species. In this selection population and altitudes should be taken into consideration for higher essential oil production. The present study was carried out by one year data and restricted populations. New studied should be carried out by large populations and periods (Avcı and Bilir, 2017).

Quality classification should be prepared based on quantity of components in essential oil isolated from seeds of the species.

ACKNOWLEDGEMENT

Authors thank to the Scientific and Technological Research Council of Turkey (TUBITAK) for the financial support (TOVAG-110O474).

REFERENCES

- Anonymous, 2017. Forest inventory of Turkey. General Directorate of Forestry of Turkey, pp. 28, Ankara, Turkey.
- Avcı, A.B., Bilir, N., 2017. Effects of growth and geographic characteristics on essential oil of Taurus cedar (*Cedrus libani* A. Rich.). FEB, 26 (6) 3919-3923.
- Avcı, A.B., 2011. Chemical variation on the essential oil of *Thymus praecox* subsp. *scorpilii* var. *laniger*. International Journal of Agriculture and Biology, 13: 607-610.
- Avcı, A.B., Bilir, N., 2009. Essential oil production and its repeatability of River red gum (*Eucalyptus camaldulensis*) in Denizli District. 1st international and 5th national Vocational Symposium. p. 46-467, SU, 27-29 May, Konya, Turkey.
- Doran, J.C., 1994. Genetic parameters and expected gains from selection for monoterpene yields in petford *Eucalyptus camaldulensis*. New Forests. 8:155-167.
- Başer, H.C., Demirçakmak, B., 1995. The Essential Oil of Taurus Cedar (*Cedrus libani* A. Rich): Recent Results. *Chemistry of Natural Compounds*, 31:16-20.
- Bilir, N., Avcı, A.B., 2013. Essential oil in Taurus cedar (*Cedrus libani* A. Rich) seeds. Journal of Essential Oil and Bearing Plants, 16 (4): 538-544.
- Boydak M. 2003. Regeneration of Lebanon cedar (*Cedrus libani* A.Rich.) on karstic lands in Turkey. Forest Ecology and Management, 178: 231-243.
- Burdon, R.D., Gaskin, R.E., Low, C.B., Zabkiewicz, J.A., 1992. Clonal Repeatability of Monoterpene Composition of Cortical Oleoresin of *Pinus radiata*. New Zealand Journal of Forestry Science. 22:299-305.
- Çetin, H., Kurt, Y., Isik, K., Yanikoglu, A., 2009. Larvicidal effect of *Cedrus libani* seed oils on Mosquito *Culex pipiens*. Pharmaceutical Biology, 47: 665-668.
- Derwich, E., Benziene, Z., Boukir, A., 2010. Chemical composition and in vitro antibacterial activity of the essential oil of *Cedrus atlantica*. International Journal of Agriculture and Biology. 12:381-385.
- Dıġrak, M., İlçim, A., Alma, M.H., 1999. Antimicrobial Activities of Several Parts of *Pinus brutia*, *Juniperus oxycedrus*, *Abies cilicia*, *Cedrus libani* and *Pinus nigra*, *Phytother. Res.*, 13:584-7.
- Esen, M., Dıġrak, M., 2009. Antimicrobial Activity of some Essential Oils which are Obtained from some Trees in Kahramanmaraş Provinces. Firat Univ. Journal of Science. 21:33-43.
- Gönüz, A., Özörgücü, B., 1999. An investigation on the morphology, anatomy and ecology of *Origanum onites* L. Turkish Journal of Botany. 23 (1): 19-32.

- Hafizoglu, H., 1987a. Studies on the chemistry of *Cedrus libani* A. Rich. I. wood extractives of *Cedrus libani*. *Holzforschung*. 41: 27-38.
- Hafizoglu, H., 1987b. Extractive constituents of *Cedrus libani*. *Journal of Forestry Faculty of Karadeniz Technical University*. 10:91-98.
- Hafizoglu, H., Holmbom, B., 1987a. Studies on the chemistry of *Cedrus libani* A. Rich. II. lipophilic constituents of the cedar bark. *Holzforschung*. 41: 73-77.
- Hafizoglu, H., Holmbom, B., 1987b. Studies on the chemistry of *Cedrus libani* A. Rich. III. oleoresin composition of cones and bark from *Cedrus libani*. *Holzforschung*. 41:141-145.
- Koski, V., Antola, J., 1993. National tree breeding and seed production programme for Turkey 1994-2003. The Research Directorate of Forest Tree Seeds and Tree Breeding, Ankara, Turkey.
- Lozzio, M.R., Saab, A., Tundis, R., Statti, G.A., Lampronti, I., Menichini, F., Gambari, R., Cinatl, J., Doerr, H. W., 2008. Phytochemical analysis and in-vitro evaluation of the biological activity against herpes simplex virus type 1 (HSV-1) of *Cedrus libani* A. Rich. *Phytomedicine*, 15 (1-2): 79-83.
- Mori, C.L.S.O., Brito, J.O., Scolforo, J.R.S., Vidal, E.J., Mendes, L.M., 2009. Influence of altitude, age and diameter on yield and alpha-bisabolol content of Candeia trees (*Eremanthus erythropappus*). *Cerne Lavras*, 15(3): 339-345.

KÜTAHYA’NIN BAZI BÖLGELERİNDE GELENEKSEL OLARAK KULLANILAN BİTKİLERİN ETNOBOTANİK ÖZELLİKLERİ

Hasan Basri KARAYEL¹

¹Dumlupınar Üniversitesi Gediz MYO.Tıbbi Ve Aromatik Bitkiler, Kütahya, TÜRKİYE

Corresponding author email: kbasri23@hotmail.com

Özet

Bu çalışma, 2016-2017 yılları arasında Kütahya merkez ve Tavşanlı, Gediz ilçelerin çevresinde tespit edilen bazı bitkilerin, etnobotanik özellikleri ve yerel isimleri araştırılmıştır. Bu bitkilerin kullanılma bilgisi bölge halkı ve aktarlarla yapılan görüşmelere göre belirlenmiştir. Araştırma alanındaki 40 bitkinin mahalli isimleri ve kullanım alanları tespit edilmiştir. Bunların 30’u tıbbî, 3’ü gıda, 7’si de diğer (yakacak, hayvan yemi vb.) alanlarda kullanıldığı sonucuna varılmıştır.

Anahtar Kelimeler: Etnobotanik, Kütahya, yerel adlar.

GİRİŞ

Doğadaki tüm hayvanlar, bitkiler ve insanlar bir dengenin ürünüdürler. Mitolojide bitkiler tanrıların insana verdiği en değerli armağan olarak ele alınmıştır. İnsanın var oluşundan itibaren bitkilerle olan ilişkisi başlamıştır (Gezgin, D ,2006). İlk çağlardan kalan arkeolojik bulgulara göre insanlar, besin elde etmek ve sağlık sorunlarını gidermek için öncelikle bitkilerden faydalanmışlardır. Deneme yanılma yoluyla elde edilen bu bilgiler, çağlar boyunca kullanım şekillerindeki bazı değişiklik ve gelişmelerle günümüze kadar gelmiştir (Koçyiğit, M. 2005). Etnobotanik, geniş anlamda “evrim süreci içinde insan-bitki ilişkileridir.” Dar anlamda ise “bir yörede yaşayan halkın çevresinde bulunan bitkilerden çeşitli gereksinimlerini karşılamak üzere yararlanma bilgisi ve o bitkiler üzerine etkileri” olarak özetlenebilir. Etnobotanik araştırmalar, deneme yanılma yoluyla edinilmiş ve uzun bir zaman süreci sonucunda nesilden nesile aktarılacak günümüze kadar ulaşan çok değerli bilgileri yansıtan içerikleri ile bitkilerin bilimsel olarak değerlendirilmelerine önemli katkıda bulunmaktadır. Zengin bir kültürel mirasa sahip olan ülkemizin de etnobotanik açıdan oldukça kapsamlı bir bilgi hazinesi mevcuttur (Kendir. G., Güvenç. A, 2010)

Ancak, kırsal kesimden kentlere olan göçlere ve gelişen teknolojiye paralel olarak, yeni nesiller bu hazinenin değerini bilmemekte ve bu bilgiler kullanılmadığı için kaybolma riski taşımaktadır. Bu nedenle çok değerli bu bilgilerimizin bir an önce yazılı hale getirilme zorunluluğu ortaya çıkmaktadır. Bu zorunluluk ülkemiz ekonomisi açısından da önemlidir. Hangi bölgelerde hangi bitkilerden yararlanılabileceğinin tespiti, ancak etnobotanik çalışmalar ışığında belirlenebilecektir ve böylece halktan alınan bilgiler halkın ekonomisine katkı sağlaması için geri dönecektir (Başaran, S,2003 Sadıkoğlu, N., 1998). İnsanoğlu tarafından geçmişten günümüze kadar gelen bu kullanım alanlarının ve kullanılan bu bitkilerin yerel isimlerinin bilinmesi, bu mirasın yeni kuşaklara aktarılması çok büyük önem taşımaktadır (Yıldırım, 2004).

Günümüzde insanlar hastalıkların tedavisinde alternatif tıpta kullanılan bitkisel droglara da başvurumaktadırlar. Ülkemizde son yıllarda yapılan etnobotanik çalışmalarda kullanılan türlerin envanterleri çıkarılmaktadır (Tümen, G. ve Sekendiz.1989, A, Honda ve ark.1996).Türkiye’de özellikle kırsal kesimde yaşayan halk tarafından toplanan bitkiler geçmişten beri süregelen inanç ve gelenekler ışığında çeşitli hastalıkların tedavisinde kullanılmaktadır. Uzun yıllar kullanılarak denenmiş bitkiler kullanım şeklini bilen aktarlardan elde edilebilmektedir (Malyer, H ve ark.2004).

Ege Bölgesi’nin İç Batı Anadolu Bölümü’nde bulunan Murat Dağı Floristik yönden araştırılmıştır. Murat Dağının Kütahya iline uzaklığı 130 km. Gediz ilçesine uzaklığı ise 30 km. dir.

Tavşanlı ilçesi Kütahya'ya uzaklığı 51 km'dir. Kütahya ve Uşak illeri sınırları içinde yer alan ve en yüksek noktası 2309 m. olan Murat Dağı kuzeybatı-güneydoğu yönünde uzanır. Akdeniz, Karadeniz ve İç Anadolu iklimleri etkisi altında bulunan araştırma bölgesi, bu özelliği nedeniyle zengin bir floraya sahiptir. 1976-78 ve 1980 yıllarında bölgeye yapılan on beş gezi sonunda Murat Dağı ve yakın çevresinde 814 taksona ait 1765 örnek toplanmıştır.

Bu çalışmada, Türkiye'nin batısında yer alan Kütahya ili Gediz ilçesi Murat dağı alanı ve çevresindeki bölgede yaşayan halkın kullandığı bitkilerin yöresel ve bilimsel adlarının tespiti, etnobotanik özelliklerinin belirlenmesi ve kayıt altına alınması amaçlanmıştır (Deniz. L. , Serteser. A. , Kargıoğlu.M.,2010). Etnobotanik özelliği olan bu bitkiler sırasıyla familya, yöresel adları, kullanılan kısımları, kullanım amacı belirlenmiştir.

MATERYAL VE METOT

Bu çalışma da Gediz (Kütahya) ilçesi florasına ait bazı bitki örnekleri ele alınmıştır. Bölgeden toplanan bitki örnekleri kurutulup herbaryum çalışmaları yapılmakta ve örneklerin teşhisi için ' Flora of Turkey' eserinde yararlanılmaktadır(Deniz. L. , Serteser. A. , Kargıoğlu.M.2010). Murat dağı çevresinde bulunan Çukurören, Gümüşlü, ve Gümele köylerinde Tavşanlı mekezde yaşayan 60 yaş üstü 11'i bayan, 25'i erkek toplam 36 kişi ile görüşülmüştür. Ayrıca Kütahya ili Gediz ve Tavşanlı ilçesinde bulunan 5 Aktarında görüşleri alındıktan sonra bitkilerin yöresel isimleri ve kullanım şekilleri hakkında bilgi edinilmiştir.

Bulgular kısmında önce Türkçe ismi, yerel ismi, Latince ismi, familyası, bitkinin kullanılan kısmı, kullanım şekli ve kullanımı çizelge 1,çizelge 2'de belirtilmiştir. Bitkilerin Latince isimleri alfabetik sırasına göre verilmiştir.

Çizelge 1. de Türkçe ismi, yerel ismi, Latince ismi, familyası, bitkinin kullanılan kısmı, kullanım şekli ve kullanımı yer almıştır.(Gediz).

TÜRKÇE ADI	YEREL ADI	LATİNCE ADI	FAMİLYA	KULLANILAN KISIM	KULLANIM ŞEKLİ	KULANIM
Anadolu Adaçayı	Elma otu	<i>Salvia triloba</i>	Lamiaceae	Yaprak ve çiçekleri	İnfüzyon	Akciğer rahatsızlıkları, gaz söktürücü, idrar artırıcı.
Alıç	Yemişen	<i>Crataegus Monogyna</i>	Rosaceae	Meyveleri, Çiçekleri ve yaprakları	İnfüzyon	Kalp kasları güçlendirir kalpdamarlarını genişletir ve kandaşımını rahatlatır.
Altın Otu	Sarı Çiçek, Arı çiçeği	<i>Helichrysum Graveolens</i>	Asteraceae	Çiçekleri	İnfüzyon	İdrar yolları iltihabında, idrar yolları kum ve taş dökmelerinde kullanılır.
Isırgan	Ciğirgen	<i>Urtica Urens</i>	Urticaceae	Bitkinin bütün kısımları	İnfüzyon, Tohumu ise öğütulmektedir	Kandaki zehirli maddeleri temizler, kanın pıhtılaşmasını önler, öksürükte, solunum yolları açmada, kansızlık tedavisinde, romatizmal hastalıklarda kullanılır.
Yüksük Otu	Yüzük otu	<i>Digilatis Purpureae</i>	Scrophulariaceae	Yaprağı	İnfüzyon	Kalbi kuvvetlendirir, damar daraltır, ödemleri giderir.
AtkuyruğuKırkkilittir	Ekli ot, Eşek otu	<i>Equisetum arvense</i>	Equisetaceae	Toprak üstü kısımları	İnfüzyon	İdrar söktürerek, fazla suyu attırarak vücuttaki romatizmal şişlikleri indirir

Ayı Gülü	Dağ gülü	<i>Paeonia Officinalis</i>	Paeonaceae	Kurutulmuş çiçekleri	İnfüzyon	Sarahastalığında,sarılıkta faydalıdır
Baldıran	Ağı otu	<i>Conium Maculatum</i>	Apiaceaea	Çiçekli dalları ve meyveleri	Haricen kullanılmak şartıyla lapası hazırlanır	Romatizmal rahatsızlıklarda haricen kullanılır, zehirli ve öldürücüdür.
Banotu	Gâvur haşhaşı	<i>Hyoscyamuss Nigor</i>	Solanaceae	Yaprakları, çiçekleri	Haricen ve dâhilen	Nefes darlığında, mide ve bağırsak spazmlarını çözmeye, yaşlılığa bağlı el ve baş titremelerinde dâhilen, romatizmal ağrılarda haricen kullanılır.
Basur Otu	Dövün otu	<i>Ranunculus Ficaria</i>	Ranunculaceae	Çiçekleri	Haricen ve dâhilen	Basur memelerini yok etmeye .haricen yara ve eziklerde kullanılır.
Böğürtlen	Kür, Karmuk	<i>Rubus Fruticosus</i>	Rosaceae	Sürgünleri, kökleri, meyva	İnfüzyon	Ağız yaralarında, gerek taze ve gerekse kurutulmuş, böğürtlenin sürgünleri ve kökleri, tansiyonu düşürür ve bedeni güçlendirir.
Civan Perçemi	Ayvadana	<i>Achillea Millefolium</i>	Asteraceae	Çiçekleri	İnfüzyon	Mide ve bağırsak ,Soğuk algınlığına, karaciğer güçlendirmede kullanılır.
Çobançantası	Çakıldak	<i>Capsella Bursa</i>	Brassicaceae	Toprak üstü kısımları	İnfüzyon	Böbrek kumu ve iltihabında, basurda, her çeşit iç ve dış kanamalarında etkilidir.
Çuha Çiçeği	Dağ marulu	<i>Primula Officinalis</i>	Primulaceae	Kökü, yaprağı, çiçeği	İnfüzyon	Çuha çiçeği iştah açıcıdır, Bronşitte ve astımda etkilidir, terleticidir, uykusuzluğa iyi gelir.
Deve Dikeni	Kenger	<i>Carduus Marianus</i>	Asteraceae	Çiçekleri ve tohumları	İnfüzyon, Tohumu ise öğütülmelidir.	Her çeşit karaciğer rahatsızlığında kullanılmalıdır. Mide güçlendiricidir, hazmı kolaylaştırır, iştah açar, sarılık gidericidir.
Deve Tabanı	Öksürük otu	<i>Tussilago Farfara</i>	Areceae	Çiçeği ve yaprağı	İnfüzyon	Öksürük ve bronşite iyi gelir. Balgam söktürücüdür. Uzun süreli devam eden öksürüklerde akciğeri rahatlatmak için kullanılır.
Ökse Otu	Burç	<i>Viscum Album</i>	Loranthaceae	Yaprakları, çiçekleri ve meyvaları	Dekoksiyon	Ökse otu kan durdurucu olarak da kullanılır, Soğuk çayı bur na çekildiğinde burun kanamalarını durdurur
Salep	Balkaymak otu	<i>Orchis</i>	Orchidaecae	Yumruları	Dekoksiyon	öksürükte faydalıdır, balgam söktürür, çocuk ishallerini önlemede etkilidir.
Papatya	Bubaşça	<i>Matircaria Chamomilla</i>	Asteraceae	Çiçeği	İnfüzyon	İştahı açar, akneye iyi gelir, diş ağrısını ve basur ağrılarını da azaltır, saçları sarartmak için kullanılır.

BULGULAR

Bitkiler her bölgede değişik amaçlarla ve yöntemlerle kullanılarak tüketilmektedir. Tedavi amaçlı kullanılan bitkilerin yaprak, gövde, kök, çiçek, tohum, filiz gibi kısımları çeşitli yöntemlerle çeşitli hastalıkların tedavisinde kullanılmıştır. Bu yöntemler; Su ile kaynatılarak (dekoksasyon) , Kaynamış su (infüzyon) ,yağ içinde bekletilerek ,lapa haline getirilerek,tohumu balla karıştırılarak kullanılmaktadır.

Gıda olarak kullanılan bitkiler; çiğ olarak, haşlayarak veya haşlayıp süzildükten sonra içine bulgur, pirinç katılarak, yumurtalı veya yumurtasız ya da sarımsaklı yoğurt ilave edilerek vb. şeklinde kullanılmaktadır. Yabani bitkiler, baharat veya çay olarak tüketilmektedir. Gıda olarak kullanılan bitkilerin tedavi amacıyla da kullanılması, toksisite değerlendirmesi bakımından büyük önem arz etmektedir. Bu özellik daha ileri çalışmalarda bitkinin olası zararlı etkilerinin veya güvenilirliğinin değerlendirilebilmesi bakımından önemlidir (Şimşek, I ., Aytekin, F . , Yeşilada, E. , Yıldırım . Ş.,2004).

TARTIŞMA VE SONUÇ

Araştırma alanındaki 43 bitkinin mahalli isimleri ve kullanım alanları tespit edilmiştir. Bunların 33'ü tıbbî, 3'ü gıda, 7'si de diğer (yakacak, hayvan yemi vb.) alanlarda kullanılmaktadır. Yörede tespit edilen bu bitkilerin, ilçe ve köylerdeki insanlar tarafından yaygın şekilde kullanıldığı gözlemlenmiştir. Yöre halkının ekonomik durumunun yetersiz olması, köylerin şehir merkezinden uzakta olması, zaman zaman ilaçların yan etkilerinin görülmesi gibi etkenler bitkilerin daha sık kullanılmasına yol açmıştır. Eskiden “kocakarı ilaçları” olarak tasvir edilen bitkisel ilaçlar, bitkilerin içeriklerinin ortaya çıkarılması ile herkes tarafından kabul edilir olmuş ve kullanılmaya başlanmıştır (Koç,H.,1999). Gerçekten de etken maddelerine bakıldığı zaman, geçmişte insanların bitkileri doğru alanlarda kullandıkları görülmektedir. Çalışma alanımız Kütahya ili Gediz ve Tavşanlı ilçesi daha çok halk tıbbında kullanılan bitkiler üzerinde tıbbi amaçlı kullanımı olan bitkiler daha çok mide ve solunum yolu rahatsızlıklarında kullanılmaktadır. Ayrıca değişik bitkilerin karıştırılıp dövülerek merhem şeklinde özellikle yara ve ağrılarda kullanılmasının da oldukça yaygın olduğu görülmüştür (Yıldırım . Ş, 2004).

Ancak, halk arasında hekim olarak görülen kişilerin, özellikle aktarların ticari amaçlarla değişik uygulamalara gitmesi, dikkat edilmesi gereken bir konudur. Babadan oğula günümüze dek ulaşan bu bilgilerin doğru kullanılması ve modern tıp gerçeğinin de göz ardı edilmemesi gerekmektedir. Ülkemizin değişik bölgelerinde birçok etnobotanik çalışma (Sayar vd., 1995; Düşen,Sümbül, 1999; Bağcı, 2000; Ertuğ, 2002; Keskin, Alpınar, 2002; Başaran, 2003; Kahraman,Tatlı, 2004; Öztürk, Dinç, 2005; Elçi,Erik, 2006; Türkan vd., 2006) göze çarparken, araştırma alanıyla ilgili çalışmaların yeterli olmadığı görülmüştür. Bu çalışmayla, çeşitli amaçlarla kullanılan bitkilerin sayısının artacağı düşünülmektedir.(*Yapıcı, İ. Ü, Hoşgören,H., Savaş,Ö, 2009*).

Özellikle ilaç-tıbbi bitkilerin sayısında görülen bu hızlı artış, modern tıbbın, yapay ilaçlar yerine doğal kaynakların kullanılmasının faydalarını kabullenmesinden sonra görülmüştür (Baytop, T., 1984). Bugün gerek dünyada ve gerekse ülkemizde ilaç ve baharat bitkileri, doğal floradan toplanarak kullanılmakta veya pazarlanmaktadır. Kontrolsüz olarak toplanan bitkilerin doğal floradaki nesli giderek azalmaktadır, her yıl bu bitkilerin birçoğu da kaybolmaktadır. Özellikle kök, rizom, yumru veya çiçekleri drog olarak kullanılan bitkilerde durum kendini daha çok göstermektedir. Bitkiler ya tamamen sökülerek yok edilmekte ya da tohum evresinden önce toplandıkları için nesillerini devam ettirememektedirler. Bu durum Avrupa ülkelerinde erken fark edilmiş ve bitki toplayıcılarına eğitici bilgi verilmiştir (Koç,H.,1999).

Ülkemizde ise doğal floranın yok edildiği çok geç fark edilmiş olmasına rağmen henüz ciddi önlemler alınmış değildir. Dolayısıyla iç tüketimde kullanmak veya satmak amacıyla birçok bitkinin doğadan bilinçsiz bir şekilde toplanmasına devam edilmektedir. Bunun sonucu olarak, ülkemiz florasında önceleri zengin bir şekilde bulunan birçok bitki türü günümüzde ya zor bulunur hale

gelmiştir ya da nesli tükenmiştir (Koç,H.,1999).

Çizelge 2. de Türkçe ismi, yerel ismi, Latince ismi, familyası, bitkinin kullanılan kısmı, kullanım şekli ve kullanımı yer almıştır.(Tavşanlı).

TÜRKÇE ADI	YEREL ADI	LATİNCE ADI	FAMİLYA	KULLANILAN KISIM	KULLANIM ŞEKLİ	KULANIM
Alıç	Akdiken	<i>Crataegus Monogyna</i>	Rosaceae	Meyveleri, Çiçekleri ve yaprakları	İnfüzyon	Kalp kaslarını güçlendirmesi, kalp damarlarını genişletir ve kan dolaşımını rahatlatır.
Bodur Mahmut	Acı yavşan	<i>Equisetum arvense</i>	Equisetaceae	Toprak üstü kısımları	İnfüzyon	İdrar söktürerek, fazla suyu attırarak vücuttaki romatizmal şişlikleri indirir.
Banotu	Dişotu	<i>Hyoscyamus Nigor</i>	Solanaceae	Yaprakları, çiçekleri	Haricen ve dâhilen	Nefes darlığında, yaşlılığa bağlı el ve baş titremelerinde kullanılır.
Civanperçemi	Akbaş,Ayvada ne	<i>Achillea Millefolium</i>	Asteraceae	Çiçekleri	İnfüzyon	Mide ve bağırsak gazları, Soğuk algınlığa, karaciğer güçlendirmede kullanılır.
Altınbaş	Sarıot, Arıçiçeği	<i>Salidago Virgousea</i>	Asteraceae	Yaprakları	İnfüzyon	Böbrek taşı düşürmede etkilidir, İdrar arttırıcıdır.
Altınotu	Kovanotu	<i>Helichrysum Graveolens</i>	Asteraceae	Çiçekleri	İnfüzyon	İdrar söktürücü etkisi vardır, romatizmal kullanılır.
Ardıç	Giligili	<i>Juniperus Communis</i>	Cupressaceae	Meyveleri	infüzyon	İdrar yolları iltihabında, idrar yolları kum ve taş dökmelerinde kullanılır.
Damarotu	Beşparmak	<i>Plantago Lanceolata</i>	Plantaginaceae	yaprakları	infüzyon	Egzama, Apse ve çıban tedavisinde kullanılır.
Koyunotu	Yavşan	<i>Agrimonia Eupatoria</i>	Rosaceae	Yaprakları	infüzyon	Hazımsızlık ve mide şişkinliğine, İdrar söktürücüdür iyi gelir
Çobançökerten	Demirdikeni	<i>Tribulus terrestris</i>	Zygophyllaceae	Yaprakları, çiçekleri, kökleri	infüzyon	Ruhsal ve duygusal açıdan zindelik
Adaçayı	Başşaplı, şalva	<i>Salvia officinalis</i>	Lamiaceae	Yaprakları, çiçekleri,	infüzyon	Antiseptik, mide ve barsak spazmlarını çözücü

Sonuç olarak çeşitli hastalıkların tedavisinde halk tarafından sıklıkla kullanılan ve olumlu etkileri göz ardı edilemez bir gerçek olan tıbbi bitkilerin bilinçli olarak tüketilmesi gerekmektedir. Analizi yapılmış içeriği belli olan güvenilir bir şekilde temin edilen bitki türlerinin hastalıkların alternatif tedavisinde kullanılması halk sağlığının olumsuz etkilenmemesi için önem arz etmektedir.

KAYNAKLAR

- Akçiçek, E. ve Vural, M., 2003. Kumalar Dağı (Afyon) ve Çevresindeki Bazı Bitkilerin Mahalli Adları ve Etnobotanik Özellikleri, *Ot Sistematik Botanik Dergisi*, 9 , 2, 151-162.
- Başaran, S., Elmalı Yöresinde Doğal Olarak Yetişen Bazı Bitkilerin Etnobotanik Özellikleri, *Batı Akdeniz Ormancılık Araştırma Müdürlüğü Dergisi*, Çevre ve Orman Bakanlığı Yayın No:211, Sayı:5, Antalya, 2003.
- Baytop, T., Türkiye’de Bitkiler İle Tedavi, İstanbul Üniversitesi Eczacılık Fakültesi Yayınları, İstanbul, 1984.
- Davis, P.H., 1965-1985. Flora of Turkey and the East Aegean Islands, Edinburg University Pres, Vol. 1-9, Edinburg.
- Davis, P.H., Mill. R. ve Tan, K., 1988. Flora of Turkey and the East Aegean Islands, (Supplement) Edinburg University Pres, Vol. 10, Edinburg.
- Deniz. L., Serteser. A. , Kargioğlu.M., Uşak Üniversitesi ve Yakın Çevresindeki Bazı Bitkilerin Mahalli Adları ve Etnobotanik Özellikleri. *AKÜ Fen Bilimleri Dergisi*.2010 - 01 57-72. Gezin, D., Bitki Mitosları, Sel Yayıncılık, (2006), İstanbul.
- Honda, G.,Takeda, Y., Tanaka, T., Takaishi, Y., Sezik, E.,Yeşilada, E., A Report on Medicineand Medicinal PlantsIn Turkey (1994)., Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto (1996).
- İ.Ü.Yapıcı, H.Hoşgören, Ö.Saya / Kurtalan (Siirt) İlçesinin etnobotanik özellikleri. *Dicle Üniversitesi Ziya Gökalp Eğitim Fakültesi Dergisi*, 12 (2009), 191-196 Kahraman, A., Tatlı, A.(2004). Umurbaba Dağı (Uşak-Eşme) ve Çevresindeki Bazı Bitkilerin Mahalli Adları ve Kullanım Alanları, *Ot Sistematik Botanik Dergisi*, 11,2, 147-154.

- Kendir, G., Güvenç, A. Etnobotanik ve Türkiye’de Yapılmış Etnobotanik Çalışmalara Genel Bir Bakış. *Hacettepe Üniversitesi Eczacılık Fakültesi Dergisi* Cilt 30 / Sayı 1 / Ocak 2010 / ss. 49-80.
- Koçyiğit, M., Yalova İlinde Etnobotanik Bir Araştırma, Yüksek Lisans İstanbul Üniversitesi Sağlık Bilimleri Enstitüsü, (2005).
- Koç, H., İlaç ve Baharat Bitkileri- I -Gaziosmanpaşa üni. Yayınları No:40. Ders kitapları serisi No:18. Tokat. 1999.
- Malyer, H., Aydın, Ö. A., Tümen, G., Er, S., Tekirdağ ve Çevresindeki Aktarlarda Satılan Bazı Bitkiler ve Tıbbi Kullanım Özellikleri, Dumlupınar Üniversitesi Fen Bilimleri Enstitüsü Dergisi, Sayı 7, 103-112, Kütahya (2004).
- Sadıkoğlu, N., Cumhuriyet Dönemi Türk Etnobotanik Araştırmalar Arşivi, Yüksek Lisans Tezi, Danışman: Alpınar, K., İstanbul Üniversitesi, Sağlık Bilimleri Enstitüsü, (1998).
- Şimşek, I. , Aytekin, F. , Yeşilada, E. , Yıldırım, Ş., Anadolu’da Halk Arasında Bitkilerin Kullanılış Amaçları Üzerinde Etnobotanik Bir Çalışma Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 29-31 Mayıs 2002, Eskişehir, Eds. K.H.C.Başer ve N.Kırimer Web’de yayın tarihi: Haziran 2004 ISBN 975-94077-2-8
- Tümen, G. ve Sekendiz, A. Balıkesir ve Merkez köylerinde Halk ilacı olarak kullanılan bitkiler, Uludağ Üniversitesi Araştırma Projesi No: 86-12 Balıkesir (1989).
- Yıldırım, Ş. 2004. Etnobotanik ve Türk Etnobotanigi. Kebikeç. Alp Matbaası. Ankara. 17: 175-193

FARKLI LOKASYONLARDA YETİŞTİRİLEN BAZI ADAÇAYI TÜRLERİNİN (*Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill.)'İN UÇUCU YAĞ BİLEŞENLERİNDEKİ DEĞİŞİMLERİN İNCELENMESİ

Hasan Basri Karayel

¹Dumlupınar Üni. Gediz MYO. Tıbbi Ve Aromatik Bitkiler. Kütahya, Türkiye.

Corresponding author email kbasri23@hotmail. Com

Özet

Bu araştırma üç lokasyonda bazı Adaçayı türlerinin (*Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill.)'in uçucu yağ bileşenleri, uçucu yağ oranı ve kalitesi üzerine etkisini belirlemek amacıyla, 2015 yılında Çanakkale, Balıkesir, Kütahya lokasyonlarında eş zamanlı olarak yürütülmüştür. Tarla deneyleri tesadüf blokları deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Fideler 50 cm sıra arası ve 30 cm sıra üzeri mesafede dikilmiştir. *Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill. herbasında uçucu yağ analizi yapılmıştır. Bu bitkilerin uçucu yağları hidrodestilasyon (GC_MS/FID) yoluyla elde edilmiş ve yaklaşık olarak üç lokasyonda uçucu yağ oranları %0,20 ile %4,10 aralığında saptanmıştır. Uçucu yağda ana bileşenleri *Salvia officinalis* L., α -thujone %26,35, %38,39, %29,84, β -thujone %29,20, %12,26, %9,07, Camphor %5,60, %13,90, %23,24, *Salvia aethiopis* L., β – caryophyllene %36,22, %30,46, %35,96, α – copaene %15,06, %16,4, %16,58, Germacrene -D %13,23, %20,01, %15,20, *Salvia fruticosa* Mill., 1,8-cineole %49,60, %33,36, %56,05, Camphor %22,21, %17,51, %6,62, β -pinene %5,45, %7,52, %7,62 olarak tespit edilmiştir. En iyi uçucu yağ oranı %4,10 (*Salvia fruticosa* Mill.) ile Çanakkale lokasyonunda elde edilmiştir. Yapılan çalışma sonunda; uçucu yağ bileşenlerinin terpenler bakımından daha zengin olduğu ve uçucu yağ miktarının ekolojik faktörlere bağlı olarak değişim gösterdiği saptanmıştır.

Anahtar Kelimeler : *Salvia* türleri, Uçucu yağ, GC_MS/FID

AN INVESTIGATION OF CHANGES IN THE VOLATILE OIL COMPONENTS OF SOME SAGE SPECIES (*Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill.) GROWING IN DIFFERENT LOCATIONS

Hasan Basri Karayel¹

¹Dumlupınar Üni. Gediz Vocational School. Medical and Aromatic Plants. Kütahya, Turkey

Corresponding author email: kbasri23@hotmail. Com

Abstract

This study was conducted at three locations (Çanakkale, Balıkesir, Kütahya) to determine the effect of location on essential oil components, essential oil rate and quality of some sage species (*Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill.) in 2015. Field examinations were repeated 3 times according to trial randomized block design. The seedlings were planted by a horizontal distance of 30 cm and vertical distance of 50 cm. Volatile oil analyzes were done for the plants “*Salvia officinalis* L., *Salvia aethiopis* L., *Salvia fruticosa* Mill.”. These plants’ volatile oils were gathered by hydrodistillation method (GC_MS/FID) and the volatile oil rates in three different locations were measured between the range of %0,20 and %4,10. The basic components of the volatile oil were determined as follows; *Salvia officinalis* L., α -thujone %26,35, %38,39, %29,84, β -thujone %29,20, %12,26, %9,07, Camphor %5,60, %13,90, %23,24, *Salvia aethiopis* L., β – caryophyllene %36,22, %30,46, %35,96, α – copaene %15,06, %16,4, %16,58, Germacrene -D %13,23, %20,01, %15,20, *Salvia fruticosa* Mill., 1,8-cineole %49,60, %33,36, %56,05, Camphor %22,21, %17,51, %6,62, β -pinene %5,45, %7,52, %7,62. . The highest volatile oil rate of the Anatolian Sage was reached

in Çanakkale location by a %4.10 (*Salvia fruticosa* Mill.) rate. As a result of the study it was found out that volatile oil components are comparatively richer in terpenes and the amount of volatile oil differs according to the ecological factors.

Keywords: *Salvia* species, volatile oil, GC_MS/FID

GİRİŞ

Lamiaceae (Labiatae) familyasına bağlı olan *Salvia* cinsinin dünyanın her iki yarım küresinde, özellikle tropik ve subtropik bölgelerle, Akdeniz ve Orta Avrupa'ya dağılmış 986'den fazla türü bulunmaktadır. Bu türlerden 106 tanesi ülkemizde doğal olarak yayılış göstermekte olup, bunlardan 58 tanesi endemiktir. Türkiye, *Salvia* cinsi tür zenginliği bakımından dünyada 13. sırada yer almaktadır¹. Çok eski devirlerden beri bilinen ve önemini bugüne kadar hiç kaybetmemiş olan faydalı bitkilerin önemli bir bölümünü *Salvia* teşkil eder. Tıbbi özelliklerine ve bu bitkinin çeşitli hastalıkların tedavisinde kullanıldığına ilişkin ilk kayıtlara eski çağlardan kalma mezar ve anıtların süslü yazı ve resimlerinde rastlanmaktadır. Şifa verici özelliğinden dolayı Latince kurtarmak, iyileştirmek anlamına gelen Salveo kelimesinden kaynaklanan *Salvia* ismi verilmiştir. Cinsin uçucu yağlarının bileşiminde bulunan monoterpenler ve bunların oksijenli türevleri antiseptik etkiye sahiptir. Son 4 yılda bu bitki üzerine yapılan çalışmalarda hücre DNA sentezini yavaşlatan bileşiklerin varlığı ortaya konmuştur¹⁷. Bu bulgu kanser araştırmaları ve tedavisi açısından önem taşımaktadır.

Uçucu yağ kompozisyonlarının kimyasal yapılarının en büyük grubunu terpenler oluşturmaktadır. Bununla birlikte az miktarda alkoller, aldehitler, esterler, fenoller, azot ve kükürt içeren bileşiklerde bulunmaktadır. Terpenlerin oksitlenmesi ile meydana gelen oksijenli türevler koku, tat ve terapik özellikteki maddelerdir¹⁵. Sudan hafif olan uçucu yağların kırılma indeksleri genellikle yüksek olup, optikçe aktif özelliktedirler. Işık ve oksijenin etkisi ile reçineleştikleri için uzun süreli saklamalarda koyu renkli şişelerde ağzı kapalı olarak muhafaza edilmelidirler. Roma, Yunan ve özellikle Mısır medeniyetlerinde uçucu yağlar yaygın olarak kullanılmıştır. Son yıllarda alternatif tıbbın bir dalı olarak görülen aroma terapiye karşı duyulan ilgi, uçucu yağ kullanımını da artırmıştır. Eterik yağlar, terapiler de uygulanan masajlarda ya da rahatlatıcı banyolarda kullanılmaktadır¹⁴.

Salvia türleri tıpta çeşitli hastalıkların tedavisinde kullanılmaktadır. *Salvia* türleri sadece tıbbi alanda değil parfümeri ve gıda sanayinde tatlandırıcı olarak kullanılmaktadır⁷. Birçok *Salvia* türü likör yapımında kullanılmaktadır¹¹⁻¹². Çoğu *Salvia* türü güzel görünüşleri nedeniyle park ve bahçelerde dekoratif süs bitkisi olarak yetiştirilmektedir⁵⁻¹⁹.

Drog olarak Adaçayı'nın Yaprakları (Folia Salviae, T.K.) ve yapraklarından elde edilen uçucu yağı (Oleum Salviae) kullanılmaktadır³. Adaçayı yaprakları %0.5-2.5 oranında uçucu yağ taşımaktadır. Kodekslerde uçucu yağ oranının en az %1.5 olması istenmektedir⁵. Tıbbi olarak kabul edilen yağda α , β Thujon, 1,8 Cineol, Campher, Borneol, Bornylacetat bulunmaktadır. Bazı uçucu yağların Thymol ve Carvacrol da taşıdığı bildirilmektedir⁴⁰. Uçucu yağında thujon oranı %30-50, cineol oranı %15, borneol oranı %10 olarak belirtilmektedir⁴.

Araştırma konumuzu oluşturan *Salvia aethiopis* L. türü üzerinde yapılan çalışmalarda bitkiden elde edilen aethiopinone adlı terpenin *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* ve *Bordetella bronchiseptica* üzerinde antibakteriyal ve sitotoksik etki yaptığı kanıtlanmıştır¹³. *Salvia aethiopis* üzerinde yapılan bir başka çalışmada ise türün antioksidan etkiye sahip olduğu tespit edilmiştir³⁷. Cinsin kimyasal özellikleri ile ilgili çok sayıda araştırma olmasına rağmen, cinsin tanıtımına yardımcı olacak morfolojik ve anatomik çalışmalar sınırlıdır²³.

Salvia aethiopis L. tohum yağ asitleri ve bunların bazı mikroorganizmalara karşı göstermiş olduğu etkileri incelemişlerdir. Sonuçta, tohum yağ asitlerinin linoleik asit bakımından zengin olduğu ve mikroorganizmalara karşı farklı düzeylerde antimikrobiyal aktivite gösterdikleri saptanmıştır¹⁷.

S. aethiopis L. (Yünlü adaçayı): Türkiye'de yaygın bir tür olan bu bitkinin yaprak ve çiçek durumları mideyi ve uyarıcı olarak antik çağdan beri bilinmektedir⁴. Bolu yöresinde kızılık denir ve merhem halinde yara iyileştirici olarak kullanılır³³.

Salvia cinsi bitkileri, uzun yıllardan beri sindirim kolaylaştırıcı, yatıştırıcı, diüretik, terlemeyi önleyici, ağrı kesici vb. etkilerinden dolayı insan sağlığında önemli bir yer almıştır. Tıbbi adaçayının kullanım alanı oldukça geniştir. İlaç, gıda, içki, parfüm ve kozmetik sanayinde kullanılmaktadır. Günümüzde ağız yıkama ve gargara preparatlarının imalinde, saç kuvvetlendirici şampuanların yapımında, deodorantlarda, saç boyası ve cilt kremi yapımında kullanılmakta, şifalı ve dinlendirici etkisi ile çay olarak tüketilmektedir³⁸. Türkiye, mevcut bitkisel çeşitliliği yönünden oldukça dikkate değer ve zengin bir floraya sahiptir. Bu zenginlik; üç fitocoğrafik bölgenin kesiştiği bölgede bulunması, Güney Avrupa ile Güney Batı Asya floraları arasında köprü olması, pek çok cins ve sekişyonun orjin ve farklılaşım merkezlerinin Anadolu'da oluşu, muhtemel ekolojik ve fitocoğrafik farklılaşma ile ilgili olarak tür endemizminin yüksek oluşu gelmektedir³⁶.

Son yıllarda üretilen sentetik kökenli maddelerin yan etkilerinin daha fazla olması, özellikle antimikrobiyal olarak kullanılan sentetik ilaçlara karşı organizmaların direnç oluşturmaları gibi sebepler doğal bitkisel kaynakların ve bu maddeleri taşıyan tıbbi bitkilerin önemini daha çok arttırmıştır²⁰.Günümüzde de yine dünya nüfusunun çoğunluğu ilaç hammaddesi olarak bitkileri kullanmaktadır. Özellikle gelişmekte olan ülkelerde nüfusun % 80'i sağlık gereksinimlerini ilk etapta geleneksel tıbbi bitkilerden sağlamaktadır. Dünya nüfusunun % 80'inin gelişmekte olan ülkelere yaşadığı düşünülürse toplam nüfusun %64'ü bitkileri tedavi amaçlı kullanmaktadır¹⁰. Gelişmiş ülkelere reçete ile satılan ilaçların yaklaşık %25'i bitkisel kökenli kimyasallardır²⁵.Doğal zenginliklerimizin sürekliliği ve gelecekteki araştırmalar için adaçayı türlerinin gen kaynaklarının korunması büyük önem arz etmektedir²¹.Bu araştırma farklı lokasyonlarda adaçayının uçucu yağ miktarı ve uçucu yağındaki ana bileşen maddesinin değişimlerini belirlemek amacıyla yürütülmüştür.

Salvia fruticosa Mill. bitkisinin drog yaprak ve herba verimi ikinci yılda en yüksek olup bunu sırayla birinci, üçüncü ve dördüncü yıl verimleri izlemiştir. *Salvia fruticosa* Mill.'ın ekonomik ömrünün üç yıl olduğu sonucuna varılabilir. Zira dördüncü yıl verimi diğer yılların verimlerine göre drog yaprakta yarısından da az, drog herbada yarısına yakındır. Adaçayının kullanılan kısmı herbası, yaprakları ve uçucu yağdır. Bileşiminde tanen, acı madde ve uçucu yağ (% 1-2.5) bulunmakta olup, uçucu yağın ana bileşenleri thujon (%30-50), sineol (%15) ve borneol (%10)'dır ³.

Bu araştırma, Ege ve Marmara bölgesinde yetiştirdiğimiz *Salvia* L. cinsine ait iki *Salvia* L. türünün uçucu yağlarının belirlenmesi, ayrıca *Salvia* L. türlerinin uçucu yağ kompozisyonuyla karşılaştırılması ve uçucu yağların değerlendirilmesine yönelik bir çalışmadır. Ayrıca bu çalışmayla; bu üç türün uçucu yağlarını diğer *Salvia* türleri ile karşılaştırmak, bölgelere uygun türün belirlenmesini tespit etmek, ürün çeşitliliğine, tat ve gıda endüstrisine katkı sağlamak amaçlanmıştır.

MATERYAL VE METOT

Bitki Materyali ve Denemelerin Kurulması

Bu çalışmada materyal olarak kullanılan tohumlar Ankara Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümünde temin edilmiştir. Tohumlar 2014 yılı Aralık ayında Kütahya Belediyesinin Hekim Sinan Tıbbi Bitkiler Botanik Bahçesinde viyollere ekilerek fide yetiştirilmiştir. Araştırma 2015 yılında Çanakkale Onsekiz Mart Üniversitesi Ziraat Fakültesi Dardanos Yerleşkesi deneme alanı, Balıkesir Edremit Kale Natürel A.Ş. Tıbbi Bitkiler Bahçesi ve Kütahya Belediyesi Hekim Sinan Tıbbi Bitkiler Botanik Bahçesi olmak üzere 3 farklı lokasyonda eş zamanlı yürütülmüştür. Tohumların çimlenmesi 20-25 gün sürmüştür. Köklenen fideler 2015 yılı Nisan ayında deneme tarlalarına şaşırtılmıştır.

Tarla denemeleri tesadüf blokları deneme desenine göre 3 tekerrürlü olarak yürütülmüştür. Bitki dikim sıklığı 50 cm sıra arası, 30 cm sıra üzeri mesafelerine göre düzenlenmiş, her parsel 3 sıradan oluşmuştur. Tüm lokasyonlarda tek biçim yapılmış ve biçim zamanı olarak uçucu yağ oranının en yüksek olduğu çiçeklenme başlangıcı tercih edilmiştir. Deneme çevreleri ayrı ayrı değerlendirildiği Marmara Bölgesinde (Çanakkale, Balıkesir) Akdeniz iklimi; İç Ege Bölgesinde (Kütahya) ise karasal iklim hâkimdir. Deneme, alanın toprak yapısı genel olarak değerlendirildiğinde alkali yapılı olduğu söylenebilir.

Uçucu Yağ Elde Edilmesi

Baharat droglarında, çeşni veren ve tıbbi bitkilerde uçucu yağ miktarı tayininde uçucu yağ miktarı, metotta belirtilen şartlarda buharla sürüklenen ve kuru maddenin 100 g'ında mililitre olarak ifade edilen bütün maddelerdir. Kullanılan reaktifler analitik saflıkta, kullanılan saf su damıtık veya buna eşdeğer saflıkta olmalıdır. Bu metot, numunenin sulu süspansiyonunun damıtılması, damıtılan kısmın içerisindeki uçucu yağın sulu fazdan ayrılarak taksimatlı tüpe toplanması, toplanan uçucu yağın hacmin okunmasından sonra uçucu yağ yüzdesinin hesaplanması prensibine dayanır. Deneme uçucu yağ analizinin başlangıcında 20 g kuru materyal tartılmıştır. Tabanı yuvarlak boynu traşlı 500 ml'lik balona alınmıştır. 200 ml (örnek miktarına göre değişebilir, yaklaşık 10 kat) saf su eklenip çalkalanmış. Balon Neo -Clevenger düzeneğinin ısıtmalı ceket kısmına yerleştirilip dikey dereceli cam borunun alt kısmı cam balona, üst kısmı ise soğutucu sistemine bağlanır. Dereceli boru ve eğik boruya saf su doldurulduktan sonra soğutucu sistem çalıştırılıp, balon ısıtılmaya başlanır. 2 saat süreyle hidrodestilasyon işlemine tabi tutulur. Sistem soğuduktan sonra ve dereceli kısma toplanan uçucu yağ sulu fazdan ayrıldıktan sonra miktarı (ml) tespit edilir. Tartımı alınan örnek miktarına (g) göre 100 g örnekteki uçucu yağ miktarı uçucu yağ oranı (%) olarak hesaplanır³⁴.

Uçucu Yağ Kompozisyonunun GC-MS ile Belirlenmesi

Uçucu yağ bileşenlerine ait analizler Batı Akdeniz Tarımsal Araştırma Enstitüsü Araştırma Laboratuvarında yapılmıştır. Örnekler 1:100 oranında hekzan ile seyreltilip Gaz kromatografisi (Agilent 7890A) cihazına 1 µl olarak 40:1 split oranı ile enjekte edilmiştir. Bileşenlerin ayrımı için kapiler kolon (HP InnowaxCapillary; 60.0 m x 0.25 mm x 0.25 µm) kullanılmıştır. Kolon, bitiminde bir ayraç (splitter) yardımıyla FID ve kütle spektrometresi dedektörüne (Agilent 5975C) akış 1:1 oranında olacak şekilde ikiye ayrılmıştır. Analizde taşıyıcı gaz olarak 0.8 ml/dk akış hızında helyum kullanılmıştır. Enjektör sıcaklığı 250°C'de tutulmuş, kolon sıcaklık programı - 60°C'de 10 dakika, 60°C'den 220°C'ye 4°C/dakika (40 dakika) ve 220°C'de 10 dakika - toplamda 60 dakika olacak şekilde ayarlanmıştır. Kütle dedektörü için tarama aralığı (m/z) 35-450 atomik kütle ünitesi ve elektron bombardımanı iyonizasyon enerjisi 70 eV'dir. Uçucu yağın bileşenlerinin teşhisinde OIL ADAMS, WILEY ve NIST kütüphanelerinin verileri esas alınmıştır. Uçucu yağ bileşen oranlarında ise FID dedektörünün verileri kullanılmıştır²⁴.

BULGULAR VE TARTIŞMA

Bu çalışmada incelenen *Salvia* türlerinin uçucu yağ oranı ve kompozisyonunun lokasyonlara göre değişimi *Salvia officinalis* L., *Salvia aethiopis* L. toplam uçucu yağ verimi sırasıyla Çanakkale %0,99 - 0,53 Balıkesir - Edremit %1,23 - 0,21, Kütahya %1,85 - 0,20 olarak ölçülmüştür. *salvia officinalis* L.ve *salvia aethiopis*'in toprak üstü organlarından elde edilen uçucu yağın analizinde (Çanakkale - Balıkesir ve Kütahya) sırasıyla *salvia officinalis* L. 19 - 23 ve 21 bileşen, *salvia aethiopis* L. 15-14 ve 14 bileşen lokasyonlara göre tanımlanmıştır. Bu bileşenler toplam yağın *salvia officinalis* L. %100 - %100 ve %100, *salvia aethiopis* L. %99.98-%100 ve %100'lik kısmını oluşturmuştur. *salvia officinalis* L'in (% 0,99-%1,23 ve %1,85) toplam uçucu yağ verimi *salvia aethiopis* L. (% 0,53-%0,21 ve %0,20) göre daha verimli bulunmuştur. Uçucu yağ bileşen miktarları Tablo 1-2'de liste halinde verilmiştir. *salvia officinalis* L'dan elde edilen yağda 19 - 23 ve 21 bileşen analiz edilmiş ve buda toplam yağın %100 - %100 ve %100'ünü oluşturmuştur. *salvia aethiopis* L.'den elde edilen yağda 15-14 ve 14 bileşen analiz edilmiş ve buda toplam yağın %99.98-%100 ve %100'lik kısmını lokasyonlara göre oluşturmuştur. Uçucu yağ ana bileşenleri lokasyonlara göre; *Salvia officinalis* L., α -thujone % 26,35, % 38,39, % 29,84, β -thujone % 29,20, % 12,26, % 9,07, Camphor %5,60,%13,90, % 23,24, 1,8-cineole % 7,75, % 7,81, %6,57, Viridiflorol % 5,55, %4,38, %2,65. *Salvia aethiopis* L. bitkisinin yaprağındaki uçucu yağında β -caryophyllene % 36,22, % 30,46, % 35,96, α -copaene % 15,06, % 16,46, % 16,58, Germacrene-D %13,23,% 20,01, % 15,20, β -cubebene % 5,62, % 7,04,

%6,93, α -humulene % 8,68, %7,40, % 8,54, Caryophylleneoxide % 7,40, % 1,82, % 3,53 olarak tespit edilmiştir. Bu majör bileşenlerin oranı toplam yağın (*Salvia officinalis* L. için) Çanakkale’de %74.45’sını, Balıkesir-Edremit’te %76.74’nü, ve Kütahya’da %71.37’sini oluşturmuştur. *Salvia aethiopis* L. için; Çanakkale’de %86.21’ni, Balıkesir-Edremit’te %83.19’unu ve Kütahya’da %86.74’nü oluşturmuştur.

Bazı araştırmacılar *Salvia officinalis* L. bitkisinde uçucu yağın %0.85-2.13 (Ceylan ve ark., 1979); %1.5-2.5 (Bernath ve ark., 1991), %0.83-1.63 (Yenikalaycı ve Özgüven, 2001), %1-3 (Bayram ve Sönmez, 2006), %1.5-1.7 ortalama %1.56 (Habân ve Otepka, 2007), oranlarında değiştiğini tespit etmişlerdir. Alman farmakopisine (DAB 8) göre, uçucu yağ oranının %1-2,5 aralığında (ort. %1.5) olması istenmektedir.

Bileşim incelendiğinde bileşimin esas maddesinin thujon olduğu görülmektedir. Bileşimi oluşturan başlıca komponentlerin oranları α -pinen % 0.92-3.76, β -pinen %1.05-5.54, myrecene %0.61-1.25, 1-8 cineole %3.23-12.35, thujon %12.62-39.29, camphor %5.06-30.97, linalool %0.39-0.74, borneol %1.11-10.56, trans-caryophyllene %2.44-13.68 aralığında yer almıştır⁹.

Miladinovic ve Miladinovic (2000), yürüttükleri bir araştırmada, *Salvia officinalis* L. bitkisinde uçucu yağ oranı ve bileşimi üzerine iklim, toprak gibi ekolojik faktörlerin etkili olduğunu, uçucu yağın ana bileşenlerinin α -thujon (%24.88), camphor (%16.03) ve 1-8 cineole (%9.79)’den oluştuğunu belirtmiştir. Salameh ve Dordevic (2000), Ürdün orijinli *Salvia officinalis* L. türünün uçucu yağ bileşiminde 29 bileşen tespit etmişlerdir. Ana bileşenlerin α -thujon (%29.9), β -thujon (%13.68), camphor (%15.74) ve 1-8 cineole (%12.31) olduğunu saptamışlardır.

Sagareishvili ve ark., (2000) ise aynı bitkide 11 farklı komponent belirlemişler ve yine ana bileşenler olarak α thujone (%31.56), β thujone (%17.55), Camphor (%16.48) ve 1,8 Cineol (%17.53)’ü tespit etmişlerdir. Delamare, Ana Paula Longaray, et al.(2007), *S. officinalis*’in yağının başlıca bileşenleri α -thujone, 1,8-cineole, camphor, borneol ve β -pinene olduğu belirlemiştir.

Salvia aethiopis L. Uçucu yağ oranı ile ilgili iranda yapılan çalışmalarda elde edilen uçucu yağ veriminin konsantrasyonu % 0.07 ile 0.71 arasında değişim göstermiştir²⁷. Tajbakhsh ve arkadaşlarının *S. aethiopis* türünün çeşitli organlarındaki uçucu yağları ile olan çalışmasında; bitkinin yaprak uçucu yağında, α -copaene (%23.8), β -caryophyllene (%23.3) ve Germacrene-D (%18); çiçeklerde β -caryophyllene (%24.1), Germacrene-D (%18.4) ve α -copaene (%18.1), gövdede α -copaene (%24.9), β -caryophyllene (%20.9) ve Germacrene-D (%17.8) olarak saptamıştır³⁵.

Aynı şekilde; Morteza ve arkadaşlarının İran sarı bölgesinden rapor ettikleri *Salvia aethiopis* L. uçucu yağında ana bileşenler olarak; β -caryophyllene (%17.0), α -copaene (%16.3), Germacrene-D (%13.8), β -cubebene (%9.7), spatulenol (%8.3), δ -kadinen (%7.7) ve α -humulen (%6.9) saptanmıştır. Bu örnek çalışma ile çalışmamızda kullandığımız uçucu yağında spatulenol bileşenine üç lokasyonda da rastlanılmamıştır. Rustaiyan, Abdolhossein, et al.(1999), *Salvia* L. türleri üzerinde yapmış oldukları araştırmalarda *S. aethiopis* yağının ana bileşenleri β -caryophyllene (% 24.6), α -copaene (% 15.5) ve germacrene D (% 13.5) arasında değişim göstermiştir.

Salvia fruticosa Mill. toprak üstü organlarından elde edilen uçucu yağın analizinde 24 bileşen tanımlanmıştır. Bu bileşenler toplam yağın Çanakkale %95.9, Balıkesir-Edremit %97.28, Kütahya %98.7’lik kısmını oluşturmuştur. 1.8-cineole’nin en yüksek oranda (%56.05 Kütahya), (%49.60 Çanakkale), (%33.36 Balıkesir-Edremit) bulunduğu ve bunu sırasıyla Camphor (Çanakkale %22.21), (Balıkesir-Edremit %17.51), (Kütahya %6.62), α -thujone (Çanakkale %2.42), (Balıkesir-Edremit %5.38), (Kütahya %1.48), β -thujone (Çanakkale %1.43), (Balıkesir-Edremit %3.47), (Kütahya %2.57), Borneol (Çanakkale %1.19), (Balıkesir-Edremit %1.65), (Kütahya %1.39) takip ettiği ve bu majör bileşenlerin toplam yağın %75.66’ini Çanakkale,%59.72’nin Balıkesir-Edremit, %66.72 Kütahya oluşturduğu tespit edilmiştir.

Uçucu yağ oranı konusunda yapılan çalışmalarda; Antalya ve Muğla florasından toplanan *Salvia fruticosa* Mill. popülasyonlarında uçucu yağ oranları 1. Yıl %3.55-5.28, 2. yıl %3.47-5.40 aralıklarında bulunmuştur⁸⁻¹⁷. Batı Anadolu Bölgesinin değişik yörelerinden toplanan *Salvia fruticosa* Mill.’da uçucu yağ oranları %1.5-5.15 aralığında bulunmuştur¹⁵.

Başer ve Kırimmer (2006), Türkiye’de *Salvia* L. türleri üzerinde yürüttükleri kapsamlı çalışmada *Salvia* L. türlerinin uçucu yağlarındaki ana bileşenlerine göre sınıflandırmışlar, *Salvia fruticosa*

Mill.'in 1.8- cineole / Camphor grubunda yer aldığını ve uçucu yağın 1.8- cineole oranının %35-51, Camphor oranının %7-13 arasında olduğunu belirlemişlerdir. Elde ettiğimiz değerlerin bu çalışma ile örtüştüğünü görmekteyiz. Yapılan diğer çalışmalarda Uçucu yağlardan elde ettiğimiz 1,8- cineole değerlerinin %33.36- 56.05 farklı yerlerde aynı bitki ile yapılan diğer çalışmalarda elde edilen değerler ile benzerlik gösterdiği anlaşılmaktadır.

Uçucu yağ ve uçucu yağ bileşimine ait elde ettiğimiz verilerin literatürlerle uyumlu olduğunu görmekteyiz. Bu Farklılıkların, hem hasat dönemi hem de lokaliteden, iklim ve çevresel faktörlerden kaynaklanabileceğini belirtmiştir³².

Tablo 1. Lokasyonlara göre *Salvia officinalis* L'in Uçucu yağ bileşenlerine ait analizi sonuçları.

Uçucu Yağ Miktarı (%)				
(Çanakkale)		Balıkesir (Edremit)		(Kütahya)
0,99		1,23		1,85
No	Bileşen adı	Bileşen miktarı (%)		
		(Çanakkale)	Balıkesir (Edremit)	(Kütahya)
1	α -pinene	4,32	3,62	2,34
2	Camphene	2,37	3,16	3,91
3	β -pinene	2,00	2,55	1,81
4	Sabinene	-	0,47	-
5	Myrcene	1,25	1,34	1,14
6	Limonene	1,29	1,47	1,66
7	1,8-cineole	7,75	7,81	6,57
8	γ -terpinene	0,41	0,44	0,37
9	α -terpinolene	-	-	0,41
10	Cymene	-	0,32	-
11	α -thujone	26,35	38,39	29,84
12	β -thujone	29,20	12,26	9,07
13	Trans-sabinene hydrate	0,31	0,32	-
14	Camphor	5,60	13,90	23,24
15	Linalool	-	0,28	0,52
16	Bornyl acetate	-	0,32	1,19
17	Terpinen-4-ol	0,29	0,30	-
18	β -caryophyllene	3,39	2,24	3,59
19	α -humulene	3,77	2,39	4,20
20	α -terpineol	-	-	0,68
21	Borneol	0,72	0,84	2,04
22	Caryophyllene oxide	1,18	0,63	0,35

23	Humulene epoxide II	1,14	0,60	0,46
24	Viridiflorol	5,55	4,38	2,65
25	Manool	3,11	1,98	3,98

Tablo 2. Lokasyonlara göre *Salvia aethiopsis* L'un Uçucu yağ bileşenlerine ait analizi sonuçları.

Uçucu Yağ Miktarı (%)				
. (Çanakkale)		<i>Balıkesir</i> (Edremit)		(Kütahya)
0,53		0,21		0,20
No	Bileşen adı	Bileşen miktarı (%)		
		. (Çanakkale)	Balıkesir (Edremit)	(Kütahya)
1	Bicycloelemene	-	1,27	-
2	Pentadecane	0,61	-	-
3	α -copaene	15,06	16,46	16,58
4	β -cubebene	5,62	7,04	6,93
5	β -ylangene	1,11	1,66	1,31
6	β -elemene	2,08	3,01	2,30
7	β -caryophyllene	36,22	30,46	35,96
8	α -humulene	8,68	7,40	8,54
9	Germacrene-D	13,23	20,01	15,20
10	β -bisabolene	-	-	0,78
11	Bicyclogermacrene	0,77	2,55	0,85
12	δ -cadinene	4,81	5,73	4,85
13	α -cubebene	0,79	0,81	0,88
14	cis-Muurool-5-en-4-beta-ol	1,27	1,00	1,06
15	Caryophyllene oxide	7,40	1,82	3,53
16	Humulene epoxide II	1,07	-	-
17	Viridiflorol	-	0,80	-
18	Carvacrol	1,26	-	1,23

Çizelge 3. Lokasyonlara göre *Salvia fruticosa* Mill'in Uçucu yağ bileşenlerine ait analizi sonuçları.

Uçucu Yağ Miktarı (%)				
(Çanakkale)		<i>Balıkesir</i> (Edremit)		(Kütahya)
4.10		2.72		1.93
No	Bileşen adı	Bileşen miktarı (%)		
		(Çanakkale)	Balıkesir (Edremit)	(Kütahya)
1	α -pinene	3.41	4.82	3.57

2	Camphene	4.09	6.90	2.34
3	β -pinene	5.45	7.53	7.64
4	Sabinene	0.25	1.31	-
5	Myrcene	2.25	4.15	5.95
6	Limonene	1.86	1.80	1.31
7	1,8-cineole	49.60	33.36	56.05
8	γ -terpinene	0.42	0.98	0.50
9	α -terpinolene	0.30	-	0.23
10	α -thujone	2.42	5.38	1.48
11	β -thujone	1.43	3.47	2.57
12	Trans-sabinenehydrate	0.45	0.68	0.65
13	Camphor	22.21	17.51	6.62
14	Linalool	0.27	1.08	0.23
15	.Cis-sabinenehydrate	-	-	0.24
16	Bornylacetate	0.30	1.34	3.07
17	Terpinen-4-ol	0.53	0.80	0.34
18	β -caryophyllene	2.31	3.31	3.20
19	δ -terpineol	-	-	0.43
20	α -humulene	0.64	1.58	1.23
21	α -terpineol	0.23	1.03	0.73
22	γ -terpineol	-	0.65	-
23	Borneol1,	1.39	-	-
24	Viridiflorol	0.42	0.69	0.23

SONUÇ

Yürütülen çalışmalara göre test edilen bitki türlerinin uçucu yağ ana bileşenleri ile oranları bakımından diğer çalışmalara göre farklılıklar saptanmıştır. *salvia*, bitkisinin çiçeklenme zamanı, yetiştiği coğrafik ve iklimsel faktörlere bağlı olarak uçucu yağ kompozisyonunda da değişkenlik olacağını bildirmiştir. Ayrıca tıbbi ve aromatik bitkilerde var olan biyoaktif maddelerin çeşitliliği, miktarı aynı zamanda bitkinin kullanılan kısmına, hasat sonrası işlemler ile kullanılan uçucu yağ elde ve analiz metodlarına göre de farklılıklar gösterebilmektedir³¹.

Çanakkale, Balıkesir, Kütahya lokasyonlarında yapılan çalışmalarda farklı toprak yapısı ve iklimsel özelliklere sahip bölgelerden yetiştirilen *Salvia officinalis* L. ve *Salvia aethiopis* L.'un uçucu yağ bileşenleri bakımından değişkenlik göstermiştir. *Salvia officinalis* L. en yüksek uçucu yağ ana bileşenleri sırasıyla α -thujone %38,39 Balıkesir, %29,84 Kütahya, %26,35 Çanakkale ve *Salvia aethiopis* L.'un β -caryophyllene %36,22 Çanakkale, %35,96 Kütahya, %30,46 Balıkesir olarak tespit edilmiştir. En fazla uçucu yağ bileşeni 23 bileşenle Balıkesir(*Salvia officinalis* L.) ve 15 bileşenle Çanakkale(*Salvia aethiopis* L.) lokasyonunda belirlenmiştir. Anadolu Adaçayı (*Salvia fruticosa* Mill.) sırasıyla 1.8-cineole %56.05 ile Kütahya, %49.60 ile Çanakkale, %33.36 Balıkesir- Edremit olarak tespit edilmiştir. Çevresel faktörlere göre uçucu yağ bileşenlerinin de farklılık gösterdiği düşünüldüğünde *Salvia* türlerinin kimyasal yapısının ortaya konulabilmesi için *Salvia* türleri açısından zengin olan ülkemizin farklı lokasyonlarından farklı *Salvia* türleri üzerinde daha fazla çalışmaların yürütülmesi gerekmektedir³⁹. Yapılan çalışmanın diğer çalışmalarla farklılık göstermesi uçucu yağ miktarı ve uçucu yağdaki ana bileşen maddesinin değişimleri, genetik ve çevresel faktörlere bağlı olarak değişim göstermiştir.

KAYNAKLAR

- [1] Arslan, N., Gürbüz, B. ve Yılmaz, G. 1995. Adaçayı (*Salvia officinalis* L.)'nda Tohum Tutma Oranı ve İndol Butirik Asitin (IBA) Gövde Çeliklerinin Köklenmesine Etkileri Üzerinde Araştırmalar. Tr. J. of Agriculture and Forestry, Tübitak, 19: 83-87
- [2] Başer, K.H.C., N. Kirimer. 2006. Essential oils of Lamiaceae plants of Turkey. Acta Horticulture, 723:163-172
- [3] Baytop, T., 1963, Türkiye'nin Tıbbi ve Zehirli Bitkileri, İ.Ü. Yayınları, İstanbul No:1039, Tıp Fakültesi, No:59, S.351.
- [4] Baytop, T. 1999 Türkiye'de Bitkiler İle Tedavi Geçmişte ve Bugün (II. Basım). Nobel Tıp Kitapevleri.
- [5] Bhattacharya, S., Study Of Some Indian Members Of The Genus *Salvia* With References To The Cytological Behaviour, Cytologia 43: 317-324, 1978.
- [6] Ceylan, A. 1996, Tıbbi Bitkiler-II (Uçucu Yağ Bitkileri) E.Ü.Z.F. Yayınları No:481, Bornova, İzmir, ISBN:975-483-362-1, S.225-240.
- [7] Clive, A. and Stace, C., Plant Taxonomy and Biosystematics, London, 1980.
- [8] Delamare, Ana Paula Longaray, et al. "Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil." Food chemistry 100.2 (2007): 603-608.
- [9] Ekren, S., Ark. Farklı Biçim Yüksekliklerinin Adaçayı (*Salvia officinalis* L.) Genotiplerinde Agronomik ve Teknolojik Özelliklere Etkisinin Belirlenmesi. Ege Üniv. Ziraat Fak. Derg., 2007, 44 (1): 55-70 .izmir.
- [10] Farnworth, N. R. (1990) The Role of Ethnopharmacology in Drug Development. In Bioactive Compounds From Plants, CIBA Foundation Symposium 154., pp.2-21. Chichester, New York Brisbane, Toronto, Singapore: John Wiley & Sons.
- [11] Formacek, V., and Kubecka, K. H., Essential Oil Analysis by GC and 13NMR Spectroscopy, Heyden son, London. 1981,
- [12] Furia, T. E. and Bellance, N., Eds. Fenarolis Hand Book of Flavour Ingredients, 2nd ed. CRC Press, Cleveland, Ohio 1975.
- [13] Hernandez-Perez, M., Rabanal, R. M., Arias A., De La Torre, M.C. and Rodriguez, B., Antibacterial and Cytotoxic Agent from *Salvia aethiopis* Roots, Pharmaceutical Biology, 37:1, 7-21, 1999.
- [14] Kılıç, A., Uçucu Yağ Elde Etme Yöntemleri, Bartın Orman Fakültesi Dergisi Yıl: 2008 Cilt:10 Sayı:13
- [15] Linskens, H.F., Jackson, J.F, 1997a Modern Methods of Plant Analysis, Vol. 19: Plant Volatile Analysis, Springer, Germany.

- [16] Miladinovic, D., Lj. Miladinovic, 2000. Antimicrobial Activity of Essential oil of Sage from Serbia. Physics, chemistry and Technology. Vol. 2, n 2, 2000, pp. 97-100.
- [17] Morteza-Semnani, K., Goodarzi, A., Azadbakht, M. (2005). The essential oil of *Salvia aethiopis* L.. Journal of Essential Oil Research, 17, 274-275.
- [18] Nakipoglu, M. (1989) Bazı Adaçayı (Salvia) Türleri ve Bu Türlerin Ekonomik Önemi. Buca Eğitim Fakültesi, Fen Bil. Eg. Bölümü., izmir.
- [19] Nakipoğlu, M., Şifalı Çay Olarak Kullanılan Bitkiler ve Adaçayı, Eğitim Bil. Dergisi, Buca Eğitim Fakültesi Yayın Organı 2(91-94), 1993.
- [20] Nakipoglu, M. ve Otan, H. (1992) Tıbbi Bitkilerin Flavonoidleri, J. of AARI, 4(1):70-93, NCCLS (National Committee for Clinical Laboratory Standards) (1999) Performance Standards For Antimicrobial Susceptibility Testing, 9th International Supplement. M100-S9.
- [21] Özgüven, M. ve Kırıcı, S. 1988. Tıbbi Bitkilerin Kültürü ve Karşılaşılan Sorunlar. I. Orman Tali Ürünleri Sempozyumu, Ormancılık ve Tabiatı Koruma Vakfı, Ankara.
- [22] Özkan, M., Senel, G. (2006). Karyotype analysis of some *Salvia* (Lamiaceae) species. Botanica Lithuanica, 12, 169-174.
- [23] Özkan, M., ŞENEL, G., *Salvia aethiopis* L. (Lamiaceae) Üzerinde Morfolojik Ve Anatomik Bir Araştırma, Doğu Anadolu Bölgesi Araştırmaları; 2006
- [24] Özek, T., Tabanca, N., Demirci, F., David E. Wedge and K. Hüsnü Can Başer. Enantiomeric Distribution of Some Linalool Containing Essential Oils and Their Biological Activities. Rec. Nat. Prod. 4:4 (2010) 180-192.
- [25] Principe, P. P. (1991) Valuing the Biodiversity of Medical Plants. In Conservation of Medicinal Plants. Eds. Akerele, O., Heywood, V. & Synge, H., pp. 79-124. Cambridge: Cambridge University Press.
- [26] Rajabi, Zahra, et al. "Compositions and yield variation of essential oils among and within nine *Salvia* species from various areas of Iran." Industrial Crops and Products 61 (2014): 233-239.
- [27] Rustaiyan, Abdolhossein, et al. "Volatile constituents of three *Salvia* species grown wild in Iran." Flavour and fragrance journal 14.5 (1999): 276-278.
- [28] Sagareishvili, T. G., B.L. Grigolava, N. E. Gelashvili, E.P. Kemertelidze. 2000. Composition of Essential Oil from *Salvia officinalis* L. Cultivated in Georgia. Chemistry of Natural Compounds. Vol: 36. N: 4. Pages: 360-361. New York.
- [29] Salameh A., S. Dordevic, 2000, The Investigation of the Quality of Sage (*Salvia officinalis* L.) from Jordan, UDC 582.824, University of Niš The Scientific Journal Facta Universitatis, Series: Working and Living Environmental Protection Vol.1, No 5, pp.103-108.
- [30] Senatore, F., De Fusco, R. ve De Feo, V. 1997. Essential oils from *Salvia* spp. (Lamiaceae). I. Chemical composition of the essential oils from *Salvia glutinosa* L. growing wild in Southern Italy. Journal of Essential Oil Research, 9: 151-157.
- [31] Senatore, F., Arnold, N.A., Piozzi, F. (2004). Chemical composition of the essential oil of *Salvia multicaulis* Vahl. Var. *simplicifolia* Boiss. Growing wild in Lebanon. Journal of chromatography A, 1052, 237-240.
- [32] Sezik, E. ve Yeşilada, E. (1999). Uçucu Yağ Taşıyan Türk Halk İlaçları. Essential Oils, Eds: Kırimer, N., Mat, A., Anadolu Üniversitesi Basımevi, Eskişehir, ss. 93-131.
- [33] Skoula, M., J. E. Abbes, C. B. Johnson. 2000. Genetic variation of volatile essential oil and rosmarinic acid in populations of *Salvia fruticosa* Mill, growing in Crete. Journal of Biochemical Systematics and Ecology, 28:551-561.
- [34] Tajbakhsh, M., Rineh, A., Khalilzadeh, M.A., Eslami, B (2007). Chemical constituents of the essential oils from leaves, flowers, stem and aerial parts of *Salvia aethiopis* L. from Iran. Journal of Essential Oil Research, 9, 569-571.
- [35] Tan, A. (1992) Türkiye’de Bitkisel Çeşitlilik ve Bitki Genetik Kaynakları. J. of AARI, 2: 50-64.
- [36] Tepe, B., Sökmen, M., Akpulat, A. H. and Sökmen, A., Screening of the antioxidant potential of six *Salvia* species from Turkey, Food Chemistry, 90, 333-340, 2005.
- [37] Yenikalaycı, A., 1998. Çukurova Bölgesinde Doğal Adaçayı Türleri (*Salvia* spp.) ile tıbbi adaçayı (*Salvia officinalis* L.)’nın Kültürü ve Kemotaksonomik Araştırmalar, Çukurova Üni. Ziraat Fakültesi Tarla Bitkileri Anabilim Dalı Yüksek lisans Tezi.
- [38] Yılar, M., Kadioğlu, İ., Telci, İ., 2015. Tokat İlinde Yayılış Gösteren Bazı Adaçayı Türlerinin Uçucu Yağ Kompozisyonlarının Belirlenmesi. Türk Tarım ve Doğa Bilimleri Dergisi 2(4): 313–319.
- [39] Zeybek, U., N. Zeybek, 2002, Farmasötik Botanik [Kapalı Tohumlu Bitkiler (Angiospermae) Sistematigi ve Önemli Maddeleri], E.Ü. Eczacılık Fakültesi Yayınları No:3 Bornova, İzmir, S.380.

DETERMINATION OF ESSENTIAL OIL COMPOSITON OF BLANKET-LEAF (*Stachys byzantina* C. Koch.) BY MICROWAVE ASSISTED EXTRACTION COUPLED TO HEADSPACE SINGLE-DROP MICROEXTRACTION

Zahra KAFIL¹, Marzieh BABASHPOUR-ASL¹, Marzieh PIRYAEI²

¹Department of Horticulture, Maragheh Branch, Islamic Azad University, Maragheh, Iran.

²Department of Chemistry, Faculty of Science, University of Maragheh, Maragheh, Iran.

Corresponding author email: babashpour@gmail.com

Abstract

The composition of essential oils from *Stachys byzantina* was studied by simple method based on gas chromatography-mass spectrophotometry (GC-MS) following microwave assisted headspace single-drop microextraction (MA-HS-SDME) method. Several parameters affecting on MA-HS-SDME such as sample mass, solvent volume, extraction time, microwave power and the nature of extracting solvent were optimized. The MA-HS-SDME method was compared with traditional hydrodistillation (HD) method. Within the study elaborated, thirty-eight components were extracted and identified. Compared with HD, MA-HS-SDME is an easy, rapid and efficient method for the analysis of essential oils in *S. byzantina*.

Keywords: volatile oil, woundwort, blanket-leaf, MA-HS-SDME.

INTRODUCTION

Stachys L. or woundwort belonging to the Lamiaceae family, is a subcosmopolitan genus of herbs and shrubs that comprise about 200-300 species in the world (Rechinger, 1986) and is considered as one of the largest genera of the Lamiaceae. The genus is distributed in temperate and tropical regions of the world, with the exception of Australasia, and it is widespread in mountainous and moist places (Ball, 1972). Plants of this genus have been used centuries to treat genital tumors, sclerosis of the spleen, inflammatory diseases, cough, and ulcers (Zargari, 1990). Several studies have demonstrated anti-inflammatory, cytotoxic, antitoxic, antibacterial and antioxidant activities of extracts from *Stachys* spp. (Bilusic-Vundac et al., 2007). *Stachys byzantina* C. Koch. (Blanket-leaf) is a species of *Stachys*. Single-drop microextraction (SDME), using typically 1-3 µL of an organic solvent at the tip of an organic solvent at the tip of a microsyringe, has evolved from liquid phase microextraction (LPME). The headspace SDME (HS-SDME) followed by gas chromatography-mass spectrophotometry (GC-MS) has been proved a simple, sensitive and solvent-free method for the analysis of essential oils (Gholivand et al., 2013). The rapid heat transfer rates of microwave heating allow for quicker analysis of the analytes in complex samples compared to more conventional extractions (Gholivand et al., 2012). So we used microwave assisted extraction coupled to headspace single-drop microextraction (MA-HS-DSME). This article investigates the effect of MA-HS-SDME method on amount of essential oils from *S. byzantina*.

MATERIAL AND METHODES

Plant material and reagents

The plant materials used in this study (aerial parts of *S. byzantina*) were obtained from Heyran Defile (Guilan province, Iran) during June-July 2016. Voucher specimens (6513 THE) of herbal material were deposited in the Herbarium of the Faculty of Pharmacy, University of Tabriz, Tabriz, Iran.

After drying in the shade and being ground to a fine powder with particle size of 120 mesh, it was used for HD and MA-HS-SDME. n-heptadecane, n-hexadecane, n-dodecan, n-butanol, n-heptane, nitrobenzene, benzyl alcohol was purchased from Merck (Darmstadt, Germany).

Hydrodistillation (HD) procedure

The essential oils of aerial parts (100 g) of *S. byzantina* were obtained by hydrodistillation for 3 h in a Clevenger type apparatus as recommended by British Pharmacopeia. The separation from water was conducted by hexane. The extract was dried over anhydrous sodium sulfate. The essential oil stored at +4°C.

MA-HS-SDME of essential oils

Weighed amount (1-7 g) of powdered herb was transferred into a 25 ml round bottom flask. After assembling a condenser, a 10 µL micro syringe (Hamilton, USA) containing 3 µl of n-heptadecane as extracting solvent and n-hexadecane as internal standard (with a volume ratio of 1:200 v/v) was suspended on top of the flask, the herb was heated by microwave power (100-700 W). After extraction (15-300 seconds), the plunger was withdrawn, and the micro drop was retracted into the micro syringe, and finally the micro drop injected directly into the GC–MS injection port.

Apparatus and GC–MS analysis

A Hewlett-Packard Agilent 7890A series GC equipped with a split/splitless injector and an Agilent 5975C mass-selective detector system were used for determination. The MS was operated in the EI mode (70 eV). Helium (99.999%) was employed as a carrier gas, and its flow rate was adjusted to 1.1 mL min⁻¹. The separation of essential oils was performed on a 30 m×0.25 mm HP-5 MS column with 0.25 µm film thickness. The column was held at 50°C and increased to 180°C at a rate of 15°C min⁻¹ and then raised to 260°C at 20°C min⁻¹ and kept at this temperature for 5 min. The injector temperature was set at 260°C, and all injections were carried out on the splitless mode for 2 min. The GC–MS interface, ion source and quadrupole temperatures were set at 280, 230 and 150°C, respectively. Compounds were identified using the Wiley 7N (Wiley, New York, NY, USA) Mass Spectral Library.

RESULTS AND DISCUSSION

Hydrodistillation (HD)

The aerial parts of *S. byzantina* yielded 0.22% (v/w) yellowish oil with HD by Clevenger apparatus. Thirty-eight compounds were identified using GC-MS analysis and the major compounds were thymol (10.34%), para-cymene (10.31%), α-copaene (8.62%) and germacrene D (6.83%). The chemical composition of the aerial parts essential oil is given in Table 1.

Optimization of MA-HS-SDME

SDME has attracted increasing attentions. Solvent-free microwave extraction is an original combination of microwaves and dry distillation with the advantages of rapidity (Gholivand et al., 2013). The combination of microwave, dry distillation and SDME that has been introduced in this study produced a method that is simple and rapid for the analysis of the essential oil in plant samples. The studies were commenced by optimization of experimental parameters such as, sample mass, solvent volume, extraction time, microwave power and the nature of extracting solvent.

Table 1. Constituents of the essential oil of *Stachys byzantina* by hydrodistillation and MA-HS-SDME.

No.	Compounds	RI ^a	(HD) Area% ^b	(MA-HS-SDME) Area % ^c	RSD ^d
1	α -Thujene	933	0.05	0.06	12.4
2	α -Pinene	940	3.22	3.05	5.3
3	Sabinene	974	0.03	0.02	17.6
4	β -Pinene	981	3.75	2.38	15.1
5	3-Octanol	995	0.03	0	-
6	δ -3-Carene	1010	0.12	0.08	17.1
7	α -Terpinene	1015	0.70	0.82	8.5
8	Para-cymene	1023	10.31	8.88	14.7
9	1,8-Cineole	1032	0.21	0.07	14.2
10	n- Octanol	1070	0.10	0.04	10.3
11	Terpin-4-ol	1075	3.66	2.12	6.2
12	α -Terpineol	1088	1.29	1.12	10.5
13	Geraniol	1251	0.41	0.40	15.8
14	Thymol	1291	10.34	9.48	12.6
15	α -Copaene	1378	8.62	6.50	13.7
16	Geranyl acetate	1385	0.17	0.08	7.1
17	β -Cubebene	1390	5.18	2.56	10.8
18	β -Elemene	1395	2.27	1.11	16.3
19	Beta-cedrene	1415	1.22	0.84	8.2
20	E-Caryophyllene	1420	2.53	1.37	9.3
21	γ - Curcumene	1476	1.36	1.24	12.7
22	Germacrene- D	1480	6.83	7.21	17.3
23	β -Selinene	1483	0.34	0.12	11.2
24	Bicyclgermacrene	1490	2.85	1.67	16.9
25	Z- β -Bisabolene	1502	4.30	4.75	12.5
26	(E,E)- α -Farnesene	1505	1.08	0.61	13.5
27	Germacrene-B	1553	3.27	4.12	8.4
28	E-Nerolidol	1560	0.14	0.17	14.6
29	Germacrene D-4-ol	1570	1.23	1.15	13.1
30	Spathulenol	1572	4.35	3.15	11.7
31	Caryophyllene oxide	1583	0.26	0.17	10.6
32	Veridiflorol	1595	1.08	0.33	15.7
33	Epi- α -cadinol	1641	0.12	0.06	9.1
34	Epi- α -muurolol	1647	0.34	0.13	12.6
35	α -Cadinol	1657	0.11	0.05	12.1
36	Valeranone	1670	5.38	4.12	11.3
37	α -Bisabolol	1675	0.07	0.03	14.7
38	Cedren-13-ol-8	1685	4.24	2.60	11.6

Notes: ^aRetention indices. ^bRelative area (peak area relative to total peak area) for hydrodistillation (HD) method. ^cRelative area (peak area relative to total peak area except for the solvent peak) for the micro-assisted headspace single-drop microextraction (MA-HS-SDME) method. ^dRSD values for MA-HS-SDME method (relative peak area).

Selection of solvent and its volume

The choice of solvent for HS-SDME should be considered from physical properties of a low vapor pressure and high boiling point, in order to reduce vaporization of the solvent drop during the extraction process (wood et al., 2004). Five different solvents with different polarities and boiling points including n-heptane, n-dodecane, n-hexadecane and n-heptadecane and benzyl alcohol were examined for MA-HS-SDME. The experimental result (Fig. 1) indicated that n-heptadecane

presented excellent characteristic as an extraction solvent among the five organic solvents. This solvent with high boiling point has separated peak from the sample peaks and does not coelute with components of the sample. Hexadecane was added to heptadecane with a ratio of 1:200 (v/v) and utilized as an internal standard. Further experiments were carried out to determine the optimum drop volume of the organic solvent. The effect of the organic solvent volume in the range 1–3 μL was tested with microwave irradiation (420 W, 240 seconds) on a 4 g plant sample. Although a better extraction result could be obtained in a larger drop volume, the larger drop tended to be disengaged from the needle of the micro syringe due to the heavier weight. Additionally, the larger injection volumes result in band broadening in capillary GC. Thus, a microdrop volume of 3.0 μL (Fig. 2) was used, as it ensured a stable and reproducible formation, albeit with some penalty in the form of loss of sensitivity.

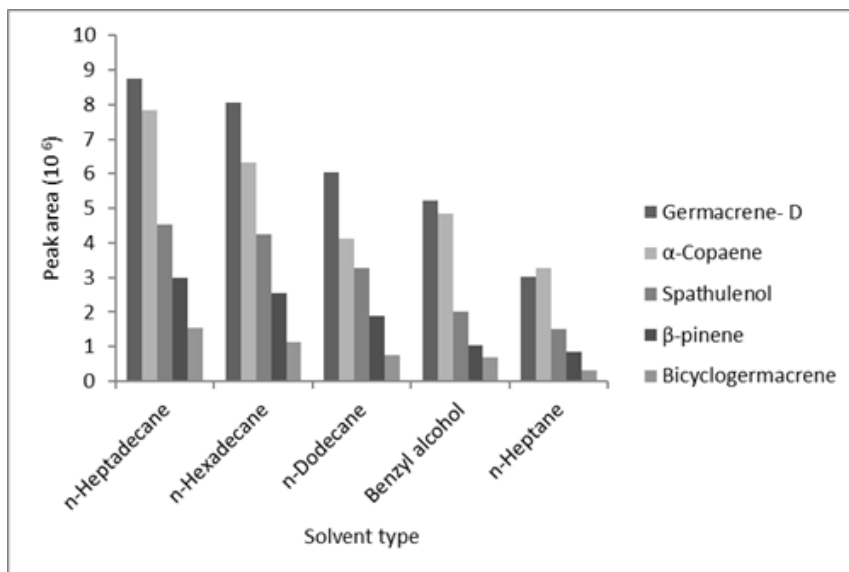


Fig 1. Effect of solvent type on the extraction efficiency. Extraction conditions: sample mass, 4 g; extraction time, 4 minutes; microwave power 420 W).

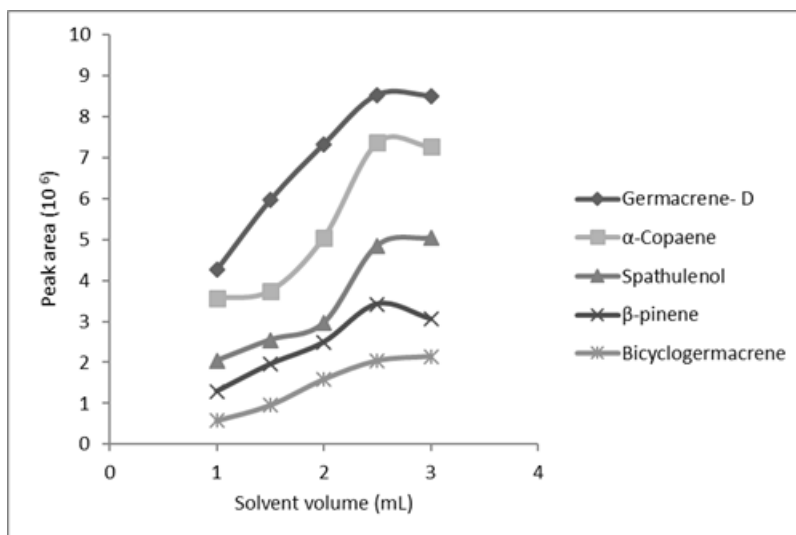


Fig 2. Effect of solvent volume on the extraction efficiency. Extraction conditions: sample mass, 4 g; extraction time, 4 minutes; microwave power 420 W.

Sample mass

The effect of sample mass on the extraction efficiency was investigated. Aerial part sample of *S. byzantina* with a mass of 1.0-7.0 g were analyzed by MA-HS-SDME at optimum conditions (microwave power, 420 W; irradiation time, 4 minutes; 3 μ L of n-heptadecane). Fig. 3 shows the total peak area and the individual peak areas of the main compounds obtained at different sample mass. As seen from Fig. 3, increasing the sample mass (from 1.0 to 7.0 g) leads to increasing the analytes concentration in the headspace, and thus enhances the extracted amount of the analytes in the microdrop solvent. Further increasing of the sample mass (>4.0 g) had no significant effect on the extracted amount of analytes in the microdrop and thus the extraction balance was achieved. Thus, based on these observations, the optimal sample mass was chosen to be 4.0 g of dried aerial parts of this plant.

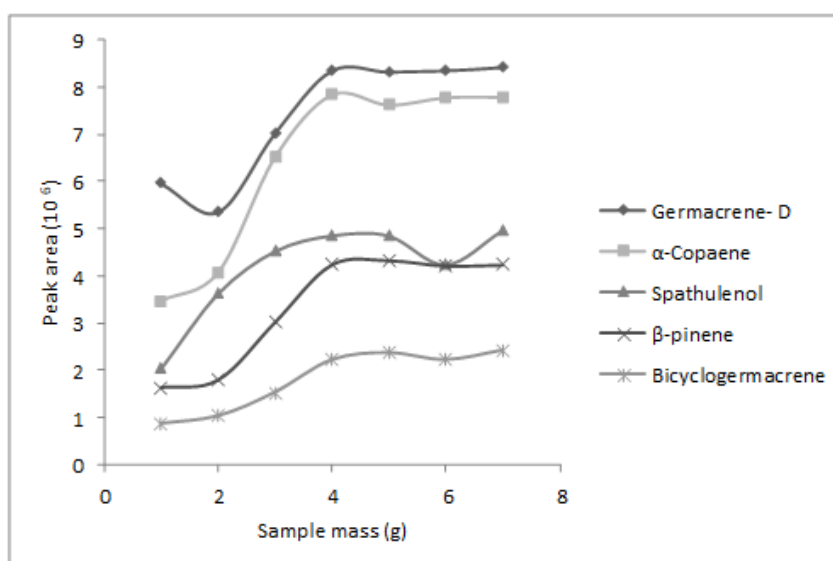


Fig 3. Effect of sample mass on the extraction efficiency. Extraction condition: extraction time, 4 minutes; 3 μ L n-heptadecane; microwave power 420 W.

Extraction time

An extraction time varying from 15 to 300 seconds was investigated and the results are shown in Fig. 4. The highest peak area was observed at 4 minutes of microdrop exposure. Longer extraction times have reversed effect on the extraction efficiency that may be attributed to solvent evaporation and back-extraction of components from the drop into the headspace. As it is obvious, microwave extraction is clearly quicker than conventional HD. The extraction takes 4 minutes, whilst 4 hours were required by HD (Table 1). An extraction time of 4 minutes with microwave provides volatile compounds comparable to those obtained after 240 minutes by means of HD, which is the most diffused method for the extraction of essential oils.

Microwave power

The effect of the microwave power ranging from 100 to 700 W was investigated. Too high microwave power would scorch the plant and it was difficult to control the temperature. Too low microwave power would consume more extraction time. The experimental results indicated that the optimum microwave power was 420 W (Fig. 5).

Comparison of HD and MA-HS-SDME methods

In a comparison study between MA-HS-SDME and HD methods almost the same number components were found (38). According to table 1 the major compounds in MA-HS-SDME method were thymol (9.48%), para-cymene 8.88%, germacrene D (7.21%) and α -copaene (6.50%). In this method the isolation of essential oils in the herb was rapidly completed, and then the isolated volatile compounds were simultaneously extracted and concentrated by a suspended microdrop (Piryaei and Nazemiyeh, 2015).

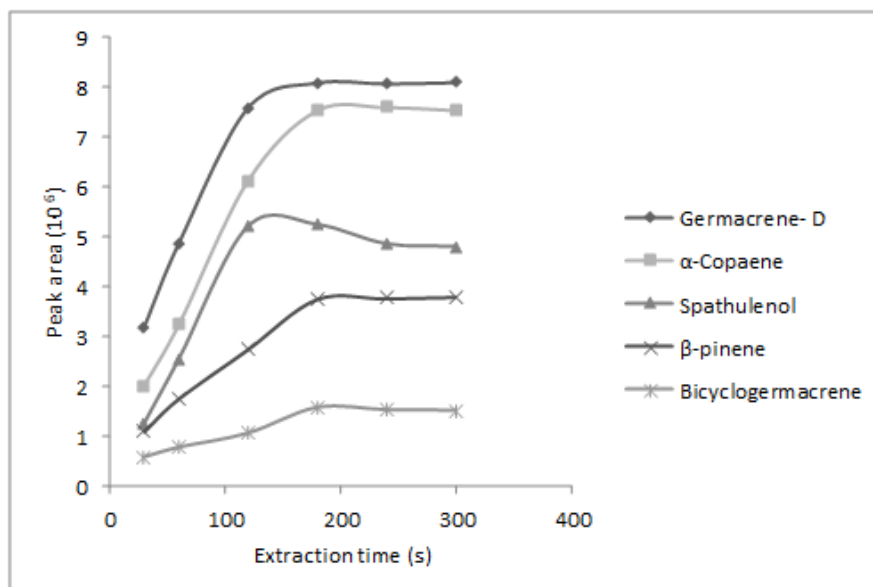


Fig 4. Effect of extraction time on the extraction efficiency. Extraction condition: sample mass, 4 g; microdrop volume, 3 μ L; microwave power 420 W.

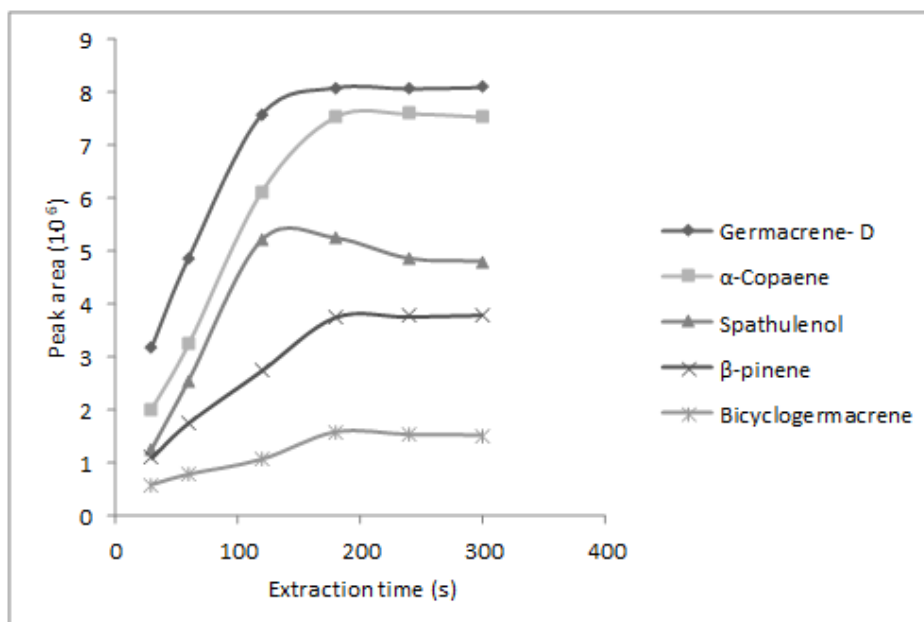


Fig 5. Effect of microwave power on the extraction efficiency. Extraction conditions: extraction time, 4 minutes; sample mass, 4 g; microdrop volume, 3 μ L.

CONCLUSION

In this study MA-HS-SDME method was successfully performed for the determination of essential oils in *S. byzantina* C. Koch. Thirty-eight compounds were identified in this plant using the proposed method. Compared with HD method MA-HS-SDME method is an easy, rapid and efficient method for the analysis of essential oils in *S. byzantina*.

REFERENCES

- Ball, P.W., 1972. *Stachys* L. In T. G. Tutin (Ed.), *Flora Europaea* (pp. 154). Cambridge: Cambridge University Press.
- Bilusic-Vundac, V., Brantner AH, Plazibat M., 2007. Content of polyphenolic constituents and antioxidant activity of some *Stachys* taxa. *Food Chem.* 104:1277–1281.
- Gholivand, M.B., Piryaee, M., Papzan, A., 2012. Evaluation effect of microwave irradiation on the amount of volatile compounds, monoterpenes and sesquiterpenoids from *Thymus kotschyanus* Boiss with four methods. *Nat. Prod. Res.*, 27:1228-1231.
- Gholivand, M.B., Piryaee, M., Abolghasemi, M.M., Popzan, A., 2013. Comparison of microwave-assisted headspace single-drop microextraction (MA-HS-SDME) with hydrodistillation for the determination of volatile compounds from *Prangos uloptera*. *J. Essen. Oil Res.*, 25:49-54.
- Piryaee, M. and Nazemiyeh, H., 2015. Fast analysis of volatile components of *Achillea tenuifolia* Lam. with microwave distillation followed by headspace single-drop microextraction coupled to gas chromatography mass spectrometry (GC–MS). *Nat. Prod. Res.*, 30:991-994.
- Rechinger, H., 1986. *Flora Iranica*, Lamiaceae, vol. 150. Akademische Druck Verlagsantalt, Graz, Austria: 360–361.
- Zargari, A., 1990. *Medicinal Plants*. Tehran University Publications, Tehran. Iran.
- Wood, D.C., Miller, J.M., Christ, I., 2004. Headspace liquid microextraction. *LC GC N. Am.*, 22:516-522.

EVALUATION OF PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITIES OF AQUEOUS AND NONAQUEOUS EXTRACT OF *Vitis vinifera* L. “WHITE SEEDLESS”

Marzieh BABASHPOUR-ASL¹, Marzieh PIRYAEI²

¹Department of Horticulture, Islamic Azad University, Maragheh Branch, Maragheh, Iran.

²Department of Chemistry, Faculty of Science, University of Maragheh, Maragheh, Iran.

Corresponding Author Email: babashpour@gmail.com

Abstract

Polyphenols have been associated with the bioactive potential of grapes due to their beneficial health effects. For this reason, the aim of this study was to determine the content of bioactive compounds (total phenolic, flavonoids, anthocyanin, and ascorbic acid contents) and in vitro antioxidant activities of selected *Vitis vinifera* “white seedless” from Maragheh region, East Azerbaijan, Iran. Antioxidant activities of the samples were determined by three testing systems namely DPPH, β -carotene/linoleic acid, and reducing power assays. In the DPPH system, the highest radical scavenging activity was seen by the polar subfraction of the methanol extract in frozen fruits ($IC_{50} = 35.67 \pm 6.5 \mu\text{g/ml}$). Comparison of two extraction methods, maceration and ultrasonic, showed that the results of maceration is better than the ultrasonic. These advantages are visible on extracting of the flavonoids, anthocyanin, total phenolic, ascorbic acid contents and antioxidant capacity.

Keywords: total phenols, anthocyanin, ascorbic acid, antioxidant, grape.

INTRODUCTION

Grapes are one of the most widely grown fruit crops throughout the world, and their composition and properties have been extensively investigated, with several reports of the presence of large amounts of phenolic compounds (Rockenbach et al, 2011). Grapes are a rich source of phenolic compounds (Vinson, 2001). Grape polyphenols range from simple compounds (monomers) to complex tannin type substances (oligomers and polymers). Several classes of antioxidant polyphenols have been identified and quantified in grapes, such as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives (resveratrol), flavan-3-ols (catechin, epicatechin), flavonols (kaempferol, quercetin, myricetin), anthocyanins (Bonilla, 1999; Borbalan, 2003; Cantos, 2002; Kallithraka, 2005).

An important field of research today is the control of ‘redox’ status by consuming foods with high antioxidant properties. Natural antioxidants present in the diet increase the resistance to oxidative stress and they may have a substantial impact on human health (Dimitrios, 2006).

Several methods have been developed to measure the free radical scavenging capacity (RSC), regardless of the individual compounds, which contribute towards the total capacity of a plant in scavenging free radicals. The methods are typically based on inhibition of the accumulation of oxidized products, since the generation of free radical species is inhibited by the addition of antioxidants and this gives rise to a reduction of the end point by scavenging free radicals. The reliable method to determine RSC involves the measurement of the disappearance of free radicals, such as the 2, 2-azino-bis 3-ethylbenzthiazoline-6-sulphonic acid radical (ABTS⁺), the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH^o) or other colored radicals, with a spectrophotometer (Gholivand, 2014; Choi et al, 2002; Miller and Rice-Evans, 1997; Sanchez-Moreno et al., 1998).

This present study, it was intended to study the total phenolics, total flavonoids, anthocyanin, ascorbic acid contents, and antioxidant activity of *Vitis vinifera* frozen and dried fruits in the polar and non-polar subfractions with two methods of extraction.

MATERIAL AND METHODES

Materials

All solvents and reagents were obtained from Merck (Darmstadt, Germany). The fruits of *Vitis vinifera* L. “white seedless” were gathered in the summer of 2015 from Maragheh region, East Azerbaijan, Iran. Fruits were stored both frozen and dried.

Maceration

Four hundred grams of frozen and dried fruits put in the methanol at 45°C for frozen and 50°C for dried fruits during 45 and 60 hours respectively. The extracts were filtered and concentrated under vacuum at 50°C by using a rotary evaporator. These extracts were suspended in water and extracted with chloroform (3 × 500 ml). The extracts were stored in darkness at 4°C until used within a maximum period of one week. The extractions separated with water (polar) and chloroform (non-polar).

Ultrasonic Extraction

Frozen and dried fruits of *Vitis vinifera* was weighed exactly (400 g) and mixed with 1000 mL of the solvent in a reagent bottle. The bottle was then closed and placed in the ultrasonic bath with temperature maintained at 45°C for frozen during 15 min and 50°C for dried fruits in 20min using ultrasonicator (42 kHz, heat power 250 W, KQ-500DE, MTH, China). The mixture was then filtered through Whatman filter paper No. 4 and the filtrate was evaporated under reduced pressure at 50°C. These extracts were suspended in water and extracted with chloroform (3 × 500 ml). The extracts were stored in darkness at 4°C until used within a maximum period of one week. The extractions separated with water (polar) and chloroform (non-polar).

Determination of Antioxidant Properties

1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH) scavenging activity assay

The free radical-scavenging activities of extract were measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Sharififar et al. (2007) with some modification.

β –Carotene/linoleic acid assay

The antioxidant activity was evaluated according to the method described by Miller (1971) with some modifications.

Reducing power assay

The reducing power of extract was determined according to the method of Jahanban-Sfahlan et al. (1965) with some modifications.

Determination of total phenolic contents

Total phenolic contents of the extracts were determined using the Folin-Ciocalteu reagent according to the method of Singleton and Rossi (1965) using gallic acid as standard, with some modifications.

Determination of total flavonoids

A modified protocol of that described by Kim et al. (2003) was employed. Total flavonoid content was calculated from a calibration curve using catechin as standard, and expressed as mg catechin equivalents (CTE) per 100 g plant (Dourtoglou et al, 2006).

Determination of ascorbic acid content

Ascorbic acid of the extracts was determined using ascorbic acid as standard, with some modifications (Pantelidis, 2007).

Determination of anthocyanins

Total anthocyanin content was measured with the pH differential absorbance method, as described by Cheng and Breen (1991).

RESULTS AND DISCUSSION

Antioxidant Properties

Free radical, DPPH, scavenging activity assay

The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability or radical-scavenging activity. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form 1, 1-diphenyl-2-picryl hydrazine (non-radical) with the loss of this violet color (Molyneux, 2004). DPPH scavenging activity is usually presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Lower IC_{50} value reflects better DPPH radical-scavenging activity.

In this study, the effect of two extraction methods ultrasound and maceration for the efficient extraction of antioxidative compounds from *Vitis vinifera* in the case of frozen and dried fruits was investigated. Methanol was chosen as the solvent because alcohols are most widely used in antioxidant extraction work. During extraction, it was seen that maximum extraction yield was achieved with maceration extraction method.

The polar subfraction in frozen fruits of methanol extract of provided the highest radical-scavenging activity with the lowest IC_{50} value of 35.67 ± 6.5 $\mu\text{g/ml}$ for maceration method. In addition, DPPH scavenging abilities of the methanolic extracts were lower than that of synthetic antioxidant BHT. Ascorbic acid and BHT were used as standards. In the figures 1 and 2 were shown a comparison between polar and non-polar subfraction in frozen and dried fruits of *Vitis vinifera* L. “white seedless” with maceration and ultrasonic extraction methods.

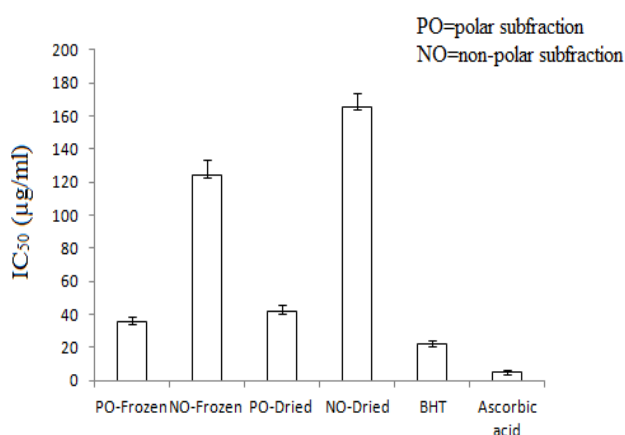


Fig 1. Comparison between polar and non-polar subfraction in frozen and dried fruits of *Vitis vinifera* L. “white seedless” with maceration.

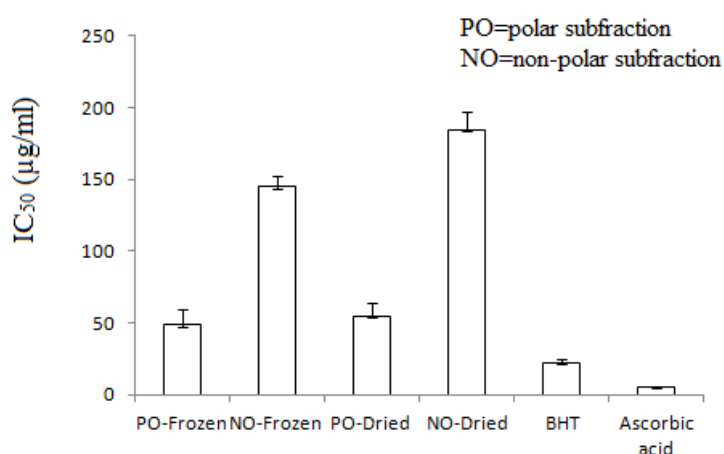


Fig 2. Comparison between polar and non-polar subfraction in frozen and dried fruits of *Vitis vinifera* L. “white seedless” with ultrasonic.

***β*-carotene/ linoleic acid assay**

The antioxidant activities of the plant extract was also evaluated by the spectrophotometric β -carotene bleaching test. In a β -carotene/ linoleic acid model system, β -carotene undergoes rapid discoloration in the absence of an antioxidant. β -carotene bleaching method is based on the loss of the yellow color of β -carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of β -carotene bleaching can be slowed down in the presence of antioxidants (Kulisic et al, 2004). The relative antioxidative activities (RAAs) of the extracts were calculated from the equation, $RAA = A \text{ sample} / A \text{ BHT}$, where A BHT is the absorbance of the control (BHT) and A sample is the absorbance of the extract. The calculated RAAs of the extract with maceration and ultrasonic methods are given in figures 3 and 4. In both assays, the polar subfraction of methanolic extract in dried fruits of *Vitis vinifera* showed better antioxidative capacity than the others subfraction (RAAs for Maceration: 88.35 % and for Ultrasonic: 71.36%).

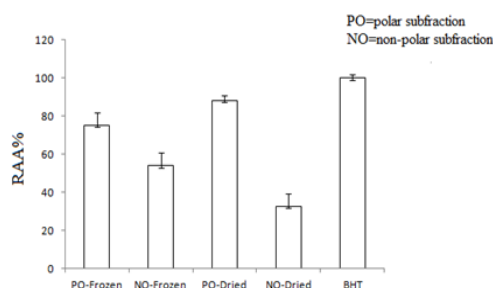


Fig 3. Relative antioxidative activities (RAAs) of the methanolic extracts of frozen and dried fruits of *Vitis vinifera* L. “white seedless” with maceration.

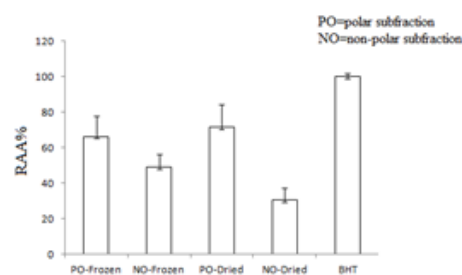


Fig 4. Relative antioxidative activities (RAAs) of the methanolic extracts of frozen and dried fruits of *Vitis vinifera* L. with ultrasonic.

Reducing power assay

Different studies have indicated that the electron donation capacity reflects the reducing power of bioactive compounds in associated with antioxidant activity. Antioxidants can be explained as reducers, and inactivation of oxidants by reducers can be described as redox reaction in which one reaction species is reduced at the expense of the oxidation of the other. Fe^{3+} reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Yildirim et al, 2001). In the reducing power assay, the presence of antioxidants in the sample would result in the reducing of Fe^{3+} – Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then being monitored by measuring the formation of Perl’s Prussian blue ($Fe_4 [Fe(CN)_6]_3$) at 700 nm. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figures 5 and 6 shows the reducing power of the methanolic extract of *Vitis vinifera* as a function of their concentrations with maceration and ultrasonic methods. It was found that the reducing power of extract increased with the increase of their concentrations.

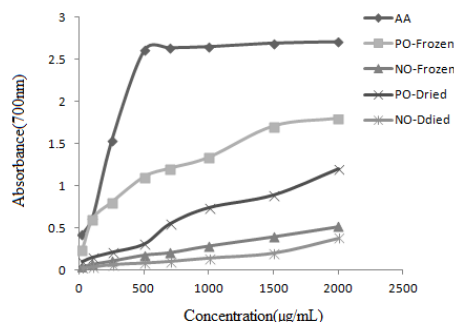


Fig 5. Reducing power for the methanolic extract of frozen and dried fruits of *Vitis vinifera* L. “white seedless” as a function of their concentrations with maceration.

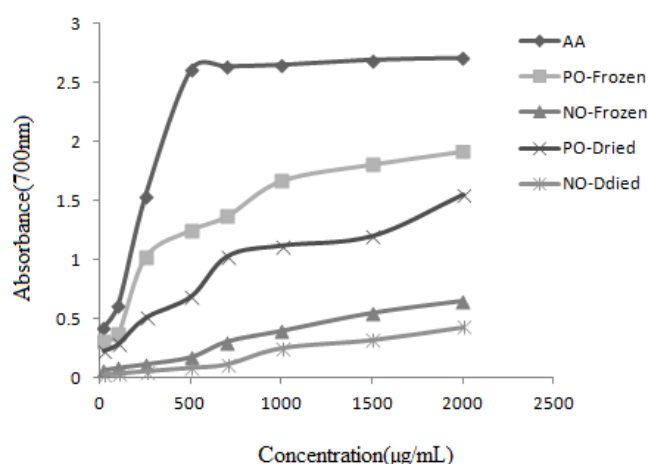


Fig 6. Reducing power for the methanolic extract of frozen and dried fruits of *Vitis vinifera* L. “white seedless” as a function of their concentrations with ultrasonic.

Total phenolic compounds

The amounts of total phenolics in the extract for frozen and dried fruits were determined spectrometrically according to the Folin–Ciocalteu procedure and calculated as gallic acid equivalents. Gallic acid is a water-soluble polyhydroxyphenolic compound that can be found in various natural plants. The standard curve equation was, y (absorbance) = $0.0003 \times \text{gallic acid } (\mu\text{g}) + 0.00534$. The absorbance value was inserted in the above equation and the total amount of phenolic compound was calculated. The amounts of total phenols found in the plant methanolic extract are shown in figures 7 and 8.

The results indicated that the polar subfraction in frozen fruits in methanolic extract with maceration method has higher total phenolic compounds than the other subfractions. In addition, according to these results, there is a relationship between total phenol contents and antioxidant activity. Phenolic compounds, biologically active components, are the main agents that can donate hydrogen to free radicals and thus break the chain reaction of lipid oxidation at the first initiation step. This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups (Sun et al, 2002).

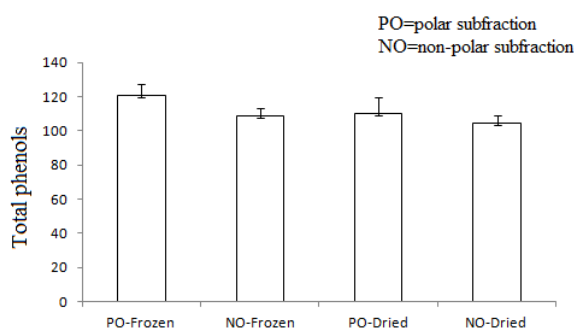


Fig 7. The amounts of total phenols found in the methanolic extracts of frozen and dried fruits of *Vitis vinifera* L. “white seedless” with maceration.

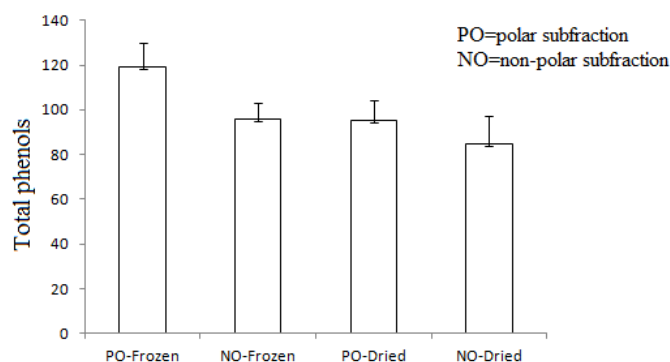


Fig 8. The amounts of total phenols found in the methanolic extracts of frozen and dried fruits of *Vitis vinifera* L. “white seedless” with ultrasonic.

Total flavonoid content

The standard curve equation for determination flavonoids with catechin is y (absorbance) $= 0.004 \times \text{catechin } (\mu\text{g}) + 0.0495$. The amounts of total flavonoids for frozen and dried fruits of *Vitis vinifera* with two extraction methods were shown in table 1.

Table 1. The amounts of total flavonoids in methanolic extract of frozen and dried fruits of *Vitis vinifera* L. “white seedless”.

Fruit type	Extraction	Flavonoids
Frozen	Maceration	82.37± 9.4
	Ultrasonic	68.57±7.6
Dried	Maceration	64.81±5.7
	Ultrasonic	52.73±3.8

Anthocyanins content

Significant differences in anthocyanin content were recorded, since these pigments are responsible for the red and blue color. The polar subfraction in frozen fruits contained the highest anthocyanin content expressed as cyanidin-3- glucoside. The results were shown in tables 2 and 3.

Ascorbic acid content

Significant differences in ascorbic acid content among the different subfraction were recorded tables 2 and 3. The polar subfraction in frozen fruits had the highest content of ascorbic acid (4.89 mg/ 100 g sw).

CONCLUSION

According to the results of this study, the methanolic extracts of frozen and dried fruits of *Vitis vinifera* may be suggested as a strong potential source of natural antioxidant. The methanolic extract in different subfraction were found to be effective antioxidants in different in vitro assays including β -carotene bleaching, DPPH radical scavenging and reducing power which can be proposed as a natural additive in food and pharmaceutical industries. There was a good correlation between total phenol content and antioxidant capacity of the extracts.

Table 2. The amounts of anthocyanin and ascorbic acid in methanolic extract of frozen and dried fruits of *Vitis vinifera* L. “white seedless” with maceration method.

Fruit type	Fraction	Ascorbic acid	Anthocyanin
Frozen	Polar-Subfraction	3.35±0.12	2.12±0.27
	Nonpolar-Subfraction	2.27±0.32	0.37±0.03
Dried	Polar-Subfraction	3.15±0.41	1.73±0.08
	Nonpolar-Subfraction	2.21±0.14	0.18±0.05

Table 3. The amounts of anthocyanin and ascorbic acid in methanolic extract of frozen and dried fruits of *Vitis vinifera* L. “white seedless” with ultrasonic method.

Fruit type	Fraction	Ascorbic acid	Anthocyanin
Frozen	Polar-Subfraction	3.24±0.68	1.21±0.07
	Nonpolar-Subfraction	1.86±0.15	0.11±0.05
Dried	Polar-Subfraction	3.21±0.30	1.15±0.12
	Nonpolar-Subfraction	1.27±0.10	0.12±0.08

REFERENCES

- Bonilla, F., Mayen, M., Merida, J., Medina, M., 1999. Extraction of phenolic compounds from red grape marc for use as food lipid antioxidants. *Food Chemistry*, 66:209–215.
- Borbalan, A.M.A., Zorro, L., Guillen, D.A., Barroso, C.G., 2003. Study of the polyphenol content of red and white grape varieties by liquid chromatography–mass spectrometry and its relationship to antioxidant power. *Journal of Chromatography A*, 1012(1):31–38.
- Cantos, E., Espin, J.C., Tomas-Barberan, F.A., 2002. Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC–DAD–MS–MS. *Journal of Agricultural and Food Chemistry*, 50(20):5691–5696.
- Cheng, G.W. and Breen, P.J., 1991. Activity of phenylalanine ammonia-lyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. *Journal of the American Society for Horticultural Science*, 116:865–869.
- Choi, C.W., Kim, S.C., Hwang, S.S., 2002. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Journal of Plant Sciences*, 163:1161–1168.
- Dimitrios B. (2006). Sources of natural phenolic antioxidants. *Trends in Food Science & Technology* 17:505–512.
- Dourdoglou, V.G., Mamalos, A., Makris, D.P., 2006. Storage of olives (*Olea europaea*) under CO₂ atmosphere: Effect on anthocyanins, phenolics, sensory attributes and *in vitro* antioxidant properties. *Food Chemistry*, 99:342–349.
- Gholivand, M.B., Piryaee, M., Maassoumi, S.M., 2014. Antioxidant activity of *Ziziphora tenuifolia* methanolic extracts and comparison of the essential oil in two stages of growth. *Chinese Journal of Natural Medicines*, 12:505–511.
- Jahanban-Sfahlan, A., Mahmoodzadeh, A., Hasanizadeh, A., 2009. Antioxidants and antiradicals in almond hull and shell (*Amygdalus communis* L.) as a function of genotype. *Food Chemistry*, 115:529–533.
- Kallithraka, S., Mohdaly, A., Makris, D.P., Kefalas, P. 2005. Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. *Journal of Food Composition and Analysis*, 18(5):375–386.
- Kim, D.O., Chun, O.K., Kim, Y.J. 2003. Quantification of polyphenolics and their antioxidant capacity in fresh plums. *Journal of Agricultural and Food Chemistry*, 51:6509–6515.
- Kulicic, T., Radonic, A., Katalinic, V., 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chemistry*, 85:633–640.
- Miller, H.E., 1971. A simplified method for the evaluation of antioxidants. *Journal of American Oil Chemists' Society*, 48:91–97.
- Miller, N.J., Rice-Evans, C.A. 1997. Factors influencing the antioxidant activity determined by the ABTS radical cation assay. *Free Radical Research*, 26 (3):195–199.
- Molyneux, P., 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal of Science and Technology*, 26:211–219.
- Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A. 2007. Antioxidant capacity, phenol, anthocyanin, and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chemistry*, 102:777–783.
- Rockenbach, II., Gonzaga, L.V., Rizelio, V.M., Gonkalves, A.E., Genovese, M.I., Fett, R., 2011. Phenolic compounds and antioxidant activity of seed and skin extracts of red grape (*Vitis vinifera* and *Vitis labrusca*) pomace from Brazilian winemaking. *Food Research International*, 44:897–901.
- Sanchez –Moreno, C., Larrauri, J.A., Saura-Calixto, F., 1998. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76(2): 270 –276.

- Sharififar, F., Moshafi, M.H., Mansouri, S.H., 2007. *In vitro* evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic *Zataria multiflora* Boiss. Food Control, 18:800–805.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16:144–158.
- Sun. J., Chu, Y.F., Wu, X., 2002. Antioxidant and antiproliferative activities of common fruits. Journal of Agricultural and Food Chemistry, 50:449–454.
- Vinson, J.A., Su, X., Zubik, L., Bose, P., 2001. Phenol antioxidant quantity and quality in foods: fruits. Journal of Agricultural and Food Chemistry, 49:5315–5321.
- Yildirim, A., Mavi, A., Kara, A., 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. Journal of Agricultural and Food Chemistry, 49:4083–4089.

EFFECTS OF DIFFERENT DRYING METHODS ON VOLATILE ORGANIC COMPOUNDS OF KADIOĞLU-12 APRICOT CULTIVAR

Mustafa KAPLAN¹, Okan LEVENT², Mehmet ALPASLAN³

¹Ministry of Food, Agriculture and Livestock, Directorate of Apricot Research Institute, Malatya, Turkey

²Inonu University, Food Engineering Department, Malatya

³Namık Kemal University, Department of Nutrition and Dietetics, Tekirdağ, Turkey

Corresponding author email: kaplanmustafa@tarim.gov.tr

Abstract

Malatya apricot is important plant fruit with medicinal and aromatic directions. Malatya apricots, which are important in terms of nutrition physiology, provide additional benefits in preventing health and illnesses on human physiology and metabolic functions beyond the inclusion of essential nutritional elements. In human nutrition, usage of food with high antioxidant capacity like apricot is being encouraged. Malatya alone has a share of 12% in world fresh apricot production and 65% in world dried apricot production. In this study, the effects of different drying methods (sun dried, sun dried then sulfurization and oven drier drying) on the volatile organic compound profile of table and dried Kadioğlu-12 apricot variety were investigated. The drying and sulphuring process has been significantly effective on volatile organic compounds. It has been determined that the amount of volatile organic compounds decreases due to sulphur application and amount. The different drying methods significantly influenced the volatile organic compounds ($p < 0,05$).

Keywords: Kadioğlu-12 apricots, volatile organic compounds, different drying methods

GİRİŞ

Meyve aroması, uçucu bileşenlerin farklı oranlarda bulunması ile meydana gelmektedir. Ayrıca meyveler arasındaki duyuşal farklılığı gösteren en belirgin faktör olan aroma bileşenleri, meyve kalitesi üzerinde önemli bir etkiye sahiptir.[6,10,12] Aroma maddelerinin oluşumu dinamik bir prosestir. Çoğu meyvenin kendine özgü aroması olgunlaşma sırasında meydana gelir. Olgunlaşma aşaması süresince meyve metabolizmasında bir çok değişim meydana gelir ve bu aşamada meyvenin bileşiminde bulunan yağ asitleri, amino asitler, şekerler, polifenoller, karotenoidlerden biyokimyasal yollarla aroma maddeleri oluşur.[3,10] Kayısı aroma maddeleri açısından oldukça zengin bir meyvedir.[3] Aroma maddeleri meyvelerde diğer bileşenlere göre çok düşük miktarlarda bulunmalarına rağmen meyvenin kendine özgü duyuşal özelliğini belirlerler.

Uçucu bileşiklerin konsantrasyonunu etkileyen başlıca faktörler çeşit, iklim koşulları, olgunlaşma, bölge ve işleme tekniğidir. Kayıslarda aroma maddeleri aldehitler, ketonlar, alkoller, esterler, uçucu asitler, çeşitli bileşikler ve terpenler gibi çeşitli kompleks gruplardan oluşur.[1,13] Çoğunluğu çiçeksi ve bazıları meyvensi kokulardan sorumlu terpen grubu bileşikler kayısının önemli aroma maddeleri arasında yer almaktadır.[6] Linalool, kayısı kokusuna çiçeksi karakter kazandıran, kayısı için özel bir aroma maddesi ve uçucu yağ olarak tanımlanmaktadır.[14] Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve hekzanoik asit olarak bildirilmiştir.[14]

Araştırmacılar β -ionon, linalool, γ -dekalakton, hekzanal, 2-hekzanal ve jeraniolün kayıslarda anahtar bileşenler olduğunu bildirmiştir. Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve hekzanoik asit olarak bildirilmiştir.[14]

Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve hekzanoik asit olarak bildirilmiştir.[14] Yüksek konsantrasyonlu aldehitler keskin bir kokuya sahipken düşük miktarlarda bulunduğunda güçlü bir aroma algısı uyandırabilmektedir. Aldehitler, meyve ve çimen kokusunu andıran uçucu fraksiyonların en baskın bileşenleridir. Aldehitler kayıslar için oldukça önemli bileşenlerdir.[2]

Kayısılardaki aroma profilinin tüketici beğenisinde oldukça önemli bir yeri bulunmaktadır.[8] Özellikle benzaldehit, linalool ve esterler kayısıların karakteristik aromasını oluşturan uçucu bileşenlerdir.[11] Kurutma sırasında gerçekleşen Maillard reaksiyonu sonucu yüksek seviyelerde furan, furfuran, imidazol gibi arzu edilmeyen bileşikler oluşmaktadır.[10]

Meyvelere ısı işlem uygulandığında çoğu kez orijinal uçucu bileşenler azalmaktayken aynı zamanda doymamış yağ asitlerinin otooksidasyonu ve termal bozulmanın yanında esmerleşme reaksiyonları veya karamelizasyon sonucunda yeni bileşenler oluşmaktadır. Örneğin Maillard reaksiyonu kurutulmuş ürünlerin aromasını olumsuz etkilemekteyken, lezzeti zenginleştiren ionon, γ -dekalakton ve butil asetat gibi bazı doğal bileşenlerin oluşması nedeniyle arzu edilebilmektedir.[9]

Bu çalışmada Güneşte gün kurusu (GK), Kükürtleme işleminden sonra güneşte kurutma (KK) ve fırın kurutucuda kurutma (FK) yöntemlerinin kayısı aromasına etkileri araştırılmıştır. Aroma maddeleri kuru kayısılarda da kaliteyi etkileyen önemli bir unsurdur. Ancak bugüne kadar ülkemizde üretilen kayısılardan sadece Şekerpare'nin farklı yöntemlerle kurutulması işleminin aroma üzerindeki etkisini incelemişlerdir.[4]

MATERYAL VE METOD

Materyal

Kayısı Araştırma Enstitüsü Ülkesel Gen Kaynakları Parsellerinde bulunan Kadioğlu-12 kayısı örneği materyal olarak seçilmiştir.

Metod

Örnek hazırlığı ve SPME koşulları

Aroma maddeleri katı faz mikro ekstraksiyon (SPME–GC–MS detection of volatile compounds in apricot varieties.) yöntemine göre elde edilmiştir. Bunun için örnekler homojen hale getirildikten sonra 3 g tartılarak 20 ml'lik SPME viallerine konulmuştur.

Örneklerin her birine iç standart olarak 10 μ L metanolde hazırlanmış 2-metil-3-heptanon çözeltisinden eklenmiştir. Ekstraksiyon için 2cm DVB/CAR/PDMS (Divinylbenzene /Carboxen /Polydimethylsiloxane; 50/30 μ m coating thickness; Supelco, Bellefonte, PA, USA) fiber kullanılmıştır.

Isıtıcı üzerinde vialler 40°C'de 30 dakika tepe boşluğuna (headspace) tutulduktan sonra fiberi vialin içerisine enjekte ederek 30 dakika adsorpsiyon işlemi gerçekleştirilmiştir.

BULGULAR VE TARTIŞMA

Toplam Aroma Bileşen Miktarları ve Bileşen Sayıları

Kurutma yöntemlerinden, güneşte kurutma yönteminde (GK), 82,00 μ g/kg uçucu bileşen miktarı ve 35 adet bileşen, kükürtlü kurutma yönteminde (KK), 47,82 μ g/kg uçucu bileşen miktarı ve 33 adet bileşen, fırın kurutma yönteminde (FK) ise 40,66 μ g/kg uçucu bileşen miktarı ve 32 adet bileşen saptanmıştır.

En yüksek değeri sırasıyla 27,80 μ g/kg ile terpenler, 13,45 μ g/kg ile uçucu asitler ve 6,49 μ g/kg miktarı ile çeşitli bileşik guruplarında saptanmıştır. Kadioğlu-12 kayısı çeşidinde aroma bileşen guruplarındaki toplam aroma bileşen miktarı ve sayısı Tablo 1' de verilmiştir.

Tablo 1. Kadioğlu-12 kayısı çeşidinde toplam aroma bileşen miktarı ve sayısı.

Aroma bileşen grupları	Kurutma yöntemleri		
	KK	GK	FK
Aldehit ve ketonlar (µg/kg)	2,12	16,85	5,54
Adet	6	11	11
Esterler (µg/kg)	2,36	4,44	1,98
Adet	2	3	2
Alkoller (µg/kg)	18,26	22,34	5,59
Adet	9	7	6
Terpenler (µg/kg)	5,14	27,80	20,48
Adet	5	8	6
Uçucu asitler (µg/kg)	13,45	8,83	1,23
Adet	6	3	3
Çeşitli bileşikler (µg/kg)	6,49	1,74	5,84
Adet	5	3	4
Toplam bileşen miktarı (µg/kg)	47,82	82,00	40,66
Toplam bileşen sayısı (Adet)	33	35	32

KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı

Aldehit ve Keton Gurubu Uçucu Aroma Bileşenleri

Yapılan çalışmada Bütanal, benzaldehit, 2-heptanal ve β -ionon bileşiklerindeki değişimler, kurutma yöntemleri üzerinde istatistiksel olarak ($p<0,05$) düzeyinde önemli değilken, diğer aldehit ve keton bileşiklerindeki değişim, kurutma yöntemleri için istatistiksel olarak ($p<0,05$) düzeyinde önemli bulunmuştur. 3-Hidroksi-2-bütanon GK örneğinde 10,83µg/kg miktar ile en fazla bulunan bileşen olarak saptanmıştır. Dihidro- β -ionon, β -ionon, hekzanal ve 2-hekzanal kayısının anahtar bileşenleridir. 2-pentanon, 3-Hidroksi-2-bütanon ve nanonal kurutuma etkisiyle oluşan yeni bileşenlerdir. Aldehit ve keton bileşenleri ile ilgili veriler Tablo 2’ de gösterilmiştir.

Ester Gurubu Uçucu Aroma Bileşenleri

Yapılan çalışmada tüm örneklerde ester bileşiklerindeki değişimler kurutma yöntemleri üzerinde istatistiksel olarak ($p<0,05$) düzeyinde önemli bulunmuştur. Ester bileşiği en fazla 4,44 µg/kg ile GK örneğinde, en düşük 1,98 µg/kg ile FK örneğinde saptanmıştır.

Etil asetat sırasıyla; 3,98 µg/kg ile GK, 1,87 µg/kg ile KK, 1,73 µg/kg ile FK örneğinde en fazla bulunan bileşen olarak belirlenmiştir. Etil asetat bileşeni, kurutma yöntemlerine göre kurutulmuş örneklerde artış meydana gelmiştir. Ester bileşenleri ile ilgili veriler Tablo 3’ de gösterilmiştir

Terpen Gurubu Uçucu Aroma Bileşenleri

Yapılan çalışmada tüm örneklerde terpen bileşiklerindeki değişimler kurutma yöntemleri üzerinde istatistiksel olarak ($p<0,05$) düzeyinde önemli bulunmuştur. Terpen bileşenlerinden limonen bileşiği en fazla 24,31 µg/kg ile GK örneğinde saptanmıştır.

Limonen bileşeni miktarında KK örneğinde azalma saptanırken, GK ve FK örneklerinde artış olduğu tespit edilmiştir. Terpen bileşenlerinde, tüm örneklerde en fazla limonen bileşiği tespit edilmiştir.

α -Terpinen, β -Pinen, sabinen β -Fellandren, styren ve p-Simen bileşenleri kurutmanın etkisiyle oluşan yeni bileşikler olarak ortaya çıkmaktadır. β -Fellandren ve p-Simen KK kükürtlü bileşikler sonucunda oluşan yeni bileşenler olarak saptanmıştır. Terpen bileşenleri ile ilgili veriler Tablo 4’ de gösterilmiştir.

Tablo 2. Kadioğlu-12 kayısı çeşidinde aldehit ve keton aroma bileşenleri (µg/kg)

Aldehit ve Keton	RI	KK	GK	FK	Kurutma
Bütanal	829	0,09±0,02	0,05±0,00	0,05±0,01	ÖD
2-Metilbütanal	880	TE	0,25±0,11	0,63±0,10	*
3-Metilbütanal	906	TE	0,62±0,27	1,34±0,01	*
Hekzanal	1073	0,19±0,04	0,81±0,35	0,48±0,02	*
3-Hidroksi-2-bütanon	1303	0,25±0,00	10,83±2,21	0,89±0,16	*
6-Metil-5-Hepten-2-On	1337	0,23±0,04	0,28±0,04	0,14±0,01	*
Benzaldehit	1545	TE	TE	TE	ÖD
Dihidro-β-ionon	1849	0,16±0,07	1,00±0,19	0,11±0,03	*
2-Bütanal	1037	TE	TE	TE	*
4-Pental	1129	TE	TE	TE	*
2-Hekzanal	1202	TE	0,54±0,13	TE	*
Heptanal	1184	TE	TE	0,09±0,01	*
2-Oktenal	1436	TE	TE	TE	*
2-Heptanal	1326	TE	TE	TE	ÖD
β-ionon	1964	TE	TE	0,03±0,04	ÖD
2,4 Hekzadienal	1420	TE	TE	TE	*
2,4 Heptadienal	1477	TE	0,07±0,02	TE	*
2-Metil-3-oktanon	1320	TE	TE	TE	*
2-Pentanon	957	1,20±0,22	2,21±1,04	1,69±0,14	*
Nanonal	1392	TE	0,19±0,05	0,09±0,02	*
Toplam (µg/kg)		2,12	16,85	5,54	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE: Tespit Edilemedi, KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı, p<0,05 düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

Tablo 3. Kadioğlu-12 kayısı çeşidinde ester bileşenleri (µg/kg)

Ester bileşenleri	RI	KK	GK	FK	Kurutma Yöntemi
3- Hekzenil asetat	1311	TE	TE	TE	*
Etil asetat	859	1,87±0,09	3,98±0,97	1,73±0,07	*
Metil bütanoat	963	0,49±0,13	0,28±0,03	0,25±0,04	*
Hekzil asetat	1264	TE	TE	TE	*
Metil hekzanoat	1176	TE	0,18±0,04	TE	*
Toplam (µg/kg)		2,36	4,44	1,98	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE: Tespit Edilemedi, KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı, p<0,05 düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

Uçucu Asit Gurubu Aroma Bileşenleri

Yapılan çalışmada tüm örneklerde uçucu asit bileşiklerindeki değişimler kurutma yöntemleri üzerinde istatistiksel olarak (p<0,05) düzeyinde önemli bulunmuştur. Örnekler içerisinde en fazla uçucu asit bileşiği 13,45 µg/kg ile KK örneğinde saptanmıştır. Asetik asit bileşeni miktarı sırasıyla; 8,39 µg/kg ile KK, 7,11 µg/kg ile GK ve 0,63 µg/kg ile FK örneğinde en fazla bulunan bileşen olarak belirlenmiştir. Bütanoik asit, hekzanoik asit ve heptanoik asit bileşenleri kurutmanın etkisiyle oluşan bileşikler olup, hekzanoik asit ve heptanoik asit bileşenleri KK örneğinde kükürtlü bileşikler sonucu belirlendiği saptanmıştır. Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve hekzanoik asit olarak bildirilmiştir.[14] Kadioğlu-12 kayısı çeşidinde uçucu asit bileşenleri Tablo 5’de gösterilmiştir.

Tablo 4. Kadioğlu-12 kayısı çeşidinde terpen bileşenleri (µg/kg).

Terpen bileşenleri	RI	KK	GK	FK	Kurutma Yöntemi
α-Pinen	1008	0,35±0,08	1,01±0,32	0,49±0,22	*
α-Terpinen	1265	0,10±0,04	0,12±0,04	0,06±0,01	*
β -Pinen	1084	TE	TE	0,07±0,01	*
Sabinen	1131	TE	0,17±0,04	0,08±0,01	*
β-Mirisen	1141	0,45±0,11	1,79±0,46	1,07±0,26	*
Limonen	1180	4,19±1,61	24,31±0,83	18,71±5,69	*
β -Fellandren	1193	TE	0,27±0,05	TE	*
Styren	1255	0,05±0,01	0,06±0,01	TE	*
p- Simen	1260	TE	0,07±0,01	TE	*
Toplam (µg/kg)		5,14	27,8	20,48	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE:Tespit Edilemedi, KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı, p<0,05 düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

Tablo 5. Kadioğlu-12 kayısı çeşidinde uçucu asit bileşenleri (µg/kg)

Uçucu asit bileşenleri	RI	KK	GK	FK	Kurutma Yöntemi
2-Metilbutanoik asit	1653	0,87±0,08	1,26±0,13	0,20±0,00	*
2-Metilpentanoik asit	1747	0,94±0,09	TE	0,41±0,10	*
Asetik asit	1471	8,39±1,12	7,11±0,44	0,62±0,20	*
Bütanoik asit	1640	0,46±0,01	0,46±0,08		*
Hekzanoik asit	1859	2,45±0,43	TE	TE	*
Heptanoik asit	1966	0,34±0,05	TE	TE	*
Toplam (µg/kg)		13,45	8,83	1,23	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE:Tespit Edilemedi, KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı, p<0,05 düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

Çeşitli Bileşenler Gurubu Aroma Bileşenleri

Yapılan çalışmada Dekan ve Tert-butil benzen bileşiklerindeki değişimler kurutma yöntemleri üzerinde istatistiksel olarak (p<0,05) düzeyinde önemli değilken, diğer çeşitli bileşiklerdeki değişimler kurutma yöntemleri için istatistiksel olarak (p<0,05) düzeyinde önemli bulunmuştur. Örnekler içerisinde en fazla çeşitli bileşenler 5,84 µg/kg ile FK örneğinde saptanmıştır.

Kurutma yöntemleri sonucunda furfural, 2-fenil furan, tert-butil benzen ve undekan bileşikleri saptanmıştır. Furfural bileşiği KK ve FK yöntemlerinde en fazla tespit edilen bileşiktir. Kadioğlu-12 kayısı çeşidinde çeşitli bileşenler Tablo 6'de gösterilmiştir.

Tablo 6. Kadioğlu-12 kayısı çeşidinde çeşitli bileşenler (µg/kg).

Çeşitli bileşenler	RI	KK	GK	FK	Kurutma Yöntemi
Dekan	976	0,23±0,13	TE	TE	ÖD
Toluen	1025	0,51±0,09	0,93±0,05	0,41±0,07	*
Undekan	1078	0,09±0,02	0,13±0,01	0,06±0,00	*
Tert-butil benzen	1259	0,10±0,01	TE	TE	ÖD
Furfural	1479	5,56±1,06	0,68±0,05	5,30±0,62	*
2-Fenil-Furan	1237	TE	TE	0,07±0,00	*
Toplam (µg/kg)		6,49	1,74	5,84	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE:Tespit Edilemedi, KK: Kükürtlü kurutulmuş kayısı, GK: Güneşte kurutulmuş kayısı, FK: Fırında kurutulmuş kayısı, p<0,05 düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

Alkol Gurubu Uçucu Aroma Bileşenleri

Yapılan çalışmada 1-Heptanol ve 2-Etil-1-hekzanol bileşiklerindeki değişimler kurutma yöntemleri üzerinde istatistiksel olarak ($p<0,05$) düzeyinde önemli değilken, diğer alkol bileşiklerindeki değişimler kurutma yöntemleri için istatistiksel olarak ($p<0,05$) düzeyinde önemli bulunmuştur.

Örnekler içerisinde en fazla alkol bileşiği 22,34 µg/kg ile GK örneğinde, en düşük alkol bileşiği 5,59 µg/kg ile FK yönteminde saptanmıştır.

Etanol bileşiği sırasıyla 15,67 µg/kg ile GK, 11,92 µg/kg ile KK ve 3,25 µg/kg ile FK yöntemlerinde en fazla bileşen olarak tespit edilmiştir.

1-Butanol ve 3-Metil-1-bütanol kurutmanın etkisiyle oluşan bileşikler olarak saptanmıştır.

Linalool bileşeni tüm örneklerde tespit edilirken, kayısıda anahtar bileşeni olarak kabul edilen linalool kurutma yöntemleri ile yapılan kurutma sonucunda artış göstermiştir. Kadioğlu-12 kayısı çeşidinde alkol bileşenleri Tablo 7' de gösterilmiştir.

Tablo 7. Kadioğlu-12 kayısı çeşidinde alkol bileşenleri (µg/kg).

Alkol bileşenleri	RI	KK	GK	FK	Kurutma Yöntemi
1-Butanol	1144	0,09±0,03	0,32±0,12	TE	*
Etanol	913	11,92±0,94	15,67±3,43	3,25±0,45	*
3-Metil-1-Butanol	1205	0,33±0,03	3,01±0,47	0,42±0,06	*
1-Pentanol	1247	0,14±0,03	0,59±0,02	0,38±0,04	*
1-Hekzanol	1352	0,32±0,02	0,71±0,04	0,20±0,01	*
2-Hekzen-1-ol	1406	0,07±0,01	TE	TE	*
3-Hekzen-1-ol	1417	TE	TE	0,06±0,00	*
1-Heptanol	1453	0,08±0,02	TE	TE	ÖD
2-Etil-1-hekzanol	1487	0,35±0,05	0,12±0,05	TE	ÖD
Linalool	1546	4,96±0,04	1,92±0,08	1,28±0,05	*
Toplam (µg/kg)		18,26	22,34	5,59	(p<0,05)

RI: Retention index (Alıkonma İndeksi), TE: Tespit Edilemedi, KK: Kükürtlü kurutulan kayısı, GK: Güneşte kurutulan kayısı, FK: Fırında kurutulan kayısı, $p<0,05$ düzeyi İstatistiksel olarak: *Önemli, ÖD: Önemli Değil

SONUÇ

Kadioğlu-12 kayısı çeşidindeki aroma bileşik sayısında yıllara ve kurutma yöntemlerinin etkisiyle azalmakta olduğu saptanmıştır. (Cabaroğlu, 2009) tarafından yapılan çalışmada, kontrol örneği Hacıhaliloğlu yaş kayısı çeşidinde 2007 yılında 33 adet, 2008 yılında 36 adet aroma bileşiği tespit etmiştir.[15] İklim, çevre ve hasat koşullarına bağlı olarak aroma miktarlarında değişimler olmaktadır. Çoğunluğu çiçeksi ve bazıları meyvemsi kokulardan sorumlu terpen grubu bileşikler kayısının önemli aroma maddeleri arasında yer almaktadır.[6] Terpen ve uçucu asit bileşenlerinde ise kurutmanın etkisiyle bileşen sayısında artışlar olduğu saptanmıştır. Kurutma ve kükürtleme işlemi aroma maddeleri üzerinde önemli derecede etkili olmuştur. Kükürt uygulaması ve dozuna bağlı olarak aroma maddesi miktarında düşüş olduğu belirlenmiştir Kurutma yöntemleri, istatistiksel olarak ($p<0,05$) düzeyinde uçucu aroma bileşen grupları için önemli bulunmuştur. Kadioğlu-12 kayısı çeşidinde lezzeti zenginleştiren β -ionon ve dihidro- β -ionon, gibi bileşenlerin kurutmanın etkisiyle azaldığı saptanmıştır.[11] Kurutmanın etkisiyle 2-pentanon, 3-Hidroksi-2-bütanon ve nanonal, metil hekzanoat, α -Terpinen, β -Pinen, sabinen β -Fellandren, styren, p-Simen. β -Fellandren, p-Simen, bütanoik asit, hekzanoik asit, heptanoik asit, furfural, 2-fenil furan, tert-butil benzen, undekan 1-Butanol ve 3-Metil-1-bütanol bileşikler saptanmıştır. Kurutma yöntemleri sırasında gerçekleşen maillard reaksiyonu sonucu furfural, 2-fenil furan, tert bütıl benzen ve undekan gibi arzu edilmeyen bileşikler tespit edilmiştir.[10] KK yönteminde ise hekzanoik asit, heptanoik, furufural, β -

Fellandren ve p- Simen bileşenleri kükürt bileşikleri sonucunda oluşan yeni bileşenler olarak saptanmıştır. Araştırma sonuçlarına göre farklı kurutma yöntemleri aroma profilini etkilemiştir.[5,7,9].

KAYNAKÇA

- Aubert, C., Chanforan, C. (2007). Postharvest changes in physicochemical properties and volatile constituents of apricot (*Prunus armeniaca* L.) characterization of 28 cultivars. *J. Agric. Food Chem.* 55, 3074-3082.
- Azodanlou, R., Darbellay, C., Luisier, J.L., Villettaz, J.C., Amado, R. (2003). Development of a model for quality assessment of tomatoes and apricots, *Lebensm.-Wiss.-U.-Tecnol.* 36, 223-233.
- Buttery, R.G. (1981). Vegetable And Fruit Flavours. In: R, Teranishi, RA, Flath, and H, Sugisawa (eds.). *Flavour Research: Recent Advances*, Marcel Dekker, New York, P. Pp:175.
- Cabaroğlu, T., Selli, S., Asma, B.M. (2009). Malatya’da yetiştirilen bazı yerli kayısı çeşitlerinde GC-MS-Olfaktometri yöntemleri ile aroma maddelerinin belirlenmesi ve kurutma işleminin aroma maddeleri üzerindeki etkisinin araştırılması. Proje No: 107 O 552, TÜBİTAK, Ankara.
- Dragovic-Uzelac, V. Delonga, Levaj, B., Djakovic, S., Pospisil, J. (2005). Phenolic profiles of raw apricots, pumpkins, and their purees in the evaluation of apricot nectar and jam authenticity. *J. Agr.Food Chem.* 53, 4836-4842.
- Gomez, E., Ledbetter, C.A. Hartsell, P.L. (1993). Volatile compounds in apricot, plum, and their interspecific hybrids. *J. Agr. Food Chem.* 41, 1669-1676.
- Greger, V., Schieberle, P. (2007). Characterization of the key aroma compounds in apricots (*Prunus armeniaca*) by application of the molecular sensory science concept. *J. Agr. Food Chem.* 55, 5221-5228.
- Gomez, E., Ledbetter, C.A. (1997). Development of volatile compounds during fruit maturation: characterization of apricot and plum×apricot hybrids. *J. Sci. Food Agr.* 74, 541-546.
- Gokbulut, İ. Karabulut, İ. (2012). SPME–GC–MS detection of volatile compounds in apricot varieties. *Food Chem.* 132, 1098-1102.
- Göğüş, F., Özel, M.Z., Lewis, A.C. (2007). The Effect of Various Drying Techniques on Apricot Volatiles Analysed Using Direct Thermal Desorption-GC–TOF/MS, *Talanta*, 73, 321-325.
- Kerler, J., Winkel, C. (2002). The basic chemistry and process conditions underpinning reaction flavour production (p. 302). In: Taylor, A.J. (Ed.), *Food Flavour Technology*, Sheffield Publ., Sheffield, U.K.
- Komes, D., Lovric, T., Kovacevic, G., Gajdos, K., Banavic, M. (2005). Trehalose improves flavour retention in dehydrated apricot puree. *Int. J. Food Sci. Technol.* 40, 425-435.
- Reineccius, G. (2006). *Flavor Chemistry and Technology*. CRC Press, Florida, USA, pp. 73-160.
- Riu-Aumatel, M., Castellari, M., E. Lopez-Tamames, Galassi, S., Buxaderas, S., (2004). Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. *Food Chem.* 87, 627-637
- Roshchina V. V., Roshchina V. D. (1993). *The excretory function of higher plants*. Springer, Berlin Heidelberg NewYork, USA. 145 p.
- Solis-Solis, H.M. Calderon-Santoyo, M., Schorr-Galindo, S., Luna-Solano, G., Ragazzo-Sanchez, J.A., (2007b). Characterization of aroma potential of apricot varieties using different extraction techniques. *Food Chem.* 105, 829–837.

DETERMINATION OF ORGANIC VOLATILE COMPOUNDS OF MALATYA KADIOĞLU-12 APRICOT CULTIVAR

Mustafa KAPLAN¹, Okan LEVENT², Mehmet ALPASLAN³

¹Ministry of Food, Agriculture and Livestock, Directorate of Apricot Research Institute, Malatya, Turkey

²Inonu University, Food Engineering Department, Malatya

³Namık Kemal University, Department of Nutrition and Dietetics, Tekirdağ, Turkey

Corresponding author email: kaplanmustafa@tarim.gov.tr

Abstract

Malatya apricot is important plant fruit with medicinal and aromatic directions. Malatya apricots, which are important in terms of nutrition physiology, provide additional benefits in preventing health and illnesses on human physiology and metabolic functions beyond the inclusion of essential nutritional elements. In human nutrition, usage of food with high antioxidant capacity like apricot is being encouraged. Malatya alone has a share of 12% in world fresh apricot production and 65% in world dried apricot production. Volatile organic compounds have an important effect on fruit quality. The main factors affecting the concentration of volatile organic compounds are variety, climatic conditions, maturity and processing techniques. The profile of the volatile organic compounds in the apricots has a very important place in consumer taste. Especially terpenes, esters, aldehydes and ketones are volatile components that form the characteristic aroma of the apricots. In this study, 33 volatile organic compounds were detected in table and dried Kadioğlu-12 apricot variety. Determined volatile organic compounds are aldehydes and ketones, esters, alcohols, terpenes, volatile acids and various compounds.

Keywords: Kadioğlu-12 apricot, volatile organic compounds, drying method

GİRİŞ

Meyve aroması, uçucu bileşenlerin farklı oranlarda bulunması ile meydana gelmektedir. Ayrıca meyveler arasındaki duyuşal farklılığı gösteren en belirgin faktör olan aroma bileşenleri, meyve kalitesi üzerinde önemli bir etkiye sahiptir.[6,10,12] Aroma maddelerinin oluşumu dinamik bir süreçtir. Çoğu meyvenin kendine özgü aroması olgunlaşma sırasında meydana gelir. Olgunlaşma aşaması süresince meyve metabolizmasında bir çok değişim meydana gelir ve bu aşamada meyvenin bileşiminde bulunan yağ asitleri, amino asitler, şekerler, polifenoller, karotenoidlerden biyokimyasal yollarla aroma maddeleri oluşur.[3,10] Kayısı aroma maddeleri açısından oldukça zengin bir meyvedir.[3] Aroma maddeleri meyvelerde diğer bileşenlere göre çok düşük miktarlarda bulunmalarına rağmen meyvenin kendine özgü duyuşal özelliğini belirlerler.

Uçucu bileşiklerin konsantrasyonunu etkileyen başlıca faktörler çeşit, iklim koşulları, olgunlaşma, bölge ve işleme tekniğidir. Kayıslarda aroma maddeleri aldehytler, ketonlar, alkoller, esterler, uçucu asitler, çeşitli bileşikler ve terpenler gibi çeşitli kompleks gruplardan oluşur.[1,13] Çoğunluğu çiçeksi ve bazıları meyvensi kokulardan sorumlu terpen grubu bileşikler kayısının önemli aroma maddeleri arasında yer almaktadır.[6] Linalool, kayısı kokusuna çiçeksi karakter kazandıran, kayısı için özel bir aroma maddesi ve uçucu yağ olarak tanımlanmaktadır.[14] Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve heksanoik asit olarak bildirilmiştir.[14] Araştırmacılar β-ionon, linalool, γ-dekalakton, heksanal, 2-heksanal ve jeraniolün kayıslarda anahtar bileşenler olduğunu bildirmiştir. Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve heksanoik asit olarak bildirilmiştir.[14] Kayıslarda bulunan önemli asitler; asetik, butanoik, 2-metilbutanoik ve heksanoik asit olarak bildirilmiştir.[14] Yüksek konsantrasyonlu aldehytler keskin bir kokuya sahipken düşük miktarlarda bulunduğu güçlü bir aroma algısı uyandırabilmektedir. Aldehytler, meyve ve çimen kokusunu andıran uçucu fraksiyonların en baskın bileşenleridir. Aldehytler kayıslar için oldukça önemli bileşenlerdir.[2] Kayıslardaki aroma profilinin tüketici beğenisinde oldukça

önemli bir yeri bulunmaktadır.[8] Özellikle benzaldehit, linalool ve esterler kayısıların karakteristik aromasını oluşturan uçucu bileşenlerdir.[11]

MATERYAL VE METOD

Materyal

Kayısı Araştırma Enstitüsü Ülkesel Gen Kaynakları Parsellerinde bulunan Kadioğlu-12 kayısı örneği materyal olarak seçilmiştir.

Metod

Örnek hazırlığı ve SPME koşulları

Aroma maddeleri katı faz mikro ekstraksiyon (SPME–GC–MS detection of volatile compounds in apricot varieties.) yöntemine göre elde edilmiştir. Bunun için örnekler homojen hale getirildikten sonra 3 g tartılarak 20 ml’lik SPME viallerine konulmuştur. Örneklerin her birine iç standart olarak 10 µL metanolde hazırlanmış 2-metil-3-heptanon çözeltisinden eklenmiştir. Ekstraksiyon için 2cm DVB/CAR/PDMS (Divinylbenzene /Carboxen /Polydimethylsiloxane; 50/30 µm coating thickness; Supelco, Bellefonte, PA, USA) fiber kullanılmıştır. Isıtıcı üzerinde vialler 40°C’de 30 dakika tepe boşluğuna (headspace) tutulduktan sonra fiberi vialin içerisine enjekte ederek 30 dakika adsorpsiyon işlemi gerçekleştirilmiştir.

BULGULAR VE TARTIŞMA

Toplam Aroma Bileşen Miktarları ve Bileşen Sayıları

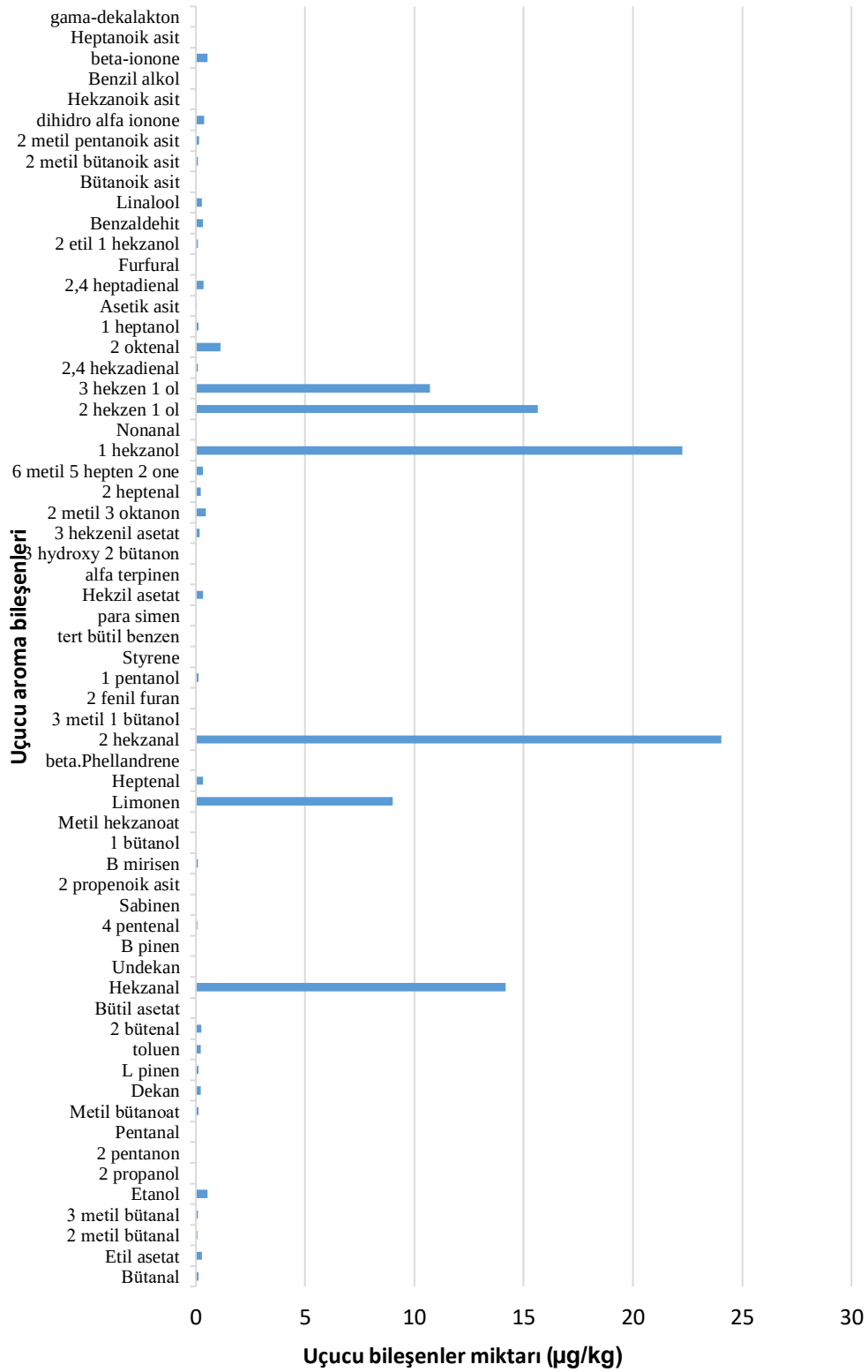
Kadioğlu-12 örneğinde toplam 37 adet (17 adet aldehit ve keton bileşiği, 4 adet ester, 8 adet alkol, 3 adet terpen, 3 adet uçucu asitler ve 2 adet çeşitli bileşikler) uçucu aroma bileşikler saptanmıştır. Kadioğlu-12 yaş kayısı çeşidinde toplam 103,70 µg/kg bileşik miktarı ve 37 adet uçucu bileşen tespit edilmiştir.

En yüksek değerler sırasıyla 49,75 µg/kg ile alkoller, 43,03 µg/kg ile aldehit ve ketonlar, 9,25 µg/kg miktarı ile terpen guruplarında saptanmıştır.

Kadioğlu-12 kayısı çeşidinde aroma bileşen guruplarındaki toplam aroma bileşen miktarı ve sayısı Tablo 1 ve Şekil 1’ de verilmiştir.

Tablo 1. Kadioğlu-12 kayısı çeşidinde toplam aroma bileşen miktarı ve sayısı

Aroma bileşen grupları	Yaş kayısı YK
Aldehit ve ketonlar (µg/kg)	43,03
Adet	17
Esterler (µg/kg)	0,92
Adet	4
Alkoller (µg/kg)	49,75
Adet	8
Terpenler (µg/kg)	9,25
Adet	3
Uçucu asitler (µg/kg)	0,33
Adet	3
Çeşitli bileşikler (µg/kg)	0,45
Adet	2
Toplam bileşen miktarı (µg/kg)	103,70
Toplam bileşen sayısı (Adet)	37



Şekil 1. Kadıoğlu-12 kayısı çeşidinde aroma uçucu bileşenleri ve miktarları (µg/kg)

Aldehit ve Keton Gurubu Uçucu Aroma Bileşenleri

YK örneğinde aldehit ve keton bileşiği toplamda 43,03 µg/kg miktarı tespit edilmiştir. 24,05 µg/kg ile 2-hekzanal ve 14,16 µg/kg ile hekzanal en fazla bulunan bileşenler olarak saptanmıştır.

Dihidro- β -ionon, β -ionon, hekzanal ve 2-hekzanal kayısının anahtar bileşenleridir. Aldehit ve keton bileşenleri ile ilgili veriler Tablo 2’ de gösterilmiştir.

Tablo 2. Kadioğlu-12 kayısı çeşidinde aldehit ve keton aroma bileşenleri ($\mu\text{g/kg}$)

Aldehit ve Keton bileşenleri	RI	YK
Bütanal	829	0,13 $\pm$ 0,00
2-Metilbütanal	880	0,07 $\pm$ 0,02
3-Metilbütanal	906	0,11 $\pm$ 0,01
Hekzanal	1073	14,16 $\pm$ 1,10
6-Metil-5-Hepten-2-On	1337	0,33 $\pm$ 0,06
Benzaldehit	1545	0,34 $\pm$ 0,08
Dihidro- β -ionon	1849	0,38 $\pm$ 0,11
2-Bütanal	1037	0,26 $\pm$ 0,01
4-Pental	1129	0,07 $\pm$ 0,01
2-Hekzanal	1202	24,05$\pm$1,20
Heptanal	1184	0,32 $\pm$ 0,11
2-Oktenal	1436	1,12 $\pm$ 0,02
2-Heptanal	1326	0,23 $\pm$ 0,05
β -ionon	1964	0,53 $\pm$ 0,05
2,4 Hekzadienal	1420	0,10 $\pm$ 0,02
2,4 Heptadienal	1477	0,36 $\pm$ 0,14
2-Metil-3-oktanon	1320	0,47 $\pm$ 0,17
Toplam ($\mu\text{g/kg}$)		43,03

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

Ester Gurubu Uçucu Aroma Bileşenleri

Ester bileşiği 0,92 $\mu\text{g/kg}$ olarak saptanmıştır. Hekzil asetat 0,34 $\mu\text{g/kg}$ ve etil asetat 0,27 $\mu\text{g/kg}$ miktarları ile en fazla tespit edilen ester bileşenleri olmuştur. Ester bileşenleri ile ilgili veriler Tablo 3’ de gösterilmiştir.

Tablo 3. Kadioğlu-12 kayısı çeşidinde ester bileşenleri ($\mu\text{g/kg}$).

Ester bileşenleri	RI	YK
3- Hekzenil asetat	1311	0,18 $\pm$ 0,06
Etil asetat	859	0,27 $\pm$ 0,03
Metil bütanoat	963	0,13 $\pm$ 0,02
Hekzil asetat	1264	0,34$\pm$0,01
Metil hekzanoat	1176	TE
Toplam ($\mu\text{g/kg}$)		0,92

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

Terpen Gurubu Uçucu Aroma Bileşenleri

Terpen bileşenleri toplamda 9,25 $\mu\text{g/kg}$ ve üç adet terpen bileşeni tespit edilirken, terpen bileşenlerinden limonen bileşiği 9,02 $\mu\text{g/kg}$ en fazla tespit edilen bileşen olmuştur. Terpen bileşenleri ile ilgili veriler Tablo 4’ de gösterilmiştir.

Tablo 4. Kadioğlu-12 kayısı çeşidinde terpen bileşenleri (µg/kg)

Terpen bileşenleri	RI	YK
α-Pinen	1008	0,13±0,01
β-Mirisen	1141	0,10±0,03
Limonen	1180	9,02±1,86
Toplam (µg/kg)		9,25

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

Uçucu Asit Gurubu Aroma Bileşenleri

Uçucu asit bileşenleri toplamda 0,33 µg/kg ve üç adet uçucu asit bileşeni tespit edilirken, uçucu asit bileşenlerinden 2-Metilpentanoik asit bileşiği 0,16 µg/kg en fazla tespit edilen bileşen olmuştur. Kadioğlu-12 kayısı çeşidinde uçucu asit bileşenleri Tablo 5’de gösterilmiştir.

Tablo 5. Kadioğlu-12 kayısı çeşidinde uçucu asit bileşenleri (µg/kg)

Uçucu asit bileşenleri	RI	YK
2-Metilbutanoik asit	1653	0,11±0,00
2-Metilpentanoik asit	1747	0,16±0,00
Asetik asit	1471	0,06±0,01
Toplam (µg/kg)		0,33

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

Çeşitli Bileşenler Gurubu Aroma Bileşenleri

Çeşitli bileşenler toplamda 0,45 µg/kg ve iki adet uçucu çeşitli bileşeni tespit edilirken, çeşitli bileşenlerinden dekan bileşiği 0,23 ve tolüen 0,22 µg/kg olarak tespit edilmiştir. Kadioğlu-12 kayısı çeşidinde çeşitli bileşenler Tablo 6’de gösterilmiştir.

Tablo 6. Kadioğlu-12 kayısı çeşidinde çeşitli bileşenler (µg/kg).

Çeşitli bileşenler	RI	YK
Dekan	976	0,23±0,06
Toluen	1025	0,22±0,06
Toplam (µg/kg)		0,45

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

Alkol Gurubu Uçucu Aroma Bileşenleri

Alkol bileşenleri toplamda 49,75 µg/kg ve sekiz adet alkol bileşenleri tespit edilirken, alkol bileşenlerinden 1-Hekzanol bileşiği 22,27 µg/kg ile en fazla tespit edilen bileşen olmuştur. Kadioğlu-12 kayısı çeşidinde alkol bileşenleri Tablo 7’ de gösterilmiştir.

Tablo 7. Kadioğlu-12 kayısı çeşidinde alkol bileşenleri (µg/kg)

Alkol bileşenleri	RI	YK
Etanol	913	0,54±0,00
1-Pentanol	1247	0,13±0,01
1-Hekzanol	1352	22,27±3,18
2-Hekzen-1-ol	1406	15,64±1,91
3-Hekzen-1-ol	1417	10,70±1,23
1-Heptanol	1453	0,11±0,09
2-Etil-1-hekzanol	1487	0,09±0,01
Linalool	1546	0,27±0,06
Toplam (µg/kg)		49,75

RI: Retention index (Alıkonma İneksi), TE:Tespit Edilemedi, YK: Yaş kayısı

SONUÇ

Uçucu bileşenlerin konsantrasyonunu çeşit, iklim koşulları, olgunlaşma, bölge ve işleme tekniği gibi faktörler etkilemektedir. Literatür çalışmalarında Hacıhaliloğlu yaş kayısı çeşidinde; 2007 yılında aldehit ve keton bileşikler miktarı 133,4 bulunurken, 2008 yılında 1042,71 µg/kg bulunmuştur.[4] Ketonlar yağ asidi metabolizması ile oluşan ve kayısı aromasına katkısı olan bileşiklerdir.[5] Çoğunluğu çiçeksi ve bazıları meyvemsi kokulardan sorumlu terpen grubu bileşikler kayısının önemli aroma maddeleri arasında yer almaktadır.[6] Çiçeksi kokuya sahip olan keton bileşenlerinden Dihidro β –ionon 0,38 µg/kg ve β-ionon 0,53µg/kg miktarlar ile Kadioğlu-12 yaş kayısı çeşidinde belirlenmiştir. Kadioğlu-12 yaş kayısı örneğinde ve keton gurubunda yer alan 2-Hekzanal 24,05 µg/kg miktar ile en fazla bulunan bileşiktir. Yapılan çalışmalarda hekzanal ve 2-hekzanal miktarının mevsimsel faktörlerden ve iklim koşullarından etkilenmekte, kayısılarda oldukça fazla bulunan bu bileşenler karakteristik taze ot ve çimen kokusunu vermektedirler. Çimen kokusu ile karakterize edilen 1-hekzanal ile yeşil, taze ot kokusu ile karakterize edilen 2-hekzenal kayısılarda belirlenmiş önemli aroma bileşikleridir. Hekzil asetat bileşeni 0,32 µg/kg miktar ile ester gurubunda en fazla bulunan bileşiktir. Meyveye elma ve muz benzeri bir koku kazandırmaktadır. Meyvemsi koku kazandıran ester bileşenlerinden metil bütanoat 0,13 µg/kg ile Kadioğlu-12 çeşidinde belirlenmiştir.

KAYNAKÇA

- Aubert, C., Chanforan, C. (2007). Postharvest changes in physicochemical properties and volatile constituents of apricot (*Prunus armeniaca* L.) characterization of 28 cultivars. *J. Agric. Food Chem.* 55, 3074-3082.
- Azodanlou, R., Darbellay, C., Luisier, J.L., Villettaz, J.C., Amado, R. (2003). Development of a model for quality assessment of tomatoes and apricots, *Lebensm.-Wiss.-U.-Tecnol.* 36, 223-233.
- Buttery, R.G. (1981). Vegetable And Fruit Flavours. In: R, Teranishi, RA, Flath, and H, Sugisawa (eds.). *Flavour Research: Recent Advances*, Marcel Dekker, New York, P. Pp:175.
- Cabaroğlu, T., Selli, S., Asma, B.M. (2009). Malatya’da yetiştirilen bazı yerli kayısı çeşitlerinde GC-MS-Olfaktometri yöntemleri ile aroma maddelerinin belirlenmesi ve kurutma işleminin aroma maddeleri üzerindeki etkisinin araştırılması. Proje No: 107 O 552, TÜBİTAK, Ankara.
- Dragovic-Uzelac, V. Delonga, Levaj, B., Djakovic, S., Pospisil, J. (2005). Phenolic profiles of raw apricots, pumpkins, and their purees in the evaluation of apricot nectar and jam authenticity. *J. Agr.Food Chem.* 53, 4836-4842.
- Gomez, E., Ledbetter, C.A. Hartsell, P.L. (1993). Volatile compounds in apricot, plum, and their interspecific hybrids. *J. Agr. Food Chem.* 41, 1669-1676.
- Greger, V., Schieberle, P. (2007). Characterization of the key aroma compounds in apricots (*Prunus armeniaca*) by application of the molecular sensory science concept. *J. Agr. Food Chem.* 55, 5221-5228.
- Gomez, E., Ledbetter, C.A. (1997). Development of volatile compounds during fruit maturation: characterization of apricot and plum×apricot hybrids. *J. Sci. Food Agr.* 74, 541-546.
- Gokbulut, İ. Karabulut, İ. (2012). SPME–GC–MS detection of volatile compounds in apricot varieties. *Food Chem.* 132, 1098-1102.
- Reineccius, G. (2006). *Flavor Chemistry and Technology*. CRC Press, Florida, USA, pp. 73-160.
- Riu-Aumatell, M., Castellari, M., E. Lopez-Tamames, Galassi, S., Buxaderas, S. (2004). Characterisation of volatile compounds of fruit juices and nectars by HS/SPME and GC/MS. *Food Chem.* 87, 627-637
- Roshchina V. V., Roshchina V. D. (1993). *The excretory function of higher plants*. Springer, Berlin Heidelberg NewYork, USA. 145 p.
- Solis-Solis, H.M. Calderon-Santoyo, M., Schorr-Galindo, S., Luna-Solano, G., Ragazzo-Sanchez, J.A. (2007b). Characterization of aroma potential of apricot varieties using different extraction techniques. *Food Chem.* 105, 829–837.

EXTRACTION OF MEDICAL AND AROMATIC PLANTS: ULTRASOUND

Seydi YIKMIŞ¹, Mustafa KAPLAN², Harun AKSU³, Mehmet ALPASLAN¹

¹Namık Kemal University, Department of Nutrition and Dietetics, Tekirdağ, Turkey

²Ministry of Food, Agriculture and Livestock, Directorate of Apricot Research Institute, Malatya, Turkey

³Istanbul University-Cerrahpaşa, Veterinary Faculty, Department of Food Hygiene & Technology, Istanbul, Turkey

Corresponding author email: syikmis@nku.edu.tr

Abstract

Extraction in conventional methods is solvent extraction, which means collecting the extracts after a certain period of time without mechanical agitation or mechanical agitation of the materials in the various solvents. Today, different extraction methods are used thanks to new technologies. Due to the drawbacks of temperature applications, ultrasound applications, a non-thermal technology, are being used to increase efficiency in extraction methods. High energy (high power, high intensity) ultrasound uses densities higher than 1 W.cm⁻² at frequencies between 20 and 500 kHz. As a green extraction method, it is used to protect both the environment and the consumers and at the same time to make them ecologically, economically and innovative. Advantages of using ultrasound; the penetration of the solvent into the cellular material, the improvement of mass transfer, the impairment of cell wall permeability, the reduction of energy input, the reduction of temperatures and the shortening of the treatment time. Extract of phenolic acid and flavanone glycoside from the shell of Satsuma mandarin (Citrus unsbiu marc), almond oils, plant extracts (fennel, hops, marigold, mint), ginseng saponins, ginger, rosemary, carnosic acid, polyphenols, amino acids and caffeine, caffeine from the green teapot and flowers like pyrethrins. In this work, the ultrasonication method used in the extraction of medical and aromatic plants is mentioned.

Keywords: Ultrasound, extraction, medical and aromatic

INTRODUCTION

The main objective of an extraction process is to obtain the target compounds most effectively with the least amount of effect on the properties of the target compound which is minimally reducing the target compound. The effect of conventional solid-liquid extraction (SLE), including infusion and "Soxhlet" extraction,) techniques are time to consume and use too much solvent. Depending on the properties of the target compounds, various chlorinated solvents (eg, chloroform, carbon tetrachloride, tetrachlorethylene and chlorobenzene) and non-chlorinated solvents (eg, acetone, methanol and acetonitrile) are used for extraction from various matrices. When safety risks combine with toxicity and low extraction yields of certain solvents, the presence of solvent residues in the target compounds has led to the discovery of environmentally friendly (green) extraction techniques that reduce or eliminate most of the use of organic solvents. Increased commercial interest in sustainable, non-toxic extraction methods has increased due to environmentalist, toxic chemicals-free natural ingredients and increased consumer demands for environmental and health risks associated with chemical solvents.

A major difficulty in separating molecules into a complex matrix in a sustainable way is that these molecules are often embedded in the matrix. Obviously, although there is an obvious need to develop cost effective, safe, effective, ecologically-innovative extraction techniques, it is important to ensure that such techniques not only provide clean label status, but at the same time achieve a higher efficiency with minimal impact on the quality of the final product. A number of new alternatives to conventional techniques for extraction of target compounds from various matrices, including ultrasonically assisted extraction (UAE), subcritical and microwave assisted extraction (MAE), supercritical fluid extraction (SFE) and accelerated, have been proposed. solvent

pretreatment including solvent extraction (ASE) or high pressure treatment and impact electric field. While these new techniques improve process efficiency and increase extraction efficiency and quality, they offer tremendous potential to reduce or eliminate the use of toxic chemical solvents. These techniques are also known as cold extraction techniques because the temperature during the extraction process is relatively low and does not affect the stability of the extracted compounds. The new techniques can also be used in combination with environmentally friendly, safe organic solvents to enhance extraction efficiency, either as a pretreatment or by increasing cell-membrane permeability, a parameter that governs extraction efficiency.

Ultrasound is a new, clean, green extraction technology for a variety of molecules and biomaterials including polysaccharides, essential oils, proteins, peptides, fine chemicals (dyes and pigments) and bioactive molecules of commercial importance. (Tiwari, 2015).

ULTRASOUND

Ultrasound is defined as sound waves that can travel in a solid, liquid, and gas environment with high frequencies (> 20 kHz) that can not be detected by the human ear (Mar, Jose V. Garcia-Perez, Antonia, Juan ve Jose, 2017). There are two types of ultrasonic applications in the food industry. The amount of energy created by the sound waves is used to classify the ultrasound applications. This energy is calculated using the power (W), density (W.m^{-2}) or energy density (W.m^{-3}) of sound waves. The ultrasound can be divided into two groups: low energy (low power, low intensity) and high energy (high power, high intensity) (Leighton, 2007; Mason, Paniwnyk ve Chemat, 2003; Torley, P.J. ve Bhandari, 2007).

Low energy ultrasonic applications are larger than 100 kHz frequency and applications with a density less than 1 W.cm^{-2} . Because low intensity ultrasonic applications use very low power quantities, they do not usually cause physical or chemical changes in the material they are applied to. The most common use of low-energy ultrasound in the food industry is to determine the physicochemical properties of food such as composition, structure and physical state (Chemat ve diğerleri, 2017; Chemat, Zill-e-Huma ve Khan, 2011; Tao ve Sun, 2015). High-energy ultrasonic applications are applications with frequencies between 18 kHz and 100 kHz and densities greater than 1 W.cm^{-2} . High-energy ultrasound has been used for many years to produce emulsions. More recently, it has also found use in areas such as control of modification and crystallization, removal of air bubbles from liquid foods, enzyme inactivation, advanced drying, filtration and initiation of oxidation reactions (Ojha, Tiwari, O'Donnell ve Kerry, 2016; Patist ve Bates, 2008).

ULTRASOUND EFFECT MECHANISM

The ability to change the physical, biochemical and microbiological properties of foods with high gustatory acoustic waves is becoming more and more variable depending on the ultrasound wave and the properties of the applied product (Torley, P.J. ve Bhandari, 2007). The basic factor of these effects that the ultrasound process brings to food processing is acoustic cavitation. These bubbles, which are created using ultrasounds with high strength, develop rapidly and with a few fluctuations, which is called cavitation (Feng ve Yang, 2011; Torley, P.J. ve Bhandari, 2007).

Temporary cavitation bubbles will occur if the intensity of the sound waves is 10 Wcm^{-2} or higher. These bubbles expand rapidly in volume with a few acoustic oscillations, but their volume reaches a critical level in a very short time, even though the bubbles can withstand effectively. When the volumes of the bubbles reach a level that can not absorb the increasing energy, they end up with a violent collapse (Feng ve Yang, 2011; Leighton, 2007; Mason ve diğerleri, 2003)

EXTRACTION

The type of extraction to be used is important in the extraction of bioactive compounds. Direct extraction using solvents is the most common technique for obtaining extracts with high antioxidant activity. The main factors to be considered in the selection of solvent; molecular affinities between the polarity of the target compound, solvent and solubility, mass transfer, co-solvent use, environmental safety, human toxicity and financial feasibility should also be considered in the selection of solvent for bioactive compound extraction (Azmir ve diğerleri, 2013). Ultrasound technology maximizes the recovery of bioactive compounds from plants while reducing the duration of extraction and solvent consumption (Corbin ve diğerleri, 2015).

The permeability of the cell membranes and the activation of the substances in the cell are increased, so that the extraction can be carried out at low temperature and time. Loss of heat sensitive aroma compounds can be prevented (Mason ve diğerleri, 2003; Preece, Hooshyar, Krijgsman, Fryer ve Zuidam, 2017). Cavitation caused by ultrasound breaks the biological cell walls and allows cell contents to become free. The ultrasound process is used for the extraction of bioactive substances from sugar beet, sugar extraction, protein extraction from fat-extracted soya bean (Mar ve diğerleri, 2017). Some extractions are summarized in the table below (Table 1)(Chemat ve diğerleri, 2017).

Table 1. Ultrasound applied extractions.

Product	Application	Extract
Rosemary	S/L ratio = 1/20 (m/V), Solvent = EtOH/H ₂ O, 90/10 (V/V), t = 30 min, T = 40 °C	Antioxidants (carnosic acid, ursolic acid)
Pepper	MeOH/H ₂ O (0–100%), t = 2–25 min, T = 10–60 °C m = 0.2–2 g, V = 15–50 mL, solvent = MeOH, EtOH, acetonitrile,	Capsaisinoids
Caraway seeds	solvent = water, t = 30 min, Hydrodistillation, I = 1 W/cm ² , m = 150 g, V = 1.5 L,	Limonene, Carvone,
Marjoram	t = 5–15 min, T = 15–35 °C, pulse = 5 s on, 5 s off m = 0.5 g, V = 25 mL, solvent = MeOH/H ₂ O 80/20 (V/V),	Antioxidants (rosmarinic acid, carnosic acid, luteolin-7-O-glucoside, apigenin-7-O-glucoside)
Saffron	solvent = EtOH/H ₂ O 70/30, (V/V), t = 5,10,15 min, T < 40 °C .M = 50 mg, V = 1 mL, solvent = MeOH/acetonitrile 38/62, (V/V), t = 22 min	Volatile compounds
Spearmint (<i>Mentha spicata</i>)	Amplitude = 25%, m = 50 g, V = 100 mL,	Flavor volatile compounds
Tea	m = 3 g, V = 300 mL, solvent = water, t = 40 min, T = 60 °C	Aroma compounds
Pomegranate peel	ratio: water/peel, 50/1 (w/w), Pulse = 5 s on, 5 s off, IP = 59.2 W/cm ² , T = 25 °C, t = 60 min,	Antioxidant
<i>Zyzyphus lotus</i> fruit	Solvent: ethanol (50%), Pulse = 2 s on, 2 s off, T = 63 °C, t = 25 min, ratio solvent/solid: 67 mL/g,	Antioxidant
Tomato pomace	Solvent: hexane/ethanol (50:50, v/v), A = 58–145 µm, T = 25 °C, t > 10 min, m = 3 g, V = 100 mL,	Carotenoids (all-trans-lycopene, β-carotene)
Pomegranate (<i>Punica granatum</i>) rinds	V = 50 mL, solvent: water, P = 80 W, T = 25 °C, t = 30 min to 3 hours, m = 1 g,	Natural color

Jabuticaba (<i>Myrciaria cauliflora</i>) peel	(pH = 1), Ratio: 46% (v/v) T = 30 °C, t = 10 min, Solvent: ethanol:water	Phenol (ellagic acid), anthocyanin (cyaniding-3-O-glucoside)
Garlic (<i>Allium sativum</i>) cloves	Solvent: diethyl ether, hexane, ethyl acetate T = 25 °C, t = 30 min, 3 times extraction (V = 50 mL),	Aroma
Grape (<i>Vitis vinifera</i>) seeds	Solvent: ethanol, Ethanol concentration: 33–67% T = 33–67 °C, t = 16–34 min, m = 2 g, V = 100 mL,	antioxidants, anthocyanins, Phenol,
Soybean, sunflower, rape	m = 10 g, V = 100 mL, solvent = hexane, Irradiation time = 10 s (after the solvent reach the siphon height)	Oil
Almond	Solvent/sample ratio = 10–20(mg/L), solvent = hexane, t = 40–60 min, T = 40–60 °C	Oil
Papaya seed	P = 235–700 W, Sample/liquid ratio = 6/1–10/1 (V/w), solvent = hexane, t = 5–30 min, T = 25–50 °C,	Oil, antioxidants
<i>Pistacia khinjukk</i> kernel	Amplitude = 0, 25, 50%, pulse = 10 s on, 5 s off, ratio = 1/4 (w/V), solvent = hexane, T = 30, 40, 50 °C	Oil
Flaxseed	P = 50 W, ratio = 6/1 (V/w), solvent = hexane, V = 100 mL, t = 30 min, T = 30 °C	Oil
Soybean	I = 47.6 W/cm ² , solvent = hexane/isopropanol (3/2)	Oil

CONCLUSION

One of the greatest achievements of innovative extraction techniques is ultrasound technology. The use of ultrasonic technology for the extraction of food and natural extracts has been confirmed in the literature review. Ultrasonically assisted extractions can be recommended as they do not contain a residual solvent, contaminants, as compared to conventional extrusion techniques. Easily integrated into the industry and being environmentally friendly is also a positive aspect. At the end of this study it was concluded that extracting of medicinal and aromatic plants is clearly beneficial in the laboratory and industrial scale.

REFERENCES

- Azmir, J., Zaidul, I. S. M., Rahman, M. M., Sharif, K. M., Mohamed, A., Sahena, F., ... Omar, A. K. M. (2013). Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4), 426–436. doi:10.1016/J.JFOODENG.2013.01.014
- Chemat, F., Rombaut, N., Sicaire, A.-G., Meullemiestre, A., Fabiano-Tixier, A.-S. ve Abert-Vian, M. (2017). Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*, 34, 540–560. doi:10.1016/J.ULTSONCH.2016.06.035
- Chemat, F., Zill-e-Huma ve Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, 18(4), 813–835. doi:10.1016/j.ultsonch.2010.11.023
- Corbin, C., Fidel, T., Leclerc, E. A., Barakzoy, E., Sagot, N., Falguières, A., ... Hano, C. (2015). Development and validation of an efficient ultrasound assisted extraction of phenolic compounds from flax (*Linum usitatissimum* L.) seeds. *Ultrasonics Sonochemistry*, 26, 176–185. doi:10.1016/J.ULTSONCH.2015.02.008
- Feng, H. ve Yang, W. (2011). Ultrasonic Processing. V. M. B. Zhang, Howard Q. , Barbosa-Cánovas, Gustavo V. Balasubramaniam ve J. T. C. Y. C. Patrick Dunne , Daniel F. Farkas (Ed.), *Nonthermal processing technologies for food içinde* . USA: Wiley-Blackwell.
- Leighton, T. G. (2007). What is ultrasound? *Progress in Biophysics and Molecular Biology*, 93, 3–83. doi:10.1016/j.pbiomolbio.2006.07.026

- Mar, V., Jose V. Garcia-Perez, Antonia, M., Juan, A. C. ve Jose, B. (2017). *Ultrasound in Food Processing: Recent Advances*. (V. Mar, Jose V. Garcia-Perez, M. Antonia, A. C. Juan ve B. Jose, Ed.) (Firs Etidi.). United Kingdom: John Wiley & Sons Ltd. <https://www.wiley.com/en-us/Ultrasound+in+Food+Processing%3A+Recent+Advances-p-9781118964187> adresinden erişildi.
- Mason, T. J., Paniwnyk, L. ve Chemat, F. (2003). Ultrasound as a preservation technology. P. Zeuthen ve L. Bøgh-Sørensen (Ed.), *Food Preservation Techniques* içinde (ss. 303–337). Woodhead Publishers. doi:10.1533/9781855737143.3.303
- Ojha, K. S., Tiwari, B. K., O'Donnell, C. ve Kerry, J. P. (2016). Emerging Nonthermal Food Preservation Technologies. C. E. Leadley (Ed.), *Innovation and Future Trends in Food Manufacturing and Supply Chain Technologies* içinde (ss. 257–274). Elsevier. doi:10.1016/B978-1-78242-447-5.00009-5
- Patist, A. ve Bates, D. (2008). Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science & Emerging Technologies*, 9(2), 147–154. doi:10.1016/J.IFSET.2007.07.004
- Preece, K. E., Hooshyar, N., Krijgsman, A., Fryer, P. J. ve Zuidam, N. J. (2017). Intensified soy protein extraction by ultrasound. *Chemical Engineering and Processing: Process Intensification*, 113, 94–101. doi:10.1016/J.CEP.2016.09.003
- Tao, Y. ve Sun, D.-W. (2015). Enhancement of Food Processes by Ultrasound: A Review. *Critical Reviews in Food Science and Nutrition*, 55(4), 570–594. doi:10.1080/10408398.2012.667849
- Tiwari, B. K. (2015). Ultrasound: A clean, green extraction technology. *TrAC Trends in Analytical Chemistry*, 71, 100–109. doi:10.1016/J.TRAC.2015.04.013
- Torley, P.J. ve Bhandari, B. R. (2007). Ultrasound in Food Processing and Preservation. S. Rahman (Ed.), *Handbook of food preservation* içinde (Second Edition., s. 713). USA: CRC Press.

DETERMINATION OF YIELD AND QUALITY CHARACTERISTICS OF *Mentha piperita* L. CLON AND VARIETIES CULTIVATED IN ISPARTA CONDITIONS

Kemal YILMAZ¹, İsa TELCİ², Hasan ASLANCAN¹, Rafet SARIBAŞ¹, Tanju TURGUT¹
Muhammed BIYIKLI²

¹Fruit Research Institute, Republic of Turkey Ministry of Food, Agriculture and Livestock – Fruit Research Institute, 32500 Eğirdir, Isparta / TURKEY

²Applied Sciences University of Isparta, Field Crops Department, Isparta/TURKEY

Corresponding author email: yilmazkemal@tarim.gov.tr

Abstract

Mentha piperita L. (Lamiaceae) is one of the important mint's species, because essential oil is valuable. In recent years, demands for producing herb and essential oils have increased. So, plant and quality properties of some *M. piperita* varieties were evaluated two location, Isparta and Aksu, in the study. Three varieties, Swiss, Multimentha, Chocolate, were used. Field studies were carried out according to random blocks design during 2016 and 2017 vegetation periods. Plant height, fresh and dried herba yields with essential oils contents and compositions was studied. Essential oils were isolated with Neo-Clevenger distillation apparatus and chemical composition was determined with GC-MS. As a result of the research, fresh herb yields were higher in Isparta location in first year of the study while values of second year are similar both locations except essential oil contents. Essential oil contents were higher in Aksu. The highest yield was obtained from Multimentha variety in both two locations. But in this variety, menthol rate was the lowest.

Keywords: Mint, mentha, yield, essential oil, menthol, Isparta.

INTRODUCTION

Mint is a general name for the *Mentha* species belonging to the Labiatae family. *Mentha* species are herbaceous plants having wide-spreading stolon (Başer, 1997). Many species of *Mentha* are used as herbal tea, spice medicine, food, cosmetic and perfumery. *M. arvensis* and *M. piperita* are commercially cultivated because of their valuable essential oil with high menthol contents. In the world, the mint species (especially *M. arvensis*) are commercially produced in countries such as China, India and the USA (Telci, 2001). *Mentha* species are the second most important essential oil plants after *Citrus* species (Telci et al. 2011)

The Isparta located Göller Region of Turkey is major essential oil producer of Turkey due to rose and lavender. There are more than 20 factories that process essential oils, including more rose oil. This is chance for the production and processing of other essential oil plants in the region.

In recent years, studies medical and aromatic plant areas are increasing as a result of their growing importance. Grower of Isparta is looking for new plant species such as mint. And there are no reach on new mint cultivar in the region. So, it was aimed to determine yield and quality characteristics of *Mentha piperita* L. cultivar (Swiss, Multimentha, Chocolate) two different locations, Isparta and Aksu (Isparta).

MATERIAL AND METHOD

The study was carried out at two different locations, Isparta and Aksu. Fields studies of Isparta were conducted Agricultural Research Center (TARUM) in Suleyman Demirel University, while the study of Aksu in Karağ1 Village.

The locations are between the Mediterranean and Central Anatolian climates, and the continental climate is dominant in both locations. The altitudes of the locations are 1035 m and 1200 m in Isparta and Aksu, respectively.

Table.1 Varieties used in the studies.

Number	Cultivar	Species	Origin
1	Swiss	<i>M. piperita</i>	Switzerland
2	Multimentha	<i>M. piperita</i>	Germany
3	Chocolate	<i>M. piperita</i>	USA

Three different commercial varieties were used in the study (Table 1). Cuttings from the cultivars were rooted in controlled greenhouse conditions and, field studies were conducted in the rooted cuttings. Rooted cutting of cultivars were planted in plots (3.6 m²) using “randomized block design” with three replications. The plantlets were planted on parcels with a surface area of 3.6 m² (3x1,2). Plants were planted 30 cm apart, in 40 cm rows. The plants were fertilized with 50 kg/ha N and 50 kg/ha P₂O₅ (Özel, 1995, Telci, 2001). Plots were irrigated frequently with drip irrigation system. Plots were kept weed free by hand hoeing. The plants were cutting twice (the first cutting in mid-July and the second cutting lasting of August) at floral initiation period after the plant height were measured. Yields (fresh herb, dry herb and dry leaves) and quality characteristics (essential oil) were determined after cutting. Mint essential oil ratios were determined according to water distillation method with Clevenger apparatus. The essential oil compositions in cultivar were determined by GC-MS. Data were subjected to analysis of variance (ANOVA) using randomized block design with SPSS statistical software. Significance of the differences in properties were grouped with DUNCAN multiple test.

RESULTS AND DISCUSSION

Plant height, yields (dry leaf yield with fresh and dry herb yield), essential oil content and essential oil composition of *Mentha piperita* cultivars in Isparta and Aksu (Isparta) ecological conditions were evaluated during two years in 2016 and 2017.

Planth Height

Table 2. Variation of plant height values of *Mentha piperita* varieties cultivated in Isparta and Aksu conditions.

Cultivar	2016		2017				Mean Cultivar **	
	Isparta		Aksu	Isparta		Aksu		
	1. cutting	2. cutting	1. cutting	1. cutting	2. cutting	1. cutting		2. cutting
Swiss	75,8	35,4	35,3	65,8	38,2	74,8	57,3	54,6a
Multimentha	69,9	47,4	40,5	73,2	58,2	62,4	61,6	59,0a
Chocolate	45,4	46,3	31,0	39,5	20,8	51,2	44,2	39,7b
Mean**	63,7a	43,0b	35,6b	59,5a	39,1b	62,8a	54,4b	

** : p<0.01

At both locations, the plant height values of the first cutting were higher than that of the second cutting. In the study, except for the first cutting in the Aksu location, the plant height in the Multimentha variety was highest. But, plant height value of Swiss was higher than that of other cultivars in the first cutting of second year of Aksu. According to mean values, plant heights of Multimentha and Swiss were similar, statistically. Chocolate cultivar had a lower value in the research.

The plant height of first cutting was higher than that of the second cutting both years. Spring rainfall, the relative humidity, and the length of the vegetation period were caused higher plant height in first cutting (Telci and Şahbaz 2005a,b, Telci 2001). Furthermore, light intensity in summer, low relative humidity, and high temperatures shortened plant height in the second cutting (Telci and Şahbaz 2005a, b).

Fresh Herbage Yield

Table 3. Variation of fresh herb yield (t/ha) of *Mentha piperita* cultivar in Isparta and Aksu conditions.

Cultivar	2016		2017		Mean Cultivar **
	Isparta	Aksu	Isparta	Aksu	
Swiss	15.9	4.7	29.1	31.7	20.4b
Multimentha	27.0	6.8	41.4	34.9	27.6a
Chocolate	14.2	3.3	14.4	14.2	11.5c
Mean**	19.0a	4.9b	28.3	26.9	

**p<0.01

As a result of two years in the study, the mean fresh herb yields of varieties were between 11.5 and 27.6 t/ha. The highest mean value was obtained from the Multimentha with 27.6 t/ha. In addition, during the studies, maximum value was obtained from Multimentha both locations; and the data was significant statistically than data of other cultivar. When locations were compared, yields of Isparta were statistically higher than that of Aksu in first year. But, second year yields of both location were similar.

Dry Herbage Yield

Dry herbage yields in Isparta varied from 4.0 to 7.9 t/ha in the first year of study, while It ranged from 1.1 to 2.6 t/ha in Aksu . The yields of second year were higher than that of first year. The number of plants in unit area increased as a result of cuttings, and it is caused higher yields in subsequent cuttings (Telci and Şahbaz 2005a, b). Dried herb yields in the second year were varied from 4.6 to 11.0 t/ha in Isparta and from 3.5 to 11.0 t/ha in Aksu. The highest yield was obtained from the Multimentha cultivar cutting, followed by Swiss cultivar.

Table 4. Variation of dried herb yield (t/ha) of *Mentha piperita* cultivar in Isparta and Aksu conditions.

Cultivar	2016		2017		Mean Cultivar **
	Isparta	Aksu	Isparta	Aksu	
Swiss	4.1	1.6	7.7	7.9	5.3b
Multimentha	7.9	2.6	11.0	11.0	8.1a
Chocolate	4.0	1.1	4.6	3.5	3.3c
Mean**	5.3a	1.8b	7.8a	7.5a	

Dry Leaf Yields

Dry leaf yields in the first year were from 2.6 to 5.3 t/ha in Isparta and, from 0.9 to 1.6 t/ha in Aksu. Similar to fresh and dry herbs, yields were high in the second year in both locations. The second year yields were 3.2-5.7 t/ha in Isparta and 2.7- 6.4 t/ha in Aksu. In both locations, the highest yield was obtained in the Multimentha with 4.7 t/ha (Table 5).

Table 5. Variation of dried leaf yield (t/ha) of *Mentha piperita* cultivar in Isparta and Aksu conditions.

Cultivar	2016		2017		Mean Cultivar**
	Isparta	Aksu	Isparta	Aksu	
Swiss	2.6	1.5	4.4	3.9	3.0b
Multimentha	5.3	1.6	5.7	6.4	4.7a
Chocolate	2.9	0.9	3.2	2.7	2.4c
Mean**	3.6a	1.2b	4.4a	4.3a	

**p<0.01

Essential Oil Content

During the study, essential oil ratios ranged from 1.3 to 3.7%. The highest value was obtained in the second cutting of the Multimentha with 3.7% in Aksu location. During the study, the differences of mean data between the cultivar were not significant statistically while mean data of location in both years were significant significantly. Accordingly, the essential oil ratios were determined higher in Aksu town.

Table 6. Variation of essential oil values (%) of *Mentha piperita* varieties cultivated in Isparta and Aksu conditions.

Cultivar	2016		2017				Mean Cultivar**	
	Isparta		Aksu	Isparta		Aksu		
	1. cutting	2. cutting	1. cutting	1.cutting	2. cutting	1. cutting		2. cutting
Swiss	2,5	1,9	3,5	2,7	2,7	3,6	3,5	2,9a
Multimentha	2,4	2,1	3,2	3,2	2,3	3,3	3,7	2,9a
Chocolate	2,1	1,3	3,2	2,2	1,3	3,6	3,5	2,5a
Mean	2,3b	1,8b	3,3a	2,7ab	2,1b	3,5a	3,6a	

**p<0.01

Essential Oil Composition

The main compounds of cultivar were given in Table 7. According to this, while Swiss and Chocolate cultivar had high menthol content, the high proportion of Multimentha cultivar menthone is found to be high. In addition, methyl acetate, which is the third most important compound in *Mentha piperita* oil, was found 13.1% and 13.6% in Swiss and Chocolate, respectively, 3.4% in Multimentha. As a result of the study, it was determined that the value of menthol in the Swiss and Chocolate varieties is close to that of the European Pharmacopoeia in menthol, while the ratio of menthol in the high Multimentha variety was determined low.

Table 7. Main components Menthol, menthone and methyl acetate values in cultivars.

Variety	Menthol	Mentone	Methyl acetate
Swiss	48.3	20.8	13.1
Multimentha	34.2	47.3	3.4
Chocolate	48.3	17.8	13.6

CONCLUSION

It has been determined that the varieties in the experiments are suitable to the ecological conditions of Isparta and Aksu climates. The highest yield was obtained from Multimentha both locations. But menthol content was low. Mint cultivars with high menthol content were cultivated in the region. So, extra researches with additional cultivars must be done in the region for suggestion suitable cultivars for grower.

REFERENCES

- Başer, K.H.C., 1997. Tıbbi ve Aromatik Bitkilerin İlaç ve Alkollü İçki Sanayilerinde Kullanımı, İstanbul Ticaret Odası Yayın No: 1997-39, İstanbul.
- Özel, A., 1995. Harran Ovası Koşullarında Farklı Dikim Zamanlarının Bazı Nane (*Mentha* spp.) Çeşitlerinde Verim ve Kalite Kriterlerine Etkisi. Çukurova Üniv. Fen Bilimleri Enstitüsü. Doktora Tezi, Adana. 169 S.
- Telci, İ., 2001. Farklı Nane (*Mentha* spp.) Klonlarının Bazı Morfolojik, Tarımsal ve Teknolojik Özelliklerinin Belirlenmesi Üzerinde Bir Araştırma. (Doktora Tezi), Gop. U. Fen Bilimleri Ens., Tokat.
- Telci, İ., Şahbaz, N., 2005a. Determination of Agronomic and Essential Oil Properties of Peppermint (*Mentha piperita* L.) in Various Ages of Plantation. Journal of Agronomy 4 (2), s. 103-108.
- Telci, İ., and Şahbaz, N., 2005b. Variation of Yield, Essential Oil and Carvone Contents in Clones Selected from Carvone-scented Landraces of Turkish *Mentha Species*". Journal of Agronomy, 4 (2), 96-102.

- Telci, İ., Kacar, O., Bayram, Arabacı,E., Demirtaş, O., Yılmaz, I., Ozcan,G., İ. Sönmezi Göksu E. Ç 2011. The effect of ecological conditions on yield and quality traits of selected peppermint (*Mentha piperita* L.) clones Industrial Crops and Products 34 (1), 1193-1197
- Telci, İ., Kacar, O., Bayram, E., Arabacı, O., Demirtaş, İ., Yılmaz, G., Özcan, İ., Sönmez, Ç., Göksu, E. 2011. The effect of ecological conditions on yield and quality traits of selected peppermint (*Mentha piperita* L.) clones. Industrial Crops and Products 34 (1), 1193-119

EFFECT OF DIFFERENT POTASSIUM DOSES ON THE YIELD, QUALITY COMPONENTS AND NUTRIENT CONTENT OF ANISE (*Pimpinella anisum* L.)

Bülent YAĞMUR¹, Bülent OKUR¹, Nur OKUR¹

¹Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ege University, Bornova-İZMİR/TURKEY

Corresponding author email: bulent.yagmur@ege.edu.tr

Abstract

The aim of this study was to investigate the effect of different levels of K fertilizer on yield, some quality components and nutrient content of anise (*Pimpinella anisum* L.) and six different K levels (K0, K1: 4 kg/da K₂O; K2: 8 kg/da K₂O; K3: 12 kg/da K₂O; K4: 16 kg/da K₂O; K5: 20 kg/da K₂O) were applied to pot soil. The highest seed yield was obtained at K4 dose (35.38 gr). The most important active ingredient of anise essential oil has been identified as Trans-Anethole. The highest the content of trans-anethole (96.71%) and the ratio of essential oil (3.13%) were found at K3 dose. It was determined that K3 (umbrella weight, stem weight and plant height), K4 (root weight) and K5 (root height) doses were the most effective K doses on agronomic properties of anise.

Keywords: Potassium, Anise, Seed yield, Essential oil

INTRODUCTION

The emergence of new using areas of fragrant, medicinal and spice plants in recent years and the return to herbal medicines from synthetic drugs has increased the production and trade of these plants (İlisulu, 1992). In this regard, the consumption of medicinal plants shows a great increase in time. The return to nature has become a widely accepted motto in today's world and medicinal and aromatic plants also reached an important place in Turkey. Turkey, as well as being the center of many plant genes, comprises geographic regions that contain some endemic species.

Today, it is accepted that Turkey flora has over 9,000 species of plants. About 1000 of these plants are medicines and spice plants (Anonymous, 1998). Anise, which has an important place among the medicinal and aromatic plants, is a single annual aromatic plant belonging to the *Apiaceae* family and has been cultivated since the ancient times of the written history (Madaus, 1979). Its scientific name is *Pimpinella anisum*. *Pimpinella* genus has 23 species and 8 of these are distributed in Turkey, especially in Cesme, Burdur and Isparta.

Fertilization has a great importance in the cultivation of medicinal plants. Not only plant or seed yield, but the active substance or essential oil yield and high quality are the most important factors in fertilization.

High yield and quality can be achieved by a conscious and balanced fertilization. Potassium has a special place in this purpose. Because potassium is one of the nutrients needed for plant development in terms of both the amount of plant tissues and the physiological and biochemical functions in plant and uptaken too much from the soil during their growth and development periods. It is also effective on enzyme activity and photosynthesis, helps transport of plant nutrients, increases protein contain, regulates turgor, prevents water loss and wilting in plants. Potassium affects positively growth of plant and root, prevent lying, increase the resistance to cold, the efficiency of nitrogen, lignification and silicification in the plant (Aktaş, 1995). For this reason, potassium can reduce the development and damage of the parasite and increase the resistance of plants against diseases and pests.

In potassium deficiency, carbohydrate metabolism deteriorates the amount of cellulose and lignin and the thickness of the cuticle layer in the structure of outside cells of leaves and stems. The

processes such as thin cell wall, weak stem and trunk formation lead to a decrease in the resistance of plants against diseases and pests (Marshner, 1986).

The aim of this study was to determine the effect of different levels of potassium fertilizer on the yield of anise plant and some quality components.

MATERIAL AND METHODS

The study was conducted in the Ege University Faculty of Agriculture, Department of Soil Science and Plant Nutrition for 6 months. The pot experiment started on 1 February and ended on 1 August. In the experiment, Burdur anise which is 3-4mm long, 1.5-2mm wide, gray-green colored, having the best performance in hot, sunny and moderately humid climates and strong aromatic type has been used. Plant material was obtained from Savaşan Tarım Company. Table 1 shows the content of macro and micro nutrient elements of the anise seed used in the experiment and the results of essential oil analysis results.

Table1. Macro-and micro element content of anise, essential oil constituents and essential oil ratio of anise seed.

Nutrients (%)		Nutrients (ppm)	
Total N	2.842	Total Na	46.0
Total P	0.40	Total Fe	512.0
Total K	1.34	Total Cu	9.10
Total Ca	0.55	Total Zn	43.20
Total Mg	0.53	Total Mn	31.42
Essential oil constituents (%)			
	GC	GC/MS	
Trans-Anethole	90.82	86.49	
Methyl-chavicol	7.38	1.36	
Anis-aldehyd	1.80	---	
γ -Himachalene	--	0.23	
Essential oil ratio			
Essential oil ratio	(%)	2.9	

The soil-perlite mixing in the experiment was used as a growing media. Firstly, three soil samples were taken from Menemen Research and Application Farm, and their K contents have been analysed. The soil sample which has the lowest K content has been selected as it was the most convenient. The characteristics of this soil are given in Table 2.

Perlite, as a mixing material, was obtained from Etibank Perlite Company (Menderes- Izmir). The physical and chemical properties of perlite are given in Table 3.

Table 2. Some physical and chemical properties of the experiment soil.

pH		7.75	Light alkaline
Total soluble salt	(%)	0.048	No salinity problem
Lime	(%)	4.03	Calcic
Sand	(%)	85.08	
Silt	(%)	9.64	
Clay	(%)	5.28	
Texture			Loamy sand
Water holding capacity	(%)	201.08	
Organic matter	(%)	1.16	Poor
Total N	(%)	0.054	Medium
Available P	(ppm)	5.0	Sufficient
Available K	(ppm)	135	Poor
Available Ca	(ppm)	2600	Sufficient
Available Mg	(ppm)	187	Sufficient
Available Na	(ppm)	40	No problem
Available Fe	(ppm)	29.07	Sufficient
Available Cu	(ppm)	4.59	Sufficient
Available Zn	(ppm)	1.61	Sufficient
Available Mn	(ppm)	13.72	Sufficient

Table 3. Physical and chemical properties of perlite.

Density	1050 - 1250 Kg / m ³
Expansion Temperature	1100 – 1150
Melting Point	1300 - 1350 °C
Moisture	% 1 max.
pH	7 – 8
Hardness	6 (Mohs scala)
SiO ₂	% 72 min.
Al ₂ O ₃	% 12 min.
Fe ₂ O ₃	% 1.5 max.
K ₂ O + Na ₂ O	% 6.3 min.

The pot experiment was conducted in a randomized plots design with 5 replicates. Mitscherlich pots (20 cm diameter x 20 cm height) were filled with 1 kg soil and 500 gr perlite. 75 anise seeds (0.35 gr) per pot were sown at 3 cm inside of pot side. After germination, seedlings were thinned to 15 plants per pot and the measurements and analysis were made in these plants. Before sowing, all pots were watered by weight to maintain moisture content in field capacity and this weight was specified as the standard pot weight for subsequent watering. Water holding capacity (WHC) of the mixing material was determined and water moisture content was holded at 50% WHC during February-April, 60% WHC during April-May, 70% WHC during 10 May-26 May, 80% WHC during 28 May-13 June, 70% WHC during 15 June-21 June and 60% WHC during 22 June-1 July. Total amounts of water used per pot were given in Table 4.

Table 4. Total amounts of water added according to the treatments (lt).

<i>Treatments</i>	Amount of water added (lt)
K0	32±0.87
K1	35±1.28
K2	35±0.88
K3	36±1.81
K4	33±0.75
K5	32±1.08

2.5 gr (NH₄)₂SO₄ and 2gr TSP were applied to per pot in sowing on 01 February and then second fertilization was done in flowering by 1.5 gr NH₄NO₃ and 1gr MAP on 10 May. Potassium fertilizer was applied as K₂SO₄ at six different levels (K0, K1, K2, K3, K4 and K5) in sowing on 01 February (Table 5). Actara (0,1 ml/ 1 lt) was applied to all pots to control insects such as aphid (*Aphis* spp.), thrips (*Thrips* spp.) and red spider (*Tetranychus* spp.) on 12 June.

After browning of seeds, the harvest started in July and ended in August. In order to determine macro and micronutrient content of the anise plant, the leaves, roots and body samples that were taken in accordance with the procedure were first washed with tap water and then washed with pure water and dried at 65 ° C. The dried samples were milled and prepared for analysis (Kacar, 1972). In these plant samples, the analysis of total N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn were made (Kacar, 1984). In addition, agronomic parameters such as plant height, stem weight, root length, root weight, umbrella weight and seed yield were also determined (Ceylan, 1997). Volatile oil ratio and volatile oil compound of anise seeds from quality parameters were determined according to Wichtl (1971) and Ceylan (1977), respectively.

Table 5. Amounts of fertilizer used in the experiment.

<i>Treatments</i>	(NH ₄) ₂ SO ₄ (7 kgN/da)	NH ₄ NO ₃	TSP (10 kgP ₂ O ₅ /da)	MAP	K ₂ SO ₄
K0 (0 kg/da K ₂ O)	2.5 g	1.5 gr	2 gr	1 gr	0
K1 (4 kg/da K ₂ O)	2.5 gr	1.5 gr	2 gr	1 gr	1.2 gr
K2 (8 kg/da K ₂ O)	2.5 gr	1.5 gr	2 gr	1 gr	2.4 gr
K3 (12 kg/da K ₂ O)	2.5 gr	1.5 gr	2 gr	1 gr	3.6 gr
K4 (16 kg/da K ₂ O)	2.5 gr	1.5 gr	2 gr	1 gr	4.8 gr
K5 (20 kg/da K ₂ O)	2.5 gr	1.5 gr	2 gr	1 gr	6 gr

Statistical analysis was performed using the statistical software statistical analysis system TARIST (Açıkgöz et al., 1993).

RESULTS AND DISCUSSION

The Effect of Different Potassium Levels on the Seed Yield of Anise

The effect of different potassium levels on the seed yield of anise is given in Table 6. Potassium applications significantly affected ($p < 0.01$) the seed number. The highest seed yield (179.33 kg/da) was determined on plants that treated with K4 treatment (16 kg/da K₂O), whereas the lowest seed yield (103.33 kg/da) was found on plants that treated with control (K0) treatment. The seed yield of anise increased up to dose K4, but it decreased at the next dose (K5). Bayram(1992) was determined the highest seed yields (80.9 kg/da and 45.7 kg/da) with 1.5 kg/da seed amount and at 40 cm row distance for Antalya ecotype and Fethiye ecotype of anise, respectively.

Table 6. The effect of K levels on seed yield of anise.

<i>K Treatments</i>	Seed Yield	
	(gr/pot)	(kg/da)
K0	3.10 d	103.33 d
K1	4.24 bc	141.33 bc
K2	4.52 b	184.00 b
K3	4.76 ab	158.67 ab
K4	5.38 a	179.33 a
K5	3.72 c	124.00 c
LSD_{0.01}	0.693	20.14

The Effect of Different Potassium Levels on K Content of Anise

The effect of different potassium levels on K content of stem, seed, root and leaf of anise is given in Table 7. Potassium application significantly affected ($p < 0.01$) all parameters.

Table 7. The effect of K levels on K content of stem, seed, root and leaf of anise.

<i>K Treatments</i>	K concentration (%)			
	Stem	Seed	Root	Leaf
K0	1.59 e	1.29 e	0.30 c	1.40 e
K1	1.97 d	1.40 de	0.54 b	2.45 d
K2	2.32 c	1.47 cd	0.59 b	2.83 c
K3	3.01 b	1.58 bc	0.85 a	3.41 b
K4	3.28 a	1.67 ab	0.87a	3.48 ab
K5	3.39 a	1.83 a	1.00 a	3.67 a
LSD_{0.01}	0,180	0,155	0,165	0,245

The highest K contents in anise stem (3.28% and 3.39%) were determined at K4 and K5 treatments, whereas the lowest K content in anise stem (1.59%) was found at control (K0) treatment. K concentration of stem increased depending on increasing K doses (Table 7). Bayram (1992) found K concentrations in anise stem between 1.39 and 2.46%, 1.79 and 2.42 and 1.93 and 2.68 in 1988, 1989 and 1990 years, respectively.

The highest K content in anise seed (1.83%) was determined at K5 whereas the lowest K content in anise seed (1.29%) was found at control (K0) treatment. K concentration of seed increased depending on increasing K doses (Table 7). Bayram (1992) found K concentrations in anise seed between 1.23 and 1.55%, 1.53 and 1.76 and 1.62 and 1.82 in 1988, 1989 and 1990 years, respectively.

The highest K content in anise root (1.00%) was determined at K5 whereas the lowest K content in anise root (0.30%) was found at control (K0) treatment. Treatments of K3, K4 and K5 were in the same statistically group. K concentration of root increased depending on increasing K doses (Table 7).

The highest K content in anise leaf (3.67%) was determined at K5 whereas the lowest K content in anise leaf (1.40%) was found at control (K0) treatment. K concentration of leaf increased depending on increasing K doses (Table 7). Mills and Jones, (1991) found K concentrations in *Hypericum frondosum* leaf as 0.67%, in *Illicium anisatum* leaf as 0.60%, in *Illicium floridanum* between 0.57 and 0.78%, in *Illicium parviflorum* between 0.67 and 0.95%, in *Leucophyllum frutescens* as 2.28% and in *Rosmarinus officinalis* between 2.36 and 2.55%.

The Effect of Different Potassium Levels on Content of Macro-and Micro Nutrients of Anise

The effect of different potassium levels on content of macro-and micro nutrients of anise is given in Table 8. Potassium applications significantly affected the contents of N, Ca, Mg and Cu at $p < 0.01$ level, the contents of P and Fe at $p < 0.05$ level.

The highest N content in anise leaf (0.216%) was determined at K4 whereas the lowest N content in anise leaf (0.187%) was found at control (K0) treatment. N concentration of leaf increased depending on increasing K doses (Table 8). Nitrogen content of anise increased up to dose K4, but it decreased at the next dose (K5).

Table 8. The effect of K levels on the content of macro-and micro nutrients of anise.

K Treatments	N (%)	P (%)	Ca (%)	Mg (%)	Na (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
K0	0.187 d	0.37 b	2.86 a	0.73 a	632	537.3 b	10.90 c	11.12	89.93
K1	0.192 cd	0.37 b	2.72 a	0.54 b	613	561.3 b	12.10 b	11.37	91.40
K2	0.197 bcd	0.37 b	2.47 b	0.48 bc	604	699.3 ab	12.40 b	13.80	92.10
K3	0.203 abc	0.37 b	2.09 c	0.40 d	604	757.6 ab	12.60 b	11.33	90.23
K4	0.216 a	0.38 b	1.82 d	0.40 cd	589	908.6 a	13.50 a	10.41	83.52
K5	0.210 ab	0.40 a	1.62 d	0.35 d	558	916.6 a	14.10 a	9.20	81.20
LSD _{0.05}	ns	0.023	ns	ns	ns	259.605	ns	ns	ns
LSD _{0.01}	0.016	ns	0.208	0.073	ns	ns	0.832	ns	ns

The highest P content in anise leaf (0.40%) was determined at K5 treatment. The other all K treatments including control were in same statistically group (Table 8). Mills and Jones, (1991) found P concentrations in *Hypericum frondosum* leaf as 0.14%, in *Illicium anisatum* leaf as 0.19%, in *Illicium floridanum* between 0.18 and 0.29%, in *Illicium parviflorum* between 0.12 and 0.18%, in *Leucophyllum frutescens* as 0.18% and in *Rosmarinus officinalis* between 0.26 and 0.35%.

Unlike the other elements, the highest Ca concentration in anise leaf was found at K0 and K1 treatments while the lowest Ca contents in anise leaf were found at K4 and K5 treatments. Ca concentration of leaf decreased depending on increasing K doses (Table 8). Mills and Jones, (1991) found Ca concentrations in *Hypericum frondosum* leaf as 0.97%, in *Illicium anisatum* leaf as 0.53%, in *Illicium floridanum* between 0.44 and 0.61%, in *Illicium parviflorum* between 0.20 and 0.28%, in *Leucophyllum frutescens* as 0.72% and in *Rosmarinus officinalis* between 0.48 and 0.69%.

Similar to Ca, the highest Mg concentration (0.73%) in anise leaf was found at K0 treatment while the lowest Mg contents in anise leaf were found at K4 and K5 treatments. Mg concentration of leaf decreased depending on increasing K doses (Table 8). Mills and Jones, (1991) found Mg concentrations in *Hypericum frondosum* leaf as 0.18%, in *Illicium anisatum* leaf as 0.17%, in *Illicium floridanum* between 0.23 and 0.32%, in *Illicium parviflorum* between 0.11 and 0.15%, in *Leucophyllum frutescens* as 0.26% and in *Rosmarinus officinalis* between 0.17 and 0.40%.

The highest Fe contents in anise leaf were determined at K4 and K5 whereas the lowest Fe contents in anise leaf were found at K0 and K1 treatments. Fe concentration of leaf increased depending on increasing K doses (Table 8). Mills and Jones, (1991) found Fe concentrations in *Hypericum frondosum* leaf as 17 ppm, in *Illicium anisatum* leaf as 29 ppm, in *Illicium floridanum* between 23 and 87 ppm, in *Illicium parviflorum* between 19 and 25 ppm in *Leucophyllum frutescens* as 57 ppm and in *Rosmarinus officinalis* between 39 and 106 ppm.

Similar to Fe, the highest Cu contents in anise leaf were determined at K4 and K5 whereas the lowest Cu content in anise leaf was found at K0 treatment. Cu concentration of leaf increased depending on increasing K doses (Table 8). Mills and Jones, (1991) found Cu concentrations in *Hypericum frondosum* leaf as 40 ppm, in *Illicium anisatum* leaf as 2 ppm, in *Illicium floridanum* between 3 and 5 ppm, in *Illicium parviflorum* between 2 and 8 ppm in *Leucophyllum frutescens* as 6 ppm and in *Rosmarinus officinalis* between 3 and 23 ppm.

The effect of K applications on Na, Zn and Mn contents of anise leaf was not significant.

The Effect of Different Potassium Levels on Content of Essential Oil Constituents

The effect of different potassium levels on content of essential oil constituents of anise is given in Table 9.

Table 9. The effect of K levels on essential oil constituents of anise.

		<i>K Treatments</i>					
		K0	K1	K2	K3	K4	K5
GC	Trans Anethole, %	91.57	94.43	95.50	96.71	96.18	94.09
	Methyl-chavicol, %	6.68	4.12	3.40	2.47	2.76	4.51
	Anis-aldehyd, %	1.75	1.46	1.10	0.82	1.06	1.40
GC-MS	Trans Anethole, %	82.65	82.97	86.70	89.33	95.20	94.43
	Methyl-chavicol, %	1.71	1.32	1.20	1.74	1.22	1.06
	Anis-aldehyd, %	1.03	-	0.15	--	--	--
	Viridiflorol, %	--	0.16	0.24	0.65	0.49	0.46
	Trans-caryophyllene, %	--	--	--	0.39	--	--
	Borneol, %	--	--	--	0.44	--	--
	(3,3-Dimethylbicyclo (2.2.1) Hept-2-ylidene)-2-propanone, %	--	--	--	--	0.48	0.37

GC: Gas chromatography; GC-MS: Gas chromatography-Mass spectrophotometry

Essential oil constituents in anise were measured both gas chromatography and gas chromatography-mass spectrophotometry. It was determined that the largest part of the essential oil is formed by Trans-Anethole in both measurements. The highest trans-anethole ratio was found at K3 dose with 96.71% and the lowest trans-anethole ratio was found at K0 dose with 91.57% in GC (Table 8). Methyl-chavicol was the second essential oil constituent in anise seeds. The highest methyl-chavicol value was found at K0 dose with 6.68% and the lowest methyl-chavicol value was found at K3 dose with 2.47% in GC. Anis-aldehyd was the third essential oil constituent in anise seeds. K applications increased the content of trans-anethole while decreased the contents of methyl-chavicol and anis-aldehyd. The essential oil values determined in this study were similar to those of the values of the other authors (Bayram, 1992; Baser et al., 1999; Arslan et al., 2003).

The contents of trans-anethole, and methyl-chavicol measured by GC-MS showed an irregular change with potassium doses. The highest trans-anethole content was found at K4 and the lowest trans-anethole content at K5; the highest Methyl-chavicol ratio at K3 dose and the lowest methyl-chavicol ratio at K5 dose. Viridiflorol was detected in all K doses except K0 dose and the highest Viridiflorol content was found at K3 dose and the lowest at K1 dose. p-anisaldehyde was detected only at K0 and K2 doses while trans-caryophyllene and Borneol at only K3 dose. (3,3-Dimethylbicyclo (2.2.1) Hept-2-ylidene)-2-propanone was detected only K4 and K5 doses.

The effect of different potassium levels on essential oil ratio of anise

The effect of different potassium levels on essential oil ratio of anise is given in Table 10.

The effect of potassium applications on essential oil ratio of anise was statistically significant at $p < 0.01$ level. The highest oil ratio was found at K3 dose with 3.13% and the lowest oil ratios were found at K0 and K5 doses with 2.65% and 2.45%, respectively. Bayram (1992) found the highest oil ratio in Fethiye ecotype with 2.68% and the lowest oil ratio in Çeşme ecotype with 1.68%. Arslan et al. (2003) and Kevser (1982) found that oil content of anise change between 1.3% and 3.7% and

between 2.83% and 2.17%, respectively. The oil ratio of anise increased up to dose K3, but it decreased at the next doses (K4 and K5).

Table 10. The effect of K levels on essential oil ratio of anise.

<i>K Treatments</i>	Essential oil ratio (%)
K0	2.65 b
K1	2.83 ab
K2	2.85 ab
K3	3.13 a
K4	2.78 ab
K5	2.45 b
LSD_{0.01}	0.405

The Effect of Different Potassium Levels on Agronomic Properties of Anise

The effect of different potassium levels on umbrella weight, stem weight, root weight, plant height and root length of anise is given in Table 11.

Table 11. The effect of K levels on umbrella weight, stem weight, root weight, plant height and root length of anise.

<i>K Treatments</i>	Umbrella weight (gr)	Stem weight (gr)	Root weight (gr)	Plant height (cm)	Root length (cm)
K0	9.60 d	17.70	2.70	53.80 b	4.60 c
K1	11.50 bc	18.10	3.00	55.30 b	4.90 bc
K2	12.20 ab	20.40	3.60	60.20 a	5.10 bc
K3	13.40 a	21.80	3.70	60.60 a	5.60 ab
K4	10.60 cd	18.10	4.30	58.60 ab	5.90 a
K5	10.30 cd	18.10	3.30	57.50 ab	6.10 a
LSD_{0.05}	--	ns	ns	4.798	0.707
LSD_{0.01}	1.255	ns	ns	ns	ns

The applications of different K doses significantly affected umbrella weight at $p < 0.01$ level. plant height and root length of anise at $p < 0.05$ level. The highest umbrella weight was found at K3 dose with 13.40 gr and the lowest umbrella weight was found at K0 with 9.60 gr. The umbrella weight of anise decreased at K4 and K5 treatments. The applications of different K doses did not significantly affected stem weight and root weight of anise. The highest plant heights were found at K2 and K3 doses with 60.20 cm and 60.60 cm and the lowest umbrella weights were found at K0 and K1 doses with 53.80 cm and 55.30 cm. Bayram (1992) found the highest plant height in Denizli ecotype with 48.8 cm and the lowest plant height in Antalya ecotype with 43.4 cm. In the other study, the highest plant height was determined in Burdur anise with 48.7 cm and the lowest plant height in anise with 24.40 cm (Kevser,1982). The higher K applications from K3 decreased plant height of anise. The highest root height was determined at K4 (5.90 cm) and K5 (6.10 cm) treatments whereas the lowest root height was found at control (K0) treatment (4.60 cm). The root height of anise increased depending on increasing K doses. Kevser (1982) found the highest root height in Denizli anise with 31.9 cm and the lowest root height in Çeşme anise with 23.1 cm.

CONCLUSION

Potassium, one of the most important nutrients on the nutrition of plants and quality of fruit/seed; enzyme activity, plays role on photosynthesis and protein synthesis in the plant, regulates turgor, prevents water loss and fading, affects plant root development and increases the resistance to cold of plant. Due to the functions on the transport of photosynthesis products and on cell growth, our study has shown an increase in the anise yield and the ratio and the constituent of essential oil from quality parameters depending on increasing potassium doses. The most effective potassium doses were determined as K3 (12 kg/da) and K4 (16 kg/da).

ACKNOWLEDGEMENTS

The data presented in this paper is obtained from the project financially supported by Ege University (Scientific Research Project No: 2005ZRF074).

REFERENCES

- Açıkgöz, N., Akbaş, M.E., Moghaddam, A. ve Özcan, K., 1994. PC'ler için veritabanı esaslı Türkçe istatistik paketi: TARİST, Tarla Bitkileri Kongresi, 25-29 Nisan 1994, Bornova-İzmir, 131-136s.
- Aktaş, M., 1995. Bitki besleme ve toprak verimliliği. Ankara Üniversitesi Ziraat Fakültesi Toprak Bölümü, Yayın no:142, Ders Kitabı:43, 345 s.
- Anonymous, 1998. www.ordutarim.gov.tr/turetim/aromatik.htm
- Arslan, N., Gürbüz, B., Sarihan, E., 2003. Variation in essential oil content and composition in turkish anise (*Pimpinella anisum* L.) populations Turkish Journal of Agric. For, 28(2004) 173-177.
- Başer, K.H.C., Özek, T., Tabanca, N., Duman, H., 1999. Essential oil of *Pimpinella anisum* Boiss and Bal. Journal of Essential Oil Research, 11:445-446.
- Bayram, E., 1992. Türkiye kültür anasonları (*Pimpinella anisum*) üzerinde agronomik ve teknolojik araştırmalar, Ege Üniversitesi, Ziraat Fakültesi Fen Bilimleri Enstitüsü, Tarla Bitkileri Anabilim Dalı, Doktora Tezi, 136 s, İzmir.
- Ceylan, A., 1997, Tıbbi Bitkiler II, Ege Üniversitesi Ziraat Fakültesi Yayınları No:481, s 1-22.
- İlisulu, K., 1992. İlaç ve baharat bitkileri. Ankara Üniversitesi Ziraat Fakültesi Yayınları No:1256, Ders Kitabı:360, s.63-75.
- Kacar, B., 1972. Bitki ve toprağın kimyasal analizleri 1-2. Ankara Üniversitesi Ziraat Fakültesi Yayınları:468 Yardımcı Ders Kitabı :161, s.45-92.
- Kevser, K.F., 1982. Bazı Anasonların fenolojik, morfolojik ve kalite özellikleri ile çiçek biyolojisi üzerine araştırmalar. Doktora Tezi, A.Ü. Ziraat Fakültesi, Ankara
- Madaus, G., 1979. Lehrbuch der biologischen heilmittel. Band I, Georg Olms Verlag-Hildesheim, p.544-550.
- Marshner, H., 1986. Mineral nutrition of higher plants. Academic Press London, p.514-527.
- Mills, A. H., Jones, J. B., 1991. Plant analysis handbook II. Athens, Ga.: MicroMacro Publishing, 1996, pp. 155-414
- Wichtl, M., 1971. Die pharmakognostisch-chemische analys. Acad. Verlagsgesellschaft, Frankfurt, Band 12 p.479.

THE INFLUENCE OF DIFFERENT NITROGEN DOSES AND NUMBER OF HARVEST ON CERTAIN AGRICUTURAL PROPERTIES OF TARRAGON (*Artemisia dracunculus* L)

Kemalettin KARA¹, Şeyma Nur TARHAN¹

¹Atatürk Üniversitesi Ziraat Fakültesi Tarla Bitkileri Bölümü, 25040, Erzurum. Türkiye

Corresponding author email: kara@atauni.edu.tr

Abstract

This research was conducted to the determine the effects of different nitrogen doses (0, 4, 8 and 12 kg) and number of harvest (florescence and end of season) on some agricultural and quality properties of ecotypes (Kağızman and Hasankale) of tarragon (*Artemisia dracunculus*). Nitrogen doses did not have a statistical effect on other characters except for the plant height. Although there was no difference between other characters; plant height, wet herba, dog herba, drog stem and leaf yields were higher at 8 kg nitrogen dose, while volatile fat ratio was higher at 12 kg nitrogen dose. The effect of number of harvest was significant on the examined traits. Especially, the first harvest values were higher than the second ones. There was no statistical difference between the examined characters among the ecotypes. However, Hasankale ecotype was better than. Kağızman ecotype in terms of examined properties. According to the result of the present study, considering the nitrogen doses and number of reaping for both tarragon ecotypes, it was determined that applying 8 kg nitrogen dose per decare had positive effects on all examined characters except for the volatile fat amount, which was maximum at 12 kg nitrogen dose. In conclusion, 8 kg nitrogen dose and harvest at florescence phase is being recommended for both of the tarragon ecotypes under Erzurum conditions.

Keywords: *Artemisia dracunculus*, tarragon, nitrogen doses, number of harvest

INTRODUCTION

Today, medical and aromatic plants are used in traditional and modern medicine for the purpose of sustaining a healthy life, preventing and improving diseases. In addition, these plants in the food, soft drinks, perfume and cosmetics industry, as brighteners and insecticides in different branches of the industry as a result of the use of the consumption has accelerated, therefore the production obligation has emerged. The dried parts of medicinal and aromatic plants called drog are prepared to a certain extent (root, root, stem, tuber, trunk or woody structure, shell, leaf, flower, fruit, seed and herb).

Tarhun (*Artemisia dracunculus*) is one of the plants grown in the world and in our country for medicinal and aromatic purposes. Tarhun plant is very important in medicine in terms of usage. By increasing the digestive secretions that facilitate digestion, stomach and intestinal gases and diuretic, beneficial to stomach diseases. In addition, the effect of strengthening tonic antiseptic, appetite opener, cramp solvent, has a feature of reducing worm. In addition, it removes the salt and water accumulated in the body, relieves indigestion, anemia and digestive disorders. It is a good spice for those who have to eat unsalted food (Libbey and Sturts 1989; Azizak 2007).

Cultural practices are important in medicinal and aromatic plants. Fertilization and harvest times from these cultural practices affect the quality, yield and yield components of medicinal and aromatic plants. In this study, it was aimed to determine the effect of nitrogen doses and form of tarhun (*Artemisia dracunculus*) ekotypes obtained from Erzurum (Hasankale) and Kars (Kagizman) provinces on the agronomic and some quality characteristics of Erzurum ecological conditions.

One of the most important economic aspects of medicinal and armatic herbs is the timely harvesting. Thus, both the highest yield and the best quality product is obtained. The determination of harvest time in the cultivation of medicinal plants is one of the most important agricultural practices affecting drug Herba and active ingredient yield. Optimum harvest time depends on desired compounds and cultivars. If the desired is artemisin, maximum efficiency is reached at the start of

flowering in most cases. If the desired oil or oil combination and Artemisia are acid, then the full flowering period is the best harvest time (Wright 2002). Eabl (2005) it is reported that the species of Artemisia annua should be harvested immediately before flowering, and the content of the harvest should be reduced as the harvest date is delayed. Tarhun plant is harvested for the green evening at the end of June.

According to studies, the highest rate of volatile oil in Russian Estragon is the time when bud is formed. However, a certain circuit was not detected in the German Estragon. Rich in nutrients, adequate moisture can be made in three forms. Seed harvest, should be made when the fruits are brown. It is also reported that for decares herb yields ranged from 100-300 kg in the first year and 200-600 kg in the second year (Ceylan 1987).

For Kara and Mervener (2015)'s study conducted in Erzurum in 2014 with 2 tarragon ecotypes Hasankale, Kağızman) and 4 harvests (15 Haziran, 15, Temmuz 15 Ağustos and 1 Eylül/Control), harvest times were detected to be influential on other characters investigated except the dry trunk efficiency and volatile fat ratio of the breeds and fresh herb efficiency. With regards to the examined characters, the tarragon under Erzurum conditions is recommended to be harvested in 15 July, with regards to the breeds, Kağızman ecotype was recommended because of its other examined characters rather than volatile fat ratio and Hasankale ecotype was recommended because of its volatile fat ratio.

MATERIAL AND METHODS

The research was carried out in the experimental field of Faculty of agriculture in 2015. In this study, tarhun ecotypes obtained from Hasankale and Kağızman region were used. A research area of climate and soil characteristics: the research we conducted during the period where the lowest temperature (10.6°C) and June (17,0°C) was recorded in the months when the highest temperatures are July and August (21,2°C) was recorded in the months of. The total rainfall was 158,50 mm and the maximum rainfall decreased in May (81.5 mm) and at least in July (5.8 mm). The monthly average relative humidity was 47.28% during the May-September period of 2015 when the experiment was conducted. In the experiment, the highest relative humidity was recorded in May with 63.8%, and the highest average relative humidity was recorded in September with 39.2%. Soil characteristics of the research area: the structure of the experimental area soils is clay-tinned and the pH is 7,27. The amount of organic matter is 0.11%, P₂O₅ 14.2 kg/da, and K₂O 163.6 kg/da.

The research was carried out in 3 replications according to the "Split parcels" arrangement in the "exact blocks of chance" trial pattern. In this experiment, the clones used were distributed to the main parcels, 4 different nitrogen doses (0, 4, 8 and 12 kg/da) and two forms (8 June and 6 October). Each parcel consists of four ordinary, six plants in each row. The Parcel area is 2,0 m x 2,4 m: 4,8 m² and the clones are planted in rows of 50 cm, 40 cm above the row. Harvest area (1,0 m x 1,6 m) was 1.6 m². In the experiment, ammonium sulphate (21%) and triple super phosphate (45%) fertilizers were used as nitrogen fertilizer. Pure phosphorous fertilizer was applied with 6 kg account of decare. Necessary maintenance procedures have been carried out. Plant height, green herba yield (kg/da), green leaf yield (kg/da), drog herba yield (kg/da), drog leaf yield (kg/da), drog stem yield (kg/da) and volatile oil rates were investigated.

RESULTS AND DISCUSSION

Plant Height

As shown in Table 2, There was a statistically significant difference between the nitrogen doses and the number of forms of tarhun ecotypes in terms of plant size. Plant height increased to 8 kg nitrogen dose per hectare, and after this dose decreased. Plant size control was 31.50 cm, 37.12 cm in 4 kg dose of decare and 40.25 cm in 8 kg dose and 35.42 cm in 12 kg dose of decare (Table 1).

The plant height was 44.25 cm in the first form (June 8/ Florescence) and 27,90 cm in the second form (end of season). The plant height was 44.25 cm in the first form (June 8/ Florescence) and 27.90

cm in the second form (end of season). This is related to the progress of the plant (Table 1). Plant-height values of ecotypes were close to each other and there was no statistically significant difference. The plant length of kagizman ekotype was 35,92 cm and the plant length of Hasankale ekotype was 36,23 cm. The plant height difference between the ecotypes may have been sourced by the genetic structures of the ecotypes informed by Piccaglia *et al.* (1993) and Telci (2001).

Table 1. Average plant height of tarragon ecotypes (*Artemisia dracunculus* L) belonging to different nitrogen doses and number of forms (cm.)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	38,50	40,17	31,50 C
		Hasankale	41,83		
	Second (End of season)	Kagızman	20,50	22,81	
		Hasankale	25,17		
4	First (Florescence)	Kagızman	45,17	45,17	37,12 B
		Hasankale	45,17		
	Second (End of season)	Kagızman	28,00	29,08	
		Hasankale	30,17		
8	First (Florescence)	Kagızman	48,67	49,33	40,25 A
		Hasankale	50,00		
	Second (End of season)	Kagızman	30,67	31,17	
		Hasankale	31,67		
12	First (Florescence)	Kağızman	44,50	42,33	35,42 B
		Hasankale	40,17		
	Second (End of season)	Kagızman	31,33	28,50	
		Hasankale	25,67		
Harvest numbersı		Ecotipes			
First harvest (Florescence): 44,25 A Second harvest (End of season): 27,90 B		Kagızman: 35,92 Hasankale : 36,23			

Differences between the averages marked with capital letters are significant according to the $p < 0.01$ probability limit.

Age Herba Yield (kg/da)

Although there was no statistically significant difference between nitrogen doses and herba yield (Table 3 and 2), There was no statistically significant difference between nitrogen doses and herba yield (Table 3 and 2). The increase in Herba yield (770,46 kg/da), after this dose (683,01 kg/da) was observed. In the application of nitrogen doses of 0 and 4 kg, 593,28 and 666,89 kg were determined respectively (Table 3).

According to the number of forms, the difference between age and herba yields was statistically significant at $p < 0.01$. In the first form (florescence period), herba yield was 1019.61 kg, in the second form (end of season) was 337.21 kg (Table 3). The second form of among the ecotypes, there is a statistically significant difference in herba yield (Table 3).

The mean age of Hasankale ecotype (682,68 kg/da) was higher than that of Kagizman ecotype (674,14 kg/da). The mean age of Hasankale ecotype (682,68 kg/da) was higher than that of Kagizman ecotype (674,14 kg/da) (Table 3). The difference between ecotypes can be recorded by genetics to provide age Herba yield.

Table 2. Different doses of nitrogen applied and two forms of tarragon (*Artemisia dracunculus* L) ekotypes of plant height, decare age and dry weight, drog leaf and drog stem, volatile oil ratio of variance analysis results

Variation Source	D.F	F Values					
		Plant height	Age herba yield	Dry herba yield	Leaf yield	Stem yield	Volatile oil ratio
Ekotype (A)	1	0,67	0,02	0,12	0,18	0,01	2,39
Error ₁	2						
Nitrogen doses (B)	3	18,35 **	2,00	1,70	2,00	2,59	0,36
Harvest (C)	1	369.00 **	175,91 **	58,08 **	9,35*	116.19**	15,30 **
BxC	3	1,23	0,54	0,21	0,65	0,11	0,24
BxA	3	4,96 *	1,00	0,95	1,04	1,64	0,35
CxA	1	0,72	0,36	0,67	1,05	0,30	2,99
AxBxC	3	0,22	0,35	0,94	1,18	0,65	0,55
Error ₂	28						

Marked F values (p <0.05), ** Marked F values are important according to (p <0.01) probability limit.

Table 3. According to different nitrogen doses and the number of forms tarragon (*Artemisia dracunculus* L) ekotypes of herba per decar yield (kg/da)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	870,83	897,40	593,28
		Hasankale	923,97		
	Second (End of season)	Kagızman	267,17	289,17	
		Hasankale	314,17		
4	First (Florescence)	Kagızman	927,10	1033,17	666,89
		Hasankale	1139,23		
	Second (End of season)	Kagızman	274,57	300,62	
		Hasankale	326,67		
8	First (Florescence)	Kagızman	1120,00	1151,03	770,46
		Hasankale	1102,07		
	Second (End of season)	Kagızman	397,07	389,88	
		Hasankale	382,70		
12	First (Florescence)	Kağızman	1002,07	996,85	683,01
		Hasankale	991,63		
	Second (End of season)	Kagızman	457,30	369,17	
		Hasankale	281,03		
Harvest number1		Ecotypes			
First harvest (Florescence g):1019,61 A Second harvest (End of season):337,01 B		Kagızman: 674,14 Hasankale: 682,68			

Differences between upper case averages p<0.01, differences between lower case averages p<0.05 probability limits are important.

Drog Herba Yield (kg /da)

There is a numerical difference between nitrogen doses in terms of decare drog herb yield, and there is a difference between rational Statistics (Table 2). The drog herba yield increased up to 8 kg nitrogen dose per decare, and decreased after this dose. The drog herba yields were 171,70 kg, 196,74 kg, 217,12 kg and 210,044 kg respectively in decar 0, 4, 8 and 12 kg nitrogenous nitrogen (Table 4). There was a statistically significant difference between the number of forms in terms of dekara drog herb yield (p < 0.01). The first form of dekar drog herb was 257,37 kg and the second form was 140,43 kg (Table 2 and 4).

The difference between the ecotypes is tested as a statistic in terms of dekar drog herb yield (Table 2). The yield of Hasankale ecotype was 203,74 kg for Herba and 194,06 kg for kagizman ecotype (Table 4). The yield of Hasankale ecotype was 203,74 kg for Herba and 194,06 kg for kagizman ecotype (Table 4). The difference between ecotypes in terms of drog herba yield can be recorded by genetics.

Table 4. According to different nitrogen doses and the number of forms tarragon (*Artemisia dracunculus* L) ekotypes decar drog herba yields (kg /da)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	232,78	232,84	171,70
		Hasankale	232,90		
	Second (End of season)	Kagızman	81,03	110,56	
		Hasankale	140,09		
4	First (Florescence)	Kagızman	236,40	263,90	196,74
		Hasankale	291,40		
	Second (End of season)	Kagızman	119,02	129,66	
		Hasankale	140,012		
8	First (Florescence)	Kagızman	259,61	269,66	217,12
		Hasankale	279,70		
	Second (End of season)	Kagızman	176,67	164,58	
		Hasankale	152,48		
12	First (Florescence)	Kağızman	256,20	263,09	210,04
		Hasankale	269,97		
	Second (End of season)	Kagızman	190,75	157,00	
		Hasankale	123,24		
Harvest number1		Ecotypes			
First harvest (Florescence): 257,37 A Second harvest (End of season): 140,43 B		Kagızman: 194,06 Hasankale: 203,74			

Differences between the averages marked with capital letters are significant according to the $p < 0.01$ probability limit.

Drog Leaf Yield (kg /da)

Although there is a numerical difference between nitrogen doses in terms of decare drog leaf yield, there is a difference in statistics. (Table 2 and 5). Depending on the increase in nitrogen doses, there is a stable increase in decare drog leaf yield. Thus, from 0, 4, 8 and 12 kg nitrogen application of decar drog leaf yields were 67,00 kg, 73, 64 kg, 85.27 kg and 84.05 kg respectively (Table 5). Significant differences were found statistically at $p < 0.05$ in terms of decare drog leaf yield (Table 2). The first form of decar drog herb was defined as 86.95 kg and the second form as 68.07 kg (Table 5). The first form of dekar drog herb was defined as 86.95 kg and the second form was defined as 68.07 kg. The difference between ecotypes in terms of decare drog leaf yield is tested as statistically (Table 2). The leaf yield was 75.62 kg in kagizman ecotype and 79.34 kg in Hasankale ecotype, the leaf yield was 75.62 kg in kagizman ecotype (Table 5).

Drog Stem Yield (kg/da)

As can be seen in Table 6, although there is a numerical difference between nitrogen doses and decare drog stem yields, there is a statistically significant difference between nitrogen doses (Table 2). As a matter of fact, according to nitrogen doses applied to decare 0, 4, 8 and 12 kg, decare drog stem yields were 104.70 kg, 123.16 kg, 142.73 kg and 124.52 kg respectively (Table 6).

There was a significant difference between the number of forms in drug stem yield and $p < 0.01$ level (Table 2). In the first form, decar drug stem is tested as 175,80 kg and in the second form as 71,776 kg (Table 6).

Since the ecotypes are close to each other, there is a statistically significant difference (Table 6 and 2). The stem yield of Hasankale ecotype was 124,56 kg and 123,00 kg in kagizman ecotype (Table 6). The stem yield of Hasankale ecotype was 124,56 kg and the stem yield of Hasankale ecotype was 123,00 kg.

Table 5. Different nitrogen doses and harvest time according to tarragon (*Artemisia dracunculus* L) ecotypes decar drug leaf yields (kg /da)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	77,02	77,40	67,00
		Hasankale	77,78		
	Second (End of season)	Kagızman	37,76	56,51	
		Hasankale	75,27		
4	First (Florescence)	Kagızman	82,42	89,65	73,64
		Hasankale	96,88		
	Second (End of season)	Kagızman	58,24	57,63	
		Hasankale	57,02		
8	First (Florescence)	Kagızman	91,76	92,05	85,27
		Hasankale	92,34		
	Second (End of season)	Kagızman	78,60	78,50	
		Hasankale	78,39		
12	First (Florescence)	Kağızman	88,27	88,69	84,05
		Hasankale	89,11		
	Second (End of season)	Kagızman	90,89	79,40	
		Hasankale	67,91		
Harvest number		Ecotypes			
First harvest (Florescence):86,95 a Second harvest (End of season): 68,01 b		Kagızman: 75,62 Hasankale: 79,34			

Differences between the averages marked with small letters are significant according to the $p < 0.01$ probability limit.

Volatile Oil Ratios

Although there is a numerical difference between the volatile oil ratios according to nitrogen doses, the Commission will differ statistically (Table 7 and 2). The volatile oil ratios of 0, 4, 8 and 12 kg nitrogen applications were 0.081%, 0.088%, 0.065% and 0.115% respectively (Table 7). The volatile oil ratios of 0, 4, 8 and 12 kg nitrogen applications were 0.081%, 0.088%, 0.065% and 0.115% respectively (Table 7). There was a significant difference between the figures in terms of volatile oil ratios at $p < 0.01$ level as statistics (Table 2). In the first form, the ratio of volatile oil was 0.115% and in the second form 0.019% (Table 7). There was no statistical difference detected between the ecotypes in terms of volatile oil ratio (Table 2). The volatile oil ratio of the Kağızman ecotype was 0.060% and that of Hasankale ecotype was 0.115% (Table 7).

Table 6. Average values of tarragon (*Artemisia dracunculus* L) ecotypes in drog stem yield according to experimental factors (kg /da)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	155,77	154,78	104,70
		Hasankale	153,79		
	Second (End of season)	Kagızman	44,09	154,62	
		Hasankale	65,15		
4	First (Florescence)	Kagızman	152,98	174,43	123,16
		Hasankale	195,89		
	Second (End of season)	Kagızman	60,78	71,89	
		Hasankale	83,00		
8	First (Florescence)	Kagızman	210,44	199,40	142,73
		Hasankale	188,36		
	Second (End of season)	Kagızman	98,07	86,06	
		Hasankale	74,05		
12	First (Florescence)	Kağızman	168,26	174,57	124,52
		Hasankale	180,87		
	Second (End of season)	Kagızman	93,61	74,47	
		Hasankale	55,33		
Harvest number		Ekotipler			
First harvest (Florescence):175,80 A Second harvest (End of season): 71,76 B		Kagızman: 123,00 Hasankale: 124,56			

Differences between the averages marked with capital letters are significant according to the $p < 0.01$ probability limit.

Table 7. Volatile oil ratios of tarragon (*Artemisia dracunculus* L) ecotypes determined in different nitrogen doses and form numbers (%)

Nitrogen doses	Harvest numbers	Ecotypes		Average harvest time	Average nitrogen doses
0	First (Florescence)	Kagızman	0,067	0,137	0,081
		Hasankale	0,207		
	Second (End of season)	Kagızman	0,020	0,025	
		Hasankale	0,030		
4	First (Florescence)	Kagızman	0,097	0,148	0,088
		Hasankale	0,200		
	Second (End of season)	Kagızman	0,020	0,020	
		Hasankale	0,033		
8	First (Florescence)	Kagızman	0,127	0,127	0,065
		Hasankale	0,127		
	Second (End of season)	Kagızman	0,007	0,003	
		Hasankale	0,0005		
12	First (Florescence)	Kağızman	0,100	0,208	0,115
		Hasankale	0,317		
	Second (End of season)	Kagızman	0,040	0,022	
		Hasankale	0,003		
Harvest numbers		Ecotypes			
First harvest (Flowering): 0,155 A Second (End of season): 0,019 B		Kagızman: 0,060 Hasankale : 0,115			

Differences between the averages marked with capital letters are significant according to the $p < 0.01$ probability limit.

CONCLUSION

This study was carried out to examine the effects of nitrogen doses and form numbers on some agricultural properties of taragon (*Artemisia dracunculus* L) ecotypes (Kagızman and Hasankale) and

volatile oil ratios and components. Nitrogen doses had a statistically significant effect on other characters in plant height warfare. Among other characters there is a numerical difference, maximum plant height, herba, drog Herba, drog stem and leaf yields are determined at a nitrogen dose of 8 kg per decares and the volatile oil rate is determined at a nitrogen dose of 12 kg per decares. The effect of format numbers on the properties studied is an important invention. In particular, the first format values are more than the second format values. The differences between the ecotypes were determined. However, it was determined that the Hasankale ecotype was superior in terms of the characteristics studied.

REFERENCES

- Azirak S., 2007. Investigation of In Vivo Genotoxic Effects of Thymol and Carvacrol. Cukurova University, Institute of Science and Technology, Ph.D. Thesis, Adana (Unpublished)
- Ceylan A, 1987. Medicinal Plants II. Ege Univ. Faculty of Agriculture edition 481, page: 1,4, 12, 14. Bornova-İzmir.
- EABL, 2005. Manual for *Artemisia annua*. East African Botanicals Ltd. Growers' Production
- Kara, K ve Aykaç, M., 2015. Effects of harvesting times on agronomic and some quality properties of tarragon (*Artemisia dracunculus*), 11. Field Crops Congress (7-10 September 2015, Canakkale) 720
- Libbey, L. M. and Sturz, G., 1989. Unusual Essential Oils Grown in Oregon I. *Artemisia afra* Jacq. J. Ess. Öl Res., 1,29.
- Piccaglia, R., Dellacecca, V., Marotti, M., Giovanelli, E., 1993. Agronomic factors effecting the yields and the essential oil composition of peppermint (*Mentha piperita* L.) International Symposium on Medicinal and Aromatic Plants (22-25 March 1993) Israel, Acta Horticulturae, 344:29-40.
- Telci, İ., 2001. A Study on Determination of Some Morphological, Agricultural and Technological Characteristics of Different Mint (*Mentha* spp) Clones. Gaziosmanpaşa Univ. Institute of Science and Technology, Ph.D. Thesis, Tokat, p. 160.
- Wright C.W., 2002. *Artemisia*. CRC Press.

DETERMINATION OF FLOWER AND SCENT CHARACTERISTICS IN OIL-BEARING ROSE (*Rosa damascena* Mill.) GENOTYPES DERIVED FROM MUTATION BREEDING

Hasan BAYDAR¹, Sabri ERBAŞ¹, Keziban SARI¹

¹University of Applied Sciences, Faculty of Agricultural Sciences and Technologies, Department of Field Crops, Isparta, Turkey

Corresponding author email: hasanbaydar@isparta.edu.tr

Abstract

Flower and fragrance characteristics of the 27 oil-bearing rose (*Rosa damascena* Mill.) genotypes obtained by application of Cobalt-60 gamma ray at 0, 100 and 200 Gray doses were determined in the Department of Field Crops at University of Applied Sciences in Isparta, Turkey. In the oil-bearing rose genotypes with 6 years old, the number of petals ranged from 5 to 85, and the petal color varied between white and dark pink. In general, those with a low number of petals (single flowers with 5-7 petals) tended to flow earlier, those with a higher number of petals (multi-petalled flowers with >25 petals) tended to bloom later. Single-layered flowers were determined to be less weight, less fragrant, shorter petal life and easier petal casting when compared to the multi-layered flowers. The important and negative relationship ($r = -0.85^{**}$) between the number of petals and the number of anthers may be the result of the homeotic functions of MADS-box function genes such as A, B and C. It was a significant observation that the genotypes with hairless (naked) hypanthia, pedicels and sepal leaves were less fragrant, but more resistant to rose aphid (*Macrosiphum rosae* L.). According to the HS-SPME and GC-MS analysis, while the most important floral scent molecule of the fresh flower was phenylethyl alcohol, the most important molecules of distilled rose oil were monoterpenic alcohols such as citronellol and geraniol, and paraffinic hydrocarbons such as nonadecane and heneicosane. Genotypes with different floral characteristics and scent composition were identified compared to the oil-bearing population cultured in the region. It has been understood that genotypes with a higher number of flowers per plant and higher duration of flowering should be given priority for high flower yield.

Keywords: *Rosa damascena* Mill., cobalt 60, floral properties, HS-SPME, GC-MS

INTRODUCTION

The oil-bearing rose (*Rosa damascena* Mill.) is the most precious species for the perfume and cosmetic industry with its unique smell among 200 roses spreading all over the world (Guenther, 1952). Its aromatic extracts such as rose oil and rose water obtained by hydro-distillation, rose concrete and rose absolute obtained by solvent extraction of fresh rose flowers are highly valuable and expensive base materials for these industries (Lawrance, 1991). It is a natural hybrid between *Rosa gallica* L. and *Rosa phoenicia* Boiss, which are the species growing naturally in Turkey (Gudin, 2000). Oil-bearing rose is cultured in many countries in the world, especially in Turkey, Bulgaria, Iran and India. Oil-bearing rose as an agro-industrial product has been cultivated in the Lakes Region of Turkey (Isparta, Burdur, Denizli and Afyonkarahisar provinces) since the last quarter of the 19th century. In recent years, the production area of oil-bearing rose is about 30 thousand hectares and annual fresh flower production is over 12 thousand tons in the rose valley of Turkey.

Essential or volatile oils are mainly stored and secreted in the pink petal leaves of the rose flower. The odor molecules of the essential oil come from monoterpenes, sesquiterpenes, aromatic alcohols, oxides, ethers, esters and aldehydes (Dudareva and Pichersky, 2000). Each molecule together with others contribute to the formation of the characteristic rose smell (Başer, 1992). Despite great efforts, genes and enzymes responsible for the biosynthesis of odor molecules in roses are not fully enlightened (Cherri-Martin et al., 2007). However, it is believed that the main monoterpene alcohols such as geraniol, citronellol and nerol are synthesized from geranyl pyrophosphate in the same biosynthesis pathway (Oka et al., 1999). Since the international standards (ISO 9842:2003) for the characteristic odor composition of *Rosa damascena* volatile oil have been established, the present

odor composition of Bulgarian and Turkish rose oils should be protected. For this reason, the new varieties are expected to have the same or similar odor compositions as the classical rose odor composition (Rusanov et al., 2009; Baydar et al., 2016).

The molecular analyses with RAPD, AFLP, and SSR markers show that industrial oil-bearing roses ($2n=4x=28$) under cultivation in Turkey and Bulgaria have little to no genetic diversity among individuals and populations and therefore contain many genetically identical accessions (Ağaoğlu et al., 2000; Göktürk Baydar et al., 2004; Rusanov et al., 2005). Since oil-bearing roses have been propagated vegetatively for hundreds of years, the current roses on the rose valleys of Turkey and Bulgaria are most probably clonal progenitors of the first planted oil-bearing roses which have been maintaining their primitive features to date. On the other hand, the phenotypic homogeneity caused by continuous vegetative reproduction makes it possible to produce the rose oil with international standards (Rusanov et al., 2009). So far, superior or outstanding types of oil-bearing rose have not been selected through breeding in Turkey due to the lack of genetic variation among the plants under culture. Finally, Baydar and his colleagues applied Cobalt-60 gamma ray to the mature seeds in order to create genetic variation and select the outstanding types of oil-bearing rose. Also open- or self-pollinating flowers of oil-bearing rose show a high degree of outcrossing and therefore lead to a high degree of heterozygosity in the alleles or genes associated with important floral characteristics and scent molecules. So the seed propagation of clonally-propagated *R. damascena* yields a change in the genetic segregation of the alleles especially related with the petal colors and petal numbers (Baydar et al., 2016).

The ultimate goals of this research were to determine the flower and scent characteristics of *R. damascena* genotypes derived from both seed propagation and Cobalt-60 gamma ray induction for breeding and agro-industrial potentials.

MATERIAL AND METHODS

In the present research carried out in 2016, a total of 27 genotypes (9 genotypes grown by the seeds with no gamma ray irradiation coded as Rd-M0, 9 mutants grown by the seeds treated with gamma ray irradiation dose of 100 Gray coded as Rd-M100, and 9 mutants grown by the seeds with gamma ray irradiation dose of 200 Gray coded as Rd-M200) differing in petal color and petal number were used as material (Table 1). As control, *Rosa damascena* Mill. ($2n=4x=28$) genotype that locally grown and vegetatively propagated was used and coded as Rd-Isparta (pink colours and 30 rose petal leaves). The individual plant of each genotype had been planted with 1.5 m within rows and 3 m between rows with drip irrigation applied to the experimental field (an altitude of 1,050 m) at University of Applied Sciences Isparta in March 2010. In the flowering season of the year 2016, it was determined first and last flowering dates, flowering day numbers, petal color, minolta color values, flower number per plant, petal number per flower, anther number per flower, stigma number per flower, pedicel-hypanthium hairiness (hairy or hairless), flower diameter (cm), flower weight (g) per flower and petal ratio (%), the part of petal weight in the total fresh weight of a whole flower) in all genotypes with 6 years old. Petal color was assessed on recently opened flowers and measured by a portable colorimeter (Model CR-300, Minolta Camera, Ltd., Osaka, Japan) used to obtain L* (represents brightness and darkness), a* (represents greenness and redness), and b* (represents blueness and yellowness) values (Schmitzer et al., 2010).

Volatile oil was isolated by hydro-distillation using a Clevenger-type apparatus and analysed by gas chromatography/mass spectrometry (GC-MS) for volatile oil compounds. Also the scent compounds of the fresh flowers were detected by using headspace solid phase microextraction (HS-SPME) combined with gas chromatography/mass spectrometry (GC-MS) according to the methods practiced by Baydar et al. (2016) and Erbaş and Baydar (2016). The flowers subjected to SPME analyses were collected around 8:00 a.m. from flowers at stages 5 or 6 (inner and outer whorls open, reproductive organs visible), according to the proposed stages of *R. damascena* flower development as suggested by Rusanov et al. (2011).

RESULTS AND DISCUSSION

Floral Characteristics

Flowering day number, petal color and Minolta color values of oil-bearing rose genotypes were given in Table 1. During the flowering season of the year 2016, the classical oil roses (Rd-Isparta as control genotype) began to bloom on May 17 and ended on July 14 with a flowering period of 29 days. However, the oil rose mutants and genotypes were flowered between May 9 (Rd-M200-34) and June 21 (Rd-M0-54), 44 days between flowering head and flowering end (Table 1). The earliest flowering genotype was Rd-M200-34 on 9 May, while the latest flowering genotype was Rd-M200-17 on 7 June. The expansion of the flowering season is an important agricultural value in terms of prolongation of the campaign period as well as increasing the total flower yield. In general, those with a low number of petals (single-layered flowers with 5-7 petals) tend to bloom earlier than those with a higher number of petals (multi-layered flowers with over 25 petals). It was also observed that the flowering season of those with a low number of petals was shorter. This finding was important to show that there were close relationships between number of petals and flowering duration. On the other hand, the flowers of single-layered genotypes were very light and had lower odor densities. In addition, their petal leaves were not suitable for hand collection because they were swallowed very quickly.

Table 1. Flowering day number, petal color and Minolta color values of oil-bearing rose genotypes.

Genotypes	Flowering day numbers	Petal color	Minolta color values		
			L* value	a* value	b* value
Rd-M0-19	21	Pink	56.9	45.5	-12.4
Rd-M0-20	30	Pink	70.3	28.4	-7.7
Rd-M0-51	25	Dark pink	50.7	44.7	-11.9
Rd-M0-52	31	White	81.1	6.0	5.6
Rd-M0-54	33	White	87.5	6.8	-1.6
Rd-M0-61	22	White	81.7	8.2	-0.8
Rd-M0-66	28	Pink	61.1	42.9	-8.5
Rd-M0-67	31	Light pink	76.0	20.0	-5.6
Rd-M0-75	18	Pink	73.1	24.7	-5.1
Rd-M100-9	24	Light pink	79.1	15.4	-4.4
Rd-M100-15	15	Pink	71.6	29.4	-9.9
Rd-M100-28/1	32	Beyaz	85.1	2.7	6.7
Rd-M100-33	15	Pink	77.6	20.7	-9.2
Rd-M100-44	25	Pink	72.6	27.1	-9.5
Rd-M100-68	19	Pink	69.5	25.1	-6.5
Rd-M100-100	15	White	79.8	11.0	-0.8
Rd-M100-104	31	Dark pink	53.4	51.1	-10.1
Rd-M100-105	12	Pink	70.0	28.9	-7.7
Rd-M200-4	24	Pink	60.9	40.4	-9.7
Rd-M200-5	24	White	89.2	4.7	0.7
Rd-M200-10	21	Pink	58.6	39.3	-12.6
Rd-M200-17	6	Pink	68.1	19.6	-7.5
Rd-M200-21	24	Pink	62.3	42.8	-5.6
Rd-M200-25	16	Light pink	81.4	14.4	-5.1
Rd-M200-28	14	Pink	71.1	25.1	-6.5
Rd-M200-31	20	Pink	74.3	22.8	-6.6
Rd-M200-34	36	White	83.8	7.4	3.2
Rd-Isparta (Control)	29	Pink	58.6	45.3	-11.5

The coloration of rose flowers is mainly caused by the accumulation of anthocyanins such as pelargonidin and cyanidin in the petal cells (Schmitzer et al., 2010). Roses lack blue/violet flower colours owing to the deficiency of F3'5'H and therefore lack the B-ring-trihydroxylated anthocyanins based upon delphinidin (Tanaka et al., 2009). While the petal color of the classical Isparta oil-bearing rose were pink, the petal color of the genotypes changed from white to dark pink. White petal color was occurred in the Rd-M0 group (52, 54 and 61 codes), Rd-M100 group (28/1 and 100 codes) and Rd-M200 group (5 and 34 codes). The genotypes outside them gave petal colors ranging from light

pink to dark pink. L^* and b^* values decreased, and a^* values increased when the color darkened from white to dark pink (Table 1). Karami et al. (2012) found a high positive correlation ($r_{\text{Sq Linear}} = 0.812$) between essential oil content and anthocyanin concentration which can be used as an essential oil quantity index in *R. damascena*. It has been reported by Nedkov et al. (2009) that the essential oil content of white rose (*Rosa alba*) is lower than that of pink rose (*Rosa damascena*).

Petal number, anther number, stigma number and pedicel-hypanthium hairiness of oil-bearing rose genotypes were given in Table 2. The numbers of petal, anther and stigma in the flowers of Rd-Isparta population was 31.1, 77.1 and 39.6, respectively. In the genotypes, these numbers were between 5.1 and 85.7, 0.0 and 122.8, and 23.5 and 48.8, respectively (Table 2). While Rd-M200-10 and Rd-M200-25 genotypes formed too many petals over 75, they did not form an anther. It was understood that genotypes with more petals had fewer anthers, and genotypes with more anthers had fewer stigmas. The important and negative relationship ($r = -0.85^{**}$) between the number of petals and the number of anthers was detected (not tabulated). This may be the result of the homeotic functions of MADS-box function genes such as A, B and C. As known in this model, the formation of the floral organs is by three sets of functional genes from the MADS-box gene family as A (sepal and petal formation), B (petal and stamen formation), and C (stamen and carpel formation) that are expressed in certain regions of the developing flower (Causier et al., 2010).

Table 2. Petal number, anther number, sigma number and pedicel-hypanthium hairiness of oil-bearing rose genotypes.

Genotypes	Petal number per flower	Anther number per flower	Stigma number per flower	pedicel-hypanthium hairiness
Rd-M0-19	27.8±1.3	59.8±4.0	48.3±3.5	Hairy
Rd-M0-20	62.8±6.3	0.3±0.2	47.0±5.8	Hairy
Rd-M0-51	28.3±1.4	82.7±2.7	47.4±2.1	Hairy
Rd-M0-52	60.5±5.5	35.5±14.5	27.0±6.0	Hairy
Rd-M0-54	32.0±1.4	65.5±2.2	33.8±2.1	Hairless
Rd-M0-61	32.0±3.0	78.5±4.5	36.0±2.0	Hairy
Rd-M0-66	44.1±4.9	68.4±6.9	33.5±1.1	Hairless
Rd-M0-67	30.1±1.8	88.8±4.3	45.7±1.8	Hairy
Rd-M0-75	37.4±3.5	61.4±7.9	39.4±1.6	Hairy
Rd-M100-9	18.2±1.0	64.0±1.7	23.5±1.1	Hairy
Rd-M100-15	28.0±0.0	66.0±1.1	27.0±0.9	Hairy
Rd-M100-28/1	61.3±2.7	32.6±1.4	43.2±2.7	Hairy
Rd-M100-33	24.6±1.1	54.2±13.2	30.4±2.3	Hairy
Rd-M100-44	50.3±4.9	89.0±2.6	39.7±2.3	Hairy
Rd-M100-68	40.2±2.5	49.5±2.3	28.6±1.1	Hairy
Rd-M100-100	42.0±3.8	46.0±8.7	33.3±6.4	Hairy
Rd-M100-104	5.1±0.2	97.7±3.9	34.0±2.5	Hairy
Rd-M100-105	30.0±1.0	59.5±3.0	32.8±1.1	Hairy
Rd-M200-4	47.5±2.9	45.6±10.1	48.8±3.8	Hairless
Rd-M200-5	48.0±7.0	72.0±2.9	45.0±3.0	Hairless
Rd-M200-10	85.7±6.6	0.0±0.0	38.5±2.7	Hairy
Rd-M200-17	32.5±1.5	86.0±4.0	38.0±2.0	Hairy
Rd-M200-21	7.5±0.5	122.8±6.0	43.2±1.5	Hairy
Rd-M200-25	75.7±5.8	0.0±0.0	46.0±2.1	Hairless
Rd-M200-28	5.8±0.2	109.5±2.8	25.0±0.6	Hairy
Rd-M200-31	16.8±0.9	82.2±2.0	43.7±1.5	Hairy
Rd-M200-34	5.1±0.1	96.4±3.3	24.2±1.2	Hairless
Rd-Isparta (Control)	31.1±0.8	77.1±3.6	39.6±1.8	Hairy

Hairiness of the hypanthium and the pedicel is a characteristic of rose flowers. In the present study, the majority of the genotypes including Rd-Isparta population had a hairy hypanthium and a hairy pedicel, while 6 of them coded as Rd-M0-54, Rd-M0-66, Rd-M200-4, Rd-M200-5, Rd-M200-25 and Rd-M200-34 were hairless or naked (Table 2). It was a significant observation that the

genotypes with hairless (naked) hypanthia, pedicels and sepal leaves were less fragrant, but more resistant to rose aphid (*Macrosiphum rosae* L.).

Flower yield, flower number, flower weight, flower diameter and petal ratio of oil-bearing rose genotypes were given in Table 3. In the Rd-Isparta population, the flower yield per plant was 276.9 g, the flower number per plant was 147, the flower weight per plant was 1.88 g, the diameter of flower was 5.8 cm and the petal ratio was 70.0%. Genotypes Rd-M0-54, Rd-M0-67 and Rd-M200-4 gave more flower yields (951.5, 498.4 and 312.6 g per plant, respectively) and flower number (685, 308 and 160 units per plant, respectively). It was noted that the flower yield per plant showed a significant and positive correlation with the number of flowers per plant ($r = 0.98^{**}$) and the duration of flowering ($r = 0.45^{*}$) (not tabulated). The unit flower weight ranged from 0.63 g (Rd-M200-34) to 2.26 g (Rd-M0-20), and the flower diameter ranged from 3.29 cm (Rd-M200-10) to 7.20 cm (Rd-M0-61). The petal ratio between the genotypes ranged from 40.1% (Rd-M100-104) to 79.1% (Rd-M200-5). In general, the petal ratio of genotypes with higher petal number, petal diameter and flower weight was found higher (Table 3). There was a significant and positive relationship ($r = 0.50^{**}$) between the petal number and petal ratio (not tabulated).

Table 3. Flower yield, flower number, flower weight, flower diameter and petal ratio of oil-bearing rose genotypes.

Genotypes	Flower yield (g per plant)	Flower number per plant	Flower weight (g per flower)	Flower diameter (cm)	Petal ratio (%)
Rd-M0-19	65.1	34	1.94	5.8±0.2	63.7
Rd-M0-20	136.0	60	2.26	3.6±0.3	57.7
Rd-M0-51	131.4	85	1.55	5.1±0.2	60.0
Rd-M0-52	12.5	6	2.08	5.6±0.2	55.7
Rd-M0-54	951.5	685	1.39	4.5±0.3	67.1
Rd-M0-61	27.5	19	1.45	7.2±0.4	59.3
Rd-M0-66	85.9	39	2.20	5.4±0.1	67.1
Rd-M0-67	498.4	308	1.62	4.6±0.2	59.8
Rd-M0-75	47.2	24	1.97	4.0±0.2	44.3
Rd-M100-9	128.5	105	1.22	4.9±0.1	51.8
Rd-M100-15	64.8	34	1.90	3.4±0.0	48.5
Rd-M100-28/1	183.3	109	1.68	4.3±0.3	62.8
Rd-M100-33	80.9	44	1.84	5.1±0.6	70.3
Rd-100-44	37.9	19	2.00	6.4±0.4	71.1
Rd-M100-68	160.3	90	1.78	5.2±0.3	57.3
Rd-M100-100	20.8	10	2.08	6.3±0.2	70.3
Rd-M100-104	128.6	139	0.93	4.4±0.3	40.1
Rd-M100-105	18.8	13	1.45	4.3±0.1	53.9
Rd-M200-4	312.6	160	1.95	5.5±0.2	61.2
Rd-M200-5	10.7	6	1.78	6.0±0.1	79.1
Rd-M200-10	157.5	77	2.05	3.2±0.3	63.4
Rd-M200-17	8.0	5	1.59	4.1±0.7	68.0
Rd-M200-21	164.5	147	1.12	4.4±0.3	50.9
Rd-M200-25	8.8	4	2.21	4.3±0.7	66.4
Rd-M200-28	23.1	31	0.74	5.3±0.1	49.0
Rd-M200-31	188.5	116	1.63	5.6±0.1	53.8
Rd-M200-34	23.9	38	0.63	4.3±0.2	45.0
Rd-Isparta	276.9	147	1.88	5.8±0.1	70.0

Scent Characteristics

The main scent compounds (> 0.5%) of volatile oils isolated by HS-SPME and GC-MS technique from fresh flowers of oil rose genotypes were presented in Table 4. In the fresh flowers of Rd-Isparta population, the compounds were also both detected by GC-MS in the distilled rose oil and by HS-SPME. The most important compounds of rose oil are monoterpene alcohols (oleoptens) such as citronellol (24.18%) and geraniol (20.99%) and paraffinic hydrocarbons (stearoptens) such as

nonadecane (15.05%) and heneicosan (7.96), while the most important natural floral compounds of rose flower is phenethyl alcohol (43.25%) (Table 4). For this reason, the volatile oil (rose oil) obtained by distillation often does not represent the floral fragrance. The most important reason why rose oil is poor in phenylethyl alcohol compared to rose flower is that a large part of phenylethyl alcohol which is an easily soluble aromatic alcohol in water is passed to pulp water during distillation and rose water at the end of cohobation. 2-Phenylethyl β -D-glucopyranoside, the main source of phenylethyl alcohol, can be easily hydrolyzed during the distillation process when it is found in abundant amounts of essential oils of the rose flowers (Eikani et al., 2005).

It has been shown that floral scent compositions of the oil-bearing rose genotypes were different from than that of the Rd-Isparta population (parental genotype) (Table 4). For example the Rd-M0-19, Rd-M0-20, Rd-M0-67, Rd-M100-68 and Rd-M200-25 genotypes were found to contain phenylethyl alcohol at a higher rate than the parental genotype. In the genotypes, phenylethyl alcohol ranged from 16.02% (Rd-M200-10) to 67.26% (Rd-M0-19), citronellol from 0.00% (Rd-M100-9) to 39.14% (Rd-M0-66), geraniol from 0.00% (Rd-M0-19, Rd-M0-54, Rd-M100-15, Rd-M100-44) to 31.64% (Rd-M0-51), and nerol from 0.00% (Rd-M0-66, Rd-M100-105, Rd-M200-21) to 29.33% (Rd-M0-54).

Table 4. Scent compounds and their ratios (%) by HS-SPME/GC-MS in the fresh flowers of *R. damascena* genotypes.

Compounds	Rd-Isparta (Population)		Genotypes		
	Rose oil (GC/MS)	Rose flower (HS-SPME)	Rd-M0 (9 units) (HS-SPME)	Rd-M100 (9 units) (HS-SPME)	Rd-M200 (9 units) (HS-SPME)
Ethanol	-	-	0.00-0.50	0.04-0.17	0.00-2.51
Hexanol	-	0.25	0.00-2.19	0.00-0.31	0.00-2.15
α -Pinene	-	0.55	0.00-1.62	0.00-0.80	0.00-0.45
β -Myrcene	-	2.10	0.00-5.20	1.01-11.52	0.00-5.53
Limonene	-	0.30	0.08-3.00	0.33-2.97	0.36-4.22
Benzyl alcohol	-	1.15	0.16-2.24	0.36-2.32	0.00-1.84
Ocimene	-	0.51	0.00-2.28	0.00-2.81	0.48-3.39
α -Terpinolene	-	0.05	0.00-0.35	0.06-0.35	0.00-0.47
Rose-furan	-	0.10	0.00-2.72	0.00-0.61	0.00-0.78
Linalool	0.30	0.18	0.00-0.70	0.00-0.33	-
Phenylethyl alcohol	1.48	43.25	21.18-67.26	16.58-49.24	16.02-49.32
Rose oxide	-	0.08	0.00-0.10	0.00-0.11	-
Allo-Ocimene	-	-	0.00-0.74	0.00-1.90	0.00-0.90
Citronellol	24.18	16.55	7.32-39.14	0.00-27.85	5.07-34.59
Linalyl formate	-	4.47	0.00-8.36	-	0.00-14.45
Z-Citral	0.11	-	0.00-4.79	0.00-2.62	0.00-2.68
Nerol	3.20	1.48	0.00-29.33	0.00-22.03	0.00-16.46
Geraniol	20.99	10.30	0.00-31.64	0.00-27.94	1.01-30.89
Geranial	-	2.39	0.00-2.61	-	0.00-3.65
E-Citral	-	-	0.00-6.94	0.00-24.66	0.00-4.47
Citronellyl acetate	-	1.43	0.00-1.14	0.00-1.20	0.08-1.70
Neryl acetate	0.71	2.70	0.00-4.38	0.00-1.35	0.00-1.55
Geranyl acetate	-	-	0.00-4.51	0.00-3.56	0.00-1.12
Methyl eugenol	0.32	0.90	0.00-0.76	0.00-0.52	0.00-0.17
β -Caryophyllene	1.23	0.20	0.00-0.26	0.00-0.09	0.00-0.25
α -Guaiane	0.53	-	-	-	-
α -Humulene	0.60	-	-	-	-
Germacrene D	1.74	-	-	-	-
Penta/Hexa/Heptadecane	7.51	1.81	0.00-4.72	0.00-9.61	0.00-2.94
Farnesol	6.40	-	-	-	-
Octa/Nonadeca(e)ne	15.05	4.22	0.00-4.05	0.00-8.88	0.00-4.82
Eicosene/Eicosane	4.52	0.21	0.00-0.06	0.00-0.06	0.00-4.02
Heneicosane	7.96	0.40	0.00-0.09	0.00-0.30	0.00-0.45
Tri/Tetra/Pentacosane	1.45	-	-	-	-
Total compound number (>%0.05)	32	37	37-49	42-53	39-48

One of the most important quality-related ingredients in rose oil is methyl eugenol which is an undesirable phenylpropane for perfumes and cosmetics due to possible allergic and toxic effects (Harris, 2002). Baydar and Erbaş (2016) determined that the main source of methyl eugenol is stamens (male organs) while the main source of phenylethyl alcohol is petals (petals) of oil-

bearing rose flowers. This compound was not detected in 15 of the 27 rose genotypes (Table 4). However, the rate of methyl eugenol in Turkish rose oil is often over 2% (sometimes 4%). In particular, methyl eugenol content in the rose oils is higher in the rose oils distilled from the late gathered and over fermented flowers (Baydar et al., 2008, Rusanov et al., 2012).

CONCLUSION

In oil-bearing rose industry, since raw materials (rose flowers) constitute the main parts of the production costs in the basic distillation and extraction products such as rose oil, concrete and absolute, it is important to develop novel oil-bearing rose varieties with high flower yield and high volatile oil yield to reduce production costs. However, unfortunately, there are no registered varieties of oil-bearing rose in the country. Due to the lack of sufficient genetic variation among the oil-bearing roses under culture, it was not possible to select superior or outstanding types by passing through a certain breeding process in Turkey. This research was found to be useful in revealing that seed propagation and mutation breeding can be easily utilized as a way to create an effective genetic variations for the floral and scent characteristics. In conclusion, oil-bearing rose seeds with or without gamma ray irradiation would be a huge selection source to breed novel varieties.

ACKNOWLEDGEMENTS

The data presented in this paper is obtained from the project financially supported by TÜBİTAK (Scientific Research Project No: 116O047).

REFERENCES

- Ağaoğlu, Y.S., Ergül, A., Göktürk Baydar, N., 2000. Molecular analysis of genetic diversity oil rose (*Rosa damascena* Mill.) grown Isparta (Turkey) region. *Biotech Biotech Eq*, 14: 16-18.
- Başer, K.H.C., 1992. Turkish rose oil. *Perfumer and Flavorist*, 17: 45-52.
- Baydar, H., Erbaş, S., 2016. Comparison of volatile compounds using headspace solid-phase microextraction (HS-SPME) and conventional hydro-distillation method in oil-bearing rose (*Rosa damascena* Mill.). *Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 20(1): 27-36.
- Baydar, H., Erbaş, S., Kazaz, S., 2016. Variations in floral characteristics and scent composition and the breeding potential in seed-derived oil-bearing roses (*Rosa damascena* Mill.). *Tr J Agri Forest*, 40: 560-569.
- Baydar, H., Schulz, H., Kruger, H., Erbaş, S., Kineci, S., 2008. Influences of fermentation time, hydro-distillation time and fractions on essential oil composition of damask rose (*Rosa damascena* Mill.). *JEOB*, 11(3): 224-232.
- Causier, B., Schwarz-Sommer, Z., Davies, B., 2010. Floral organ identity: 20 years of ABCs. *Semin Cell Dev Biol* 21:73-79.
- Cherry, M., Jullien, F., Heizmann, P., Baudino, S., 2007. Fragrance heritability in hybrid tea roses. *Sci Hort*, 113: 177-181.
- Dudareva, N., Pichersky, E., 2000. Biochemical and molecular genetic aspects of floral scents. *Plant Physiol*, 122: 627-633.
- Eikani, M.H., Golmohammad, F., Rowshanzamir, S., Mirza, M., 2005. Recovery of water-soluble constituents of rose oil using simultaneous distillation-extraction. *Flav Frag J*, 20(6): 555-558.
- Erbaş, S., Baydar, H., 2016. Variation in scent compounds of oil-bearing rose (*Rosa damascena* Mill.) produced by headspace solid phase microextraction, hydrodistillation and solvent extraction. *Rec Natural Prod*, 10(5): 555-565.
- Göktürk Baydar, N., Baydar, H., Debener, T., 2004. Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. *J Biotechnol*, 111, 263-267.
- Gudin, S., 2000. Rose: Genetics and Breeding. *Plant Breed Reviews*, 17: 159-189.
- Guenther, E. 1952. Oil of rose. In: *The essential oils*. Kreiger, Florida, USA. pp. 3-48.
- Harris, B., 2002. Methyl eugenol-the current bete noir of aromatherapy. *Int J Aromatherapy*, 12(4): 193-201.
- Karami, A., Khosh-Khui, M., Salehi, H., Saharkhiz, J., 2012. Correlation between anthocyanin and essential oil content of Damask rose (*Rosa damascena* Mill.). *Med Pl By-Prod*, 1: 3-6.
- Lawrence, B.M., 1991. Progress in essential oils: rose oil and extracts. *Perfumer and Flavorist*, 16: 43-77.
- Nedkov, N., Dobрева, A., Kovacheva, N., Bardarov, V., Velcheva, A., 2009. Bulgarian rose oil of white oil-bearing rose. *BJAS*, 15(4): 318-322.
- Oka, N., Ohishi, H., Hatona, T., Hornberger, M., Sakata, K., Watanabe, N., 1999. Aroma evolution during flower opening in *Rosa damascena* Mill. *Zeitschrift für Naturforschung*, 54: 889-895.

- Rusanov, K., Kovacheva, N., Atanassov, A., Atanassov, I., 2009. *Rosa damascena* – genetics of a complex allotetraploid species and perspectives for molecular breeding. Biotechnol Biotech Eq 23: 594-596.
- Rusanov, K., Kovacheva, N., Rusanova, M., Atanassov, I., 2011. Traditional *Rosa damascena* flower harvesting practices evaluated through GC/MS metabolite profiling of flower volatiles. Food Chem, 129: 1851-1859.
- Rusanov, K., Kovacheva, N., Rusanova, M., Atanassov, I., 2012. Reducing methyl eugenol content in *rosa damascena* mill. rose oil by changing the traditional rose flower harvesting practices. J Euro Food Res Tech, 234: 921-926.
- Rusanov, K., Kovacheva, N., Vosman, B., Zhang, L., Rajapakse, S., Atanassov, A., Atanassov, I., 2005. Microsatellite analysis of *Rosa damascena* Mill. accessions reveals genetic similarity between genotypes used for rose oil production and old Damask rose varieties. Theor Appl Genet, 111: 804-809.
- Schmitzer, V., Veberic, R., Osterc, G., Stampar, F., 2010. Color and phenolic content changes during flower development in groundcover rose. J Amer Soc Horti Sci, 135 (3): 195-202.
- Tanaka, Y., Sasaki, N., Ohmiya, A., 2008. Biosynthesis of plant pigments. Plant J, 54: 733-749.

DETERMINATION OF SOME MORPHOLOGICAL, AGRONOMIC AND QUALITY PROPERTIES OF A CLONES WHICH GENERATED FROM A SELECTED POPULATION OF ISTANBUL OREGANO (*Origanum vulgare* subsp. *hirtum* (Link) Iestwaart)*

Olcaç ARABACI¹, Emine BAYRAM², Uğur TAN¹, Çiğdem SÖNMEZ²

¹Field Crops Department, Agricultural Faculty, Adnan Menderes University, 09100, Aydın, Turkey

²Field Crops Department, Agricultural Faculty, Ege University, 35100 İzmir, Turkey

Corresponding author email: oarabaci@adu.edu.tr

Abstract

Medicinal and aromatic plants are traditionally cultivated and collected from the flora for use and trade. Oregano is one of the plant that was introduced to agriculture in recent years by aid of natural genetic resources. Cultivation of oregano has began 20 years ago in our country with 10 acres. According to data of 2017 oregano has 121.472 acres of cultivation area, 14.477 tonne production and 119 kg/da yield. Turkey has earned 57 million dollars from exports in 2017 for oregano. Izmir oregano (*Origanum onites* L.) is one of the cultivated oregano species in Turkey. Izmir oregano varieties (Ceylan-2002 and Tayşi-2002) were developed with clone selection method and registered. However, despite that Istanbul oregano is a cultivated plant, there is no registered varieties of Istanbul oregano. The seeds of Istanbul oregano used in cultivation are obtained from the flora and carry the characteristics of population. This study was carried out to select genotypes with high rate of drug yield and essential oil ratio among different populations (A, B, C, D, E) of Istanbul oregano (*Origanum vulgare* subsp. *hirtum* (Link) Iestwaart). with clonal selection method. In this article, A population was characterized by examining some morphological, agronomic and quality characteristics of clones A. Values of A clones (plant height, drug leaf yield, essential oil ratio, essential oil yield) were found between 18-51.4 cm, 2.3-88.2 g/parcel, 1.4-7.50 and 0.03-5.64 g/plot, respectively. It has been determined that carvacrol was the most important component of the essential oils content.

Keywords: Istanbul oregano, *Origanum vulgare* subsp. *hirtum*, selection, yield, essential oil component.

*This study is part of the project of Tubitak 113O285.

INTRODUCTION

Medicinal and aromatic plants are traditionally cultivated and collected from the flora for use and trade. Oregano is one of the plant that was introduced to agriculture in recent years by aid of natural genetic resources. Cultivation of oregano has began 20 years ago in our country with 10 acres. According to data of 2017 oregano has 121.472 acres of cultivation area, 14.477 tonne production and 119 kg/da yield. Turkey has earned 57 million dollars from exports in 2017 for oregano (Anonim, 2018).

Oregano species of Turkey were *Origanum*, *Thymbra*, *Thymus*, *Coridothymus* and *Satureja* (Başer, 2001). The most valuable species of oregano were *Origanum* genus due to economic and agricultural importance in Turkey. Turkey has exported some *Origanum* species. These species were *Origanum onites* (bilyalı kekik, İzmir kekiği), *Origanum minutiflorum* (Sütçüler kekiği, yayla kekiği, Tota kekiği), *Origanum majorana* (beyaz kekik, Alanya kekiği), *Origanum syriacum* var. *bevanii* (dağ kekiği, Suriye kekiği, İsrail kekiği) and *Origanum vulgare* subsp. *hirtum* (İstanbul kekiği, kara kekik) (Başer, 2000; Kırimer et. al., 2003; Sarıhan et. al., 2006).

Izmir oregano (*Origanum onites* L.) is one of the cultivated oregano species in Turkey. Izmir oregano varieties (Ceylan-2002 and Tayşi-2002) were developed with clone selection method and registered. However, despite that Istanbul oregano is a cultivated plant, there is no registered varieties of Istanbul oregano. The seeds of Istanbul oregano used in cultivation are obtained from the flora and carry the characteristics of population.

This study was carried out to select genotypes with high rate of drug yield and essential oil ratio among different populations (A, B, C, D, E) of Istanbul oregano (*Origanum vulgare* subsp. *hirtum* (Link) Iestwaart). with clonal selection method. In this study, A population was characterized by examining some morphological, agronomic and quality characteristics of clones A.

MATERIAL AND METHODS

The field studies of the research were carried out in the research area of Adnan Menderes University, Faculty of Agriculture Research, Department of Field Crops Department. Cultivation of A populations was carried out for two years in 2013-2015 and A clones were cultivated in 2016. In this study, the research material was formed from seeds of the plants those cultivated in the ecological conditions of Aydın, which were grown naturally in the region of the southern of the 586 altitude south of the Kazdağ Mountains, with the coordinates (542066-4422267). Cultural processes and analyzes of A population and A clones were given in Table 1.

Table 1. Cultural processes and analyzes of A population and A clones on *Origanum vulgare* subsp. *hirtum* (İstanbul oregano)

Cultivation and Analysis	A Population	A Clones
Sowing	06/12/2013	Cutting; 02-09/12/2015
Emergence	17/12/2013	-
Planting	702 seedlings; 02/04/2014	129 seedlings x10=1290 seedlings; 05/04/2016, 09/04/2016
Planting Standard	50x40 cm	50x40 cm
Harvest	10-18/07/2014, 10/09/14, 04-07/11/14 24/06/2015	27-29/06/2016
Essential Oil Analysis	ADU and Ege Agr. Fac. Crops Sci. Dep.	ADU and Ege Agr. Fac. Crops Sci. Dep.
Essential Oil Rate	Wichtl (1971)	Wichtl (1971)
Essential Oil Component Analysis	SDU Lab., model GC-17 A GCMS-QP505	SHIMADZU JAPAN
Essential Oil Component Rate	Library NIST, WILEY	
Statistical analysis	SPSS and TARIST	

RESULTS AND DISCUSSION

Single plants were selected to generate A clones (129 genotype) according to fresh herbage yield (g/plant), drug herbage yield (g/plant), drug leaf yield (g/plant) and essential oil ratio (%) values from A population which grown during the vegetation periods of 2014 and 2015. These genotypes were replicated to plant on the field with vegetative organs. Some statistical values of the features obtained from the harvests of plants during flowering period were given in Table 2 and frequency distributions in Figure 1.

In terms of average values of A population, plant height 31.6 cm, fresh herbage yield 159.5 g/parcel, drug herbage yield 67.4 g/parcel, drug leaf yield 36.9 g/parcel, leaf-stem ratio 58.9%, essential oil ratio 4.65% and essential oil yield 1.779 g/parcel were determined. Plant height of clones 18-51.4 cm, fresh herbage yield 4-548.6 g/parcel, drug herbage yield 3-205.7 g/parcel, drug leaf yield 2.3-88.2 g/parcel, leaf-stem ratio 25.6-80.4%, essential oil ratio %1.4-7.5 and essential oil yield ranged between 0.03-5.64 g/parcel (Table 2).

When frequency distributions of A clones (Figure 1) were examined, it is observed that the values are concentrated within certain limits in general. According to this, the values of the plant height was 24.7-38.0 cm in %69.8 of plant, fresh herbage yield 4.0-221.8 g/parcel in 72.7% of plants, drug herbage yield 3.0-104.3 g/parcel in 78.3% of plants, drug leaf yield 2.3-53.83 g/parcel in 77.5% of plants, leaf-stem ratio %47.52-74.92 in %76.7 of plants, essential oil ratio, 3.23-6.27% in 83.7% of plants and essential oil yield 0.03-3.39 g/parcel in 93%.

Table 2. Statistical Values of Some Investigated features of A populations and A clones in *Origanum vulgare* subsp. *hirtum* (İstanbul Oregano)

Features	Total Plant	Minimum	Maximum	Mean	Variance	Standard deviation	S $\bar{x}$	CV
Plant Height (cm)	129	18.000	51.400	31.590	44.870	6.699	0.590	21.205
Fresh Herb Yield (g/parcel)	129	4.000	548.600	159.547	12869.298	113.443	9.988	71.103
Drog herb Yield (g/parcel)	129	3.000	205.700	67.388	2073.646	45.537	4.009	67.574
Drog leaf yield (g/parcel)	129	2.300	88.200	36.930	446.673	21.135	1.861	57.229
Leaf/stem rate (%)	129	25.600	80.400	58.867	130.557	11.426	1.006	19.410
Essential Oil Rate (%)	129	1.400	7.500	4.650	1.323	1.150	0.101	24.739
Essential Oil yield (g/parcel)	129	0.030	5.640	1.779	1.317	1.148	0.101	64.528

20 clones were selected to form B clones that were above average or maximum in terms of fresh herbage yield, drug herbage yield, drug leaf yield and essential oil ratio features.

A population; A-25, A-91, A-97, A-210, A-212, A-320, A-331, A-386, A-400, A-418, A-419, A-420, A-495, A-500, A-501, A-524, A-628, A-629, A-630, A-637.

The essential oil components of the 20 clones, selected from A clones are presented in Table 3. The main component of the essential oil was determined as carvacrol. In addition to the main component, it was determined that γ -terpinene, p -cymene and β -myrcene form the main odor components. The number of components varied between 7-23. The main component, carvacrol ratio was determined between 76.66-94.20%, the lowest ratio was found in the A-386 clone and the highest ratio was A-630 clone. A clones of A population were determined as carvacrol type clones. It was noted that the ratio of thymol in these clones were very low and the values varied between 0.03-0.33%, whereas the A-320, A-500 and A-629 clones contained no thymol. In the study, the ratio of γ -terpinene was reported between 1.58 and 13.26%. The lowest value for γ -terpinene was obtained by clone A-630 and the highest value was obtained from A-386 clone. Also it was found that some clones have not contain γ -terpinen at all.

The main components p -cymene and β -myrcene were determined between 2.30-5.32%, and 1.07-2.18% respectively, however it was found that some other clones not include these components. The distribution of the essential oil components of the selected A clones were given in Table 3.

The mean and maximum values of the plant height obtained from A clones were found similar with Zukauska (2001) 47-53 cm and Ahmad et. al. (2008) between 25.45-55.58 cm. As a result of cultivation of *Origanum vulgare* subsp. *hirtum* Zukauska (2001) reported that the first year 113-1050 g/m², second year 125-2135 g/m² and third year 516-2414 g/m² fresh herbage yield were obtained. It was determined that the values we obtained in the study were within the values reported by the researcher.

It has been reported by Leto and Salomone (1996) that the yield of drug herbage yield ranged between 105-110 g/plant in the native population. Our findings from the study were above the lower limit and below the upper limit of reported by the researchers.

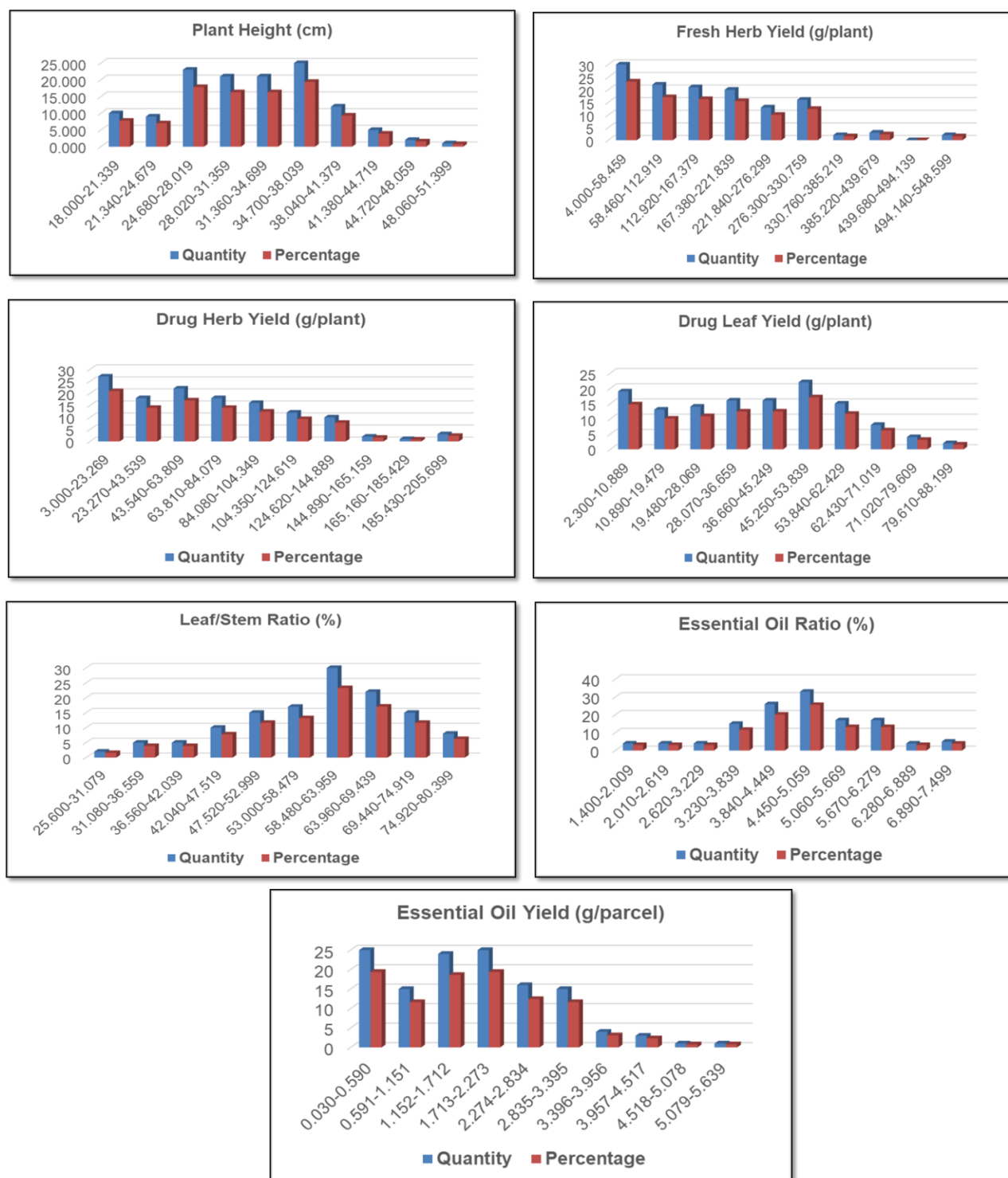


Fig. 1. Frequency distributions of A clones.

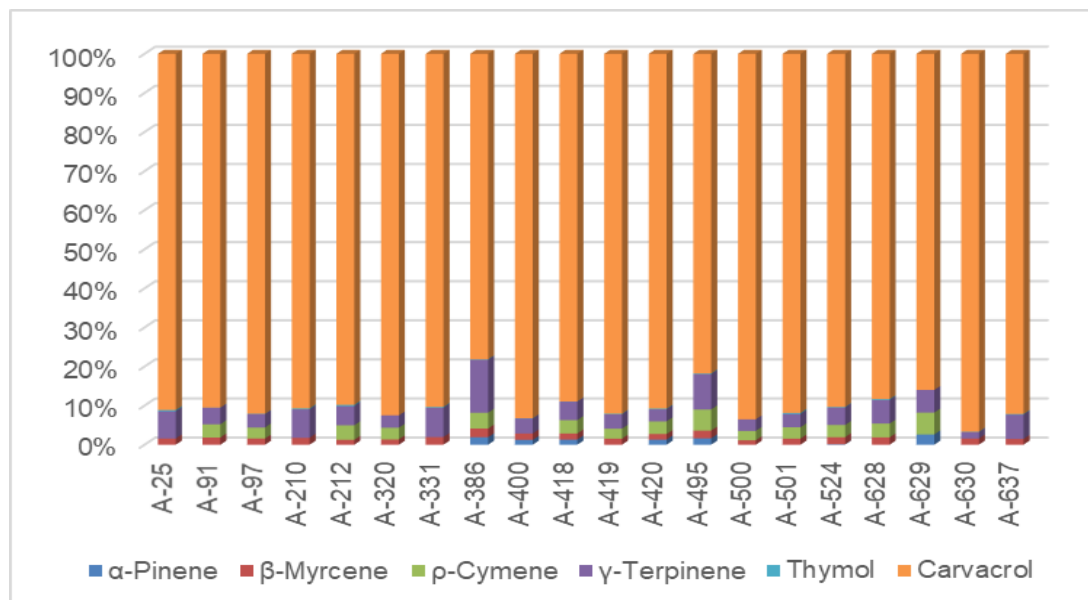
Tınmaz et. al. (2002), Baytop (1999), Başer (2001), Zeybek and Zeybek (2002) and Oflaz et. al. (2004) reported essential oil ratio of *Origanum vulgare* subsp. *hirtum* as 1-6.1%, 4-5%, 1-7%, 5% and 3.6-4.4% respectively. It was noted that our results regarding the ratio of essential oil were found above the upper limits reported by the researchers.

Said-Al Ahl et. al. (2009) stated that the essential oil yield changed between 0.070-0.072 ml/plant. Our findings were found similar to the values of the researchers.

Table 3. The distribution of the essential oil components of the selected A clones.

Clone No	Number of Components (total)	Name of Components and Ratios (%)						Essential Oil Ratios (%)
		α -Pinene	β -Myrcene	ρ -Cymene	γ -Terpinene	Thymol	Carvacrol	
A-25	12	-	1.52	-	6.63	0.33	86.93	6.10
A-91	23	-	1.75	3.20	4.00	0.03	85.68	5.40
A-97	22	-	1.50	2.68	3.25	0.08	87.39	5.80
A-210*	16	-	1.66	-	6.79	0.24	84.90	6.90
A-212	9	-	1.22	3.64	4.77	0.31	87.73	6.80
A-320	7	-	1.33	2.91	3.09	-	90.28	5.00
A-331	9	-	1.89	-	7.20	0.23	87.02	6.00
A-386	10	1.88	2.18	3.97	13.26	0.15	76.66	7.00
A-400	9	1.22	1.57	-	3.74	0.08	90.95	5.40
A-418	10	1.28	1.56	3.36	4.66	0.06	87.72	5.70
A-419	13	-	1.50	2.53	3.57	0.15	90.06	7.50
A-420	9	1.30	1.39	3.19	3.04	0.18	89.46	6.30
A-495	9	1.60	1.93	5.32	8.87	0.17	80.22	5.00
A-500	18	-	1.07	2.30	2.79	-	88.88	6.30
A-501	15	-	1.48	2.81	3.23	0.26	88.08	5.50
A-524	20	0.09	1.75	2.98	4.27	0.14	86.44	5.80
A-628	15	-	1.83	3.38	5.82	0.22	85.24	7.20
A-629**	9	2.51	-	5.32	5.49	-	81.75	7.10
A-630	9	-	1.56	-	1.58	0.10	94.20	6.00
A-637	12	-	1.42	-	6.08	0.15	89.74	6.50

*The A-210 clone also contains 3.11% limonene.

The clone A-629 also contains 2.11% 2- β -pinene.Fig. 2.** Essential oil composition of selected A clones

Sezik et. al. (1993) reported that *Origanum vulgare* subsp *hirtum* is rich in carvacrol with ratio of 70.47%. Also the main component that carvacrol ratio is reported by Baser (2001) between 23-80%. Veres et. al. (2003) were determined main components of *Origanum vulgare* subsp. *hirtum* as carvacrol (76.4%), γ -terpinene (6.69%), thymol (0.23%) and ρ -cymene (4.7%). Karik et. al. (2007) were identified *Origanum vulgare* subsp. *hirtum* components as γ -terpinene (0.46-10.7%), ρ -cymene (2.60-16.1%), linalool (0.02-1.18%), thymol (0.24-82.3%) and carvacrol (1.88-88.6%). Our results show that our values are above the maximum values reported by all previous researchers.

CONCLUSION

Selected genotypes (A and B clones) from among the studied population, wide variation were determined. Considering the minimum and maximum values of the variation, it can be concluded that genetic variability is high for breeding.

ACKNOWLEDGEMENTS

This study is part of the project that financially supported by Tubitak (Project No: 113O285).

REFERENCES

- Ahmad, S., Koukab, S., Islam, M., Ahmad, K., Aslan, S., Aminullah, Gill, A. 2008. "Germplasm evaluation of medicinal and aromatic plants in highland balochistan", Pakistan, Pak. J. Bot., 40 (4): 1473-1479.
- Anonim, 2018. TUIK, www.tuik.gov.tr (Erişim Tarihi 12.09.2018).
- Başer, K. H. C. 2000. "Uçucu Yağların Parlak Geleceği", TAB (Tıbbi ve Aromatik Bitkiler Bülteni) 15: 20-33.
- Başer, K. H. C. 2001. "Her Derde Deva Bitki Kekik", Bilim ve Teknik, Mayıs, 74-77.
- Baytop, T. 1999. Türkiye'de Bitkileri ile Tedavi Geçmişte ve Bugün (İlaveli ikinci baskı) Nobel Tıp Kitapevleri, s480.
- Karık, Ü., Tınmaz, A.B., Kürkcüoğlu, M., Başer, K.H.C., Tümen, G. 2007. "İstanbul Kekiki (*Origanum vulgare* subsp. *hirtum*) Populasyonlarında Farklı Biçim Zamanlarının Verim ve Kaliteye Etkileri", Bahçe 36 (1-2): 37-48.
- Kırimer, N., Boybağ, I., Sargın, N., Arslan, O. 2003. Ticareti Yapılan *Origanum* Türlerinin Doğadaki Durumu. TÜBİTAK projesi sonuç raporu, Proje no: TBAG-Ç.SEK/10 (101T012), s16.
- Leto C., Salamone A. 1996. Bio-agronomical behaviour in Sicilian *Origanum* ecotypes. Promoting the conservation and use of under utilized and neglected crops. 14. Oregano. Proceedings of the IPGRI International Workshop on Oregano. 8-12 May. CIHEAM, Valenzano, Bari, Italy.
- Oflaz, S., Kürkcüoğlu, M., Başer, K.H.C. 2002. *Origanum onites* ve *Origanum vulgare* subsp. *hirtum* Üzerinde Farmakognozik Araştırmalar, 14. Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 29-31 Mayıs, Eskişehir, Eds. K.H.C. Başer ve N. Kırimer, ISBN 975-94077-28.
- Said-Al Ahl, H.A.H., Omer, E.A., Naguib, N.Y. 2009. "Effect of water stress and nitrogen fertilizer on herb and essential oil of oregano", Int. Agrophysics, 23, 269-275.
- Sarıhan, E.O., İpek, A., Arslan, N., Gürbüz, B. 2006. "Farklı Sıra Arası ve Sıra Üzeri Mesafelerinin Kekik (*Origanum vulgare* var. *hirtum*)'de Verim ve Verim Öğeleri Üzerine Etkisi", Tarım Bilimleri Dergisi Ankara Üniversitesi Ziraat Fakültesi 12 (3) 246-251.
- Sezik, E., Tümen, G., Kırimer, N., Özek, T., Baser, K. H.C. 1993. "Essential Oil Composition of Four *Origanum vulgare* subspecies of Anatolian Origin" Journal of Essential Oil Research Vol. 5, Iss. 4.
- Tınmaz, A. B., Kürkcüoğlu, M., Başer, K. H. C., Öztürk, M., 2002. Marmara Bölgesindeki İstanbul Kekiki (*Origanum vulgare* subsp. *hirtum*) Populasyonlarının Kalite Özelliklerinin Belirlenmesi, 14. Bitkisel İlaç Hammaddeleri Toplantısı, Bildiriler, 29-31.05.2002, Eskişehir. ISBN: 9759407728.
- Veres, K., Varga, E., Dobos, Á., Hajdú, Z., Máthé, I., Németh, E., Szabo, K. 2003. "Investigation of the composition and stability of the essential oils of *Origanum vulgare* ssp. *vulgare* L. and *O. vulgare* ssp. *hirtum* (Link) letswaart." Chromatographia, ISSN: 0009-5893, Volume 57, Issue 1, pp 95-98.
- Zeybek, U. Zeybek, N. 2002. Farmasötik Botanik, Kapalı Tohumlu Bitkiler (Angiospermae) Sistematiki ve Önemli Maddeleri, Ege Üniv. Basımevi, Bornova
- Zukauska, I. 2001. "Agrobotanical features and productivity of wild and cultivated populations of *Origanum vulgare*", Botany, Acta Biol. Daugavp. 1(2), 107-109p.

DETERMINATION OF THE ESSENTIAL OIL COMPOSITION ON FLOWER AND SEED OF DILL (*Anethum graveolens* L.) UNDER THE ŞANLIURFA CONDITIONS

Abdulhabip ÖZEL¹, Orçun ÇINAR², İslim KOŞAR³

¹Harran University, Faculty of Agriculture, Department of Crop Sciences, Şanlıurfa, Turkey.

²Batı Akdeniz Agricultural Research Institute (BAARI), Antalya, Turkey.

³GAP Agricultural Research Institute, Şanlıurfa, Turkey

Corresponding author email: hozel@harran.edu.tr

Abstract

This study aimed to determine the essential oil ratio and composition of flower and seed of dill (Anethum graveolens L.). It was conducted at the research field of Faculty of Agriculture at Harran University, at 2013-2014 growing season. The dill plants were harvested the full bloom period and the seeds were matured in the main umbel and dried, after that essential oil ratio and essential oil components were determined. The essential oil ratio was determined 2.07% in dry flower and 3.55% in seed. The results of CS-MS showed that were obtained 19 compounds in the dry flower's essential oil and 14 compounds in the essential oil of seed. The dry flower's essential oil macro components were determined as α -Phellandrene (33.09%), Dill ether (21.42%) and Limonene (18.05%) while the seed essential oil macro components were Carvone (47.75%) and Limonene (38.07%).

Keywords: Anethum graveolens, volatile oils components, flower, seed

INTRODUCTION

Dill (*Anethum graveolens* L.) is an annual plant belongs to *Apiaceae* family and is grown for its herb and seeds in many parts of the world. While fresh dill herbs are used as green vegetable, its dried herbs and seeds are used as spice. The volatile oils extracted from its herbs and seeds are used in many fields of industry, including food, cosmetics and pharmaceuticals. Some studies showed that essential oils of dill induced antimicrobial, anti inflammatory, analgesic, gastric mucosal protective, hyperlipidaemic and many other effects (Hosseinzadeh et al., 2002; Stavri and Gibbons, 2005; Hajhashemi and Abbasi, 2008; Valady et al., 2010). The well known properties of dill from the traditional medicine, such as carminative, stomachic and diuretic have been reported (Amin and Sleem, 2007).

Dill is important for its essential oils. The quality of dill volatile oil varies depending on the herb which is including the vegetative component ratio, generative component ratio and seed ratio. Both amount and composition of essential oils in leaves, stems, umbel (inflorescence) and seed of dill show differences. Therefore, intensity of different parts of the plant (i.e. leaf, stem and umbel) on the herb may also affect both concentration and composition of the volatile oils of dill. So that, the chemical composition of dill volatile oil varies depending on the plant parts (Radulescu et al., 2010). This is determined the area of use of the dill essential oil. There are three types of the dill essential oil in the world trade (fresh herb essential oil containing less than 30-32% Carvone, a high-carvone dill herb oil and seed essential oil) is marketed. The characteristic of dill essential oil is largely dependent on the Carvone and Phellandrene content. If the Carvone ratio is less than 35%, the herba oil is dominant (Callan et al., 2007). Carvone and Limonene are the main ingredients in dill seed oil. Badoc and Lamarti (1991) were divided the three chemotype essential oils of dill seed essential oils according to Carvone and Myristicin ratios and Dillapiole whether contained or not. They reported that the dill, the European type, was not contained the Dillapiole component.

Many factors including genotype, location, ecological factors and growth techniques affect the quantity and composition of essential oils of dill (Salmasi et al., 2006; Frąszczak, 2009; Hashemzadeh et al., 2013; Khamssi, 2014; Rana and Blazquez, 2014). Some of the researches have been conducted

on the morphogenetic and ontogenetic (Huopalahti and Linko, 1983; Porter et al., 1983; Radulescu et al., 2010) variability of volatile oils of dill. Radulescu et al. (2010) reported that were identified the major compound constituted α -Phellandrene in the leaves and Limonene in the flowers, on the naturally dried plant parts.

The present study was conducted to determine the essential oil ratio and composition of flower and seed of dill (*Anethum graveolens* L.) under the Şanlıurfa conditions.

MATERIAL AND METHODS

Plant Material

The dill plants used in the present study were obtained from the three dill plots grown in the Harran Plain (South-eastern Anatolia, Turkey) in 2013-2014 growing session. The dill seeds were sowing in December 5, 2013 with 30 cm inter-row space and the seed level of 10 kg ha⁻¹. At the stage of sowing, nitrogen (50 kg ha⁻¹) and phosphorus (50 kg ha⁻¹) were fertilized, and at the beginning of April, nitrogen (50 kg ha⁻¹) was applied to all plots. The whole dill plants were harvested at the flowering stage (Jun 20, 2014) and the full maturity (Jun 30, 2014) period of seeds in the main umbel. After harvest dill plant samples were separated in flowers and seeds. Each sample were naturally dried in shadow and kept at 4°C until analysis.

Essential Oil Isolation

Each dry sample (50 g) was hydrodistilled with 500 ml water in a Clevenger type apparatus for 3h. The essential oil was stored in a dark glass bottle and kept at 4°C until analysis.

Gas Chromatography-Mass Spectrometry Analysis

Dill essential oil was analyzed by GC-MS using an Agilent 7890A equipped with an electron impact quadrupole, Agilent 5975C mass spectrometer detector. The electron ionisation energy was 70 eV, scan range 35-450 amu and scan rate 1 scans sec.⁻¹. The GC column was used an HP Innowax Capillary (A fused silica capillary column 5% phenyl-poly-dimethyl-siloxane), film thickness of 0.25 μ m, a length of 60 m, and internal diameter 0.25 mm. The carrier gas was helium with a flow rate 0.8 ml min.⁻¹. Inlet temperature was 250 °C. The GC oven temperature program was used as follows; 60 °C initial temperatures, hold for 10 min, raised at 4 °C min⁻¹ to 220 °C and finally hold at 220 °C for 10 min. A 1% (v/v) solution of the sample in n-Hexane was prepared and 1 μ l was injected using a 40:1 split ratio.

The identification of compounds was performed by comparing their mass spectra with data from Adams, US National Institute of Standards and Technology (NIST, USA) and WILEY 1996 Ed. mass spectra library. The identification of compounds was also based on the Kovats retention indices.

RESULTS AND DISCUSSION

The rates of dill essential oils were determined 2.07% in dry flower and 3.55% in seed. The seed essential oil contents, as expected, were higher than the flower essential oil contents. These findings were in harmony with Radulescu et al. (2010) who demonstrated that the ratio of essential oils in the seeds highest than the flowers.

The composition (expressed as percentage from total area) of essential oils of flower and seed of dill plants is listed in Table 1.

Table 1. The composition of essential oils at dry flower and seed dill plants.

R.T.	Compounds	Dry Flower	Seed
11.523	α -Pinene	0.97	-
11.590	α -Thujene	0.16	-
15.495	Myrcene	0.53	0.17
15.736	α -Phellandrene	33.09	0.98
16.721	Limonene	18.05	38.07
17.055	β -Phellandrene	4.57	0.20
17.233	1,3,8-p-Menthatriene	0.13	0.64
18.905	Cymene	1.68	0.13
19.239	α -Terpinolene	0.10	-
23.943	cis-Limonene oxide	-	0.18
25.891	Dill ether	21.42	0.21
28.200	trans-Dihydrocarvone	0.53	0.36
28.464	Bicyclo[3.2.1]-3-octen-6-on,4,7-dimethyl (endo)	0.27	-
28.694	Dihydrocarvone	4.01	6.49
31.179	Carvone	7.21	47.75
31.849	iso-Dihydrocarveol	0.15	0.22
32.411	neoiso-Dihydrocarveol	0.21	0.33
32.821	p-Menth-1-en-9-yl acetate	0.17	-
40.306	Cinnamyl alcohol	0.45	-
42.650	Myristicin	5.90	4.23
Total Area (%)		99.60	99.96

The results of CS-MS showed that the essential oil from *Anethum graveolens* were identified 19 compounds in the dry flower, adding up to 99.60% of the total area. The essential oil from *Anethum graveolens* were identified 14 compounds in the seed, adding up to 99.96% of the total area.

The major constituent the essential oil of dill flowers were detected the α -Phellandrene (33.09%) as the main component, Dill ether (21.42%), Limonene (18.05%) and Myristicin (5.90%). However, in the seed essential oil were detected the Carvone (47.75%) as the main component, Limonene (38.07%), Dihydrocarvone (6.49%) and Myristicin (4.23%) (Table 1). It is seen that the ratio of macro components were changed to depending on the flower and seed materials. In general, Carvone, Limonene and Dihydrocarvone ratios in the seed essential oils were increased compared to the flower essential oils. The α -Phellandrene, Dill ether, Myristicin and β -Phellandrene ratios were decreased compared to the flower essential oils.

Radulescu et al. (2010) were reported that in the flower essential oil Limonene ratio was 33.22% and the Dill Ether ratio 22.00%, while in the seed essential oil the Carvone ratio 75.21% of and the Limonene ratio 21.56%. Our findings were different from those reported. These differences may be due to differences in genetic diversity and ecological conditions. Thus, Bado Badoc and Lamarti (1991) were reported that the composition of the volatile oil varies depending on the cultivars. In addition, some researchers were reported that the composition of the dill essential oil was varied depending on the plant density (Callan et al., 2007) and light quality (Hälvä et al., 1992; Frąszczak, 2009).

CONCLUSIONS

The seed essential oil contents were higher than the flower essential oil contents. The chemical composition of dill essential oil was varied depending on the flower and seed. The main component of the flower essential oils was determined as α -Phellandrene, Dill ether and Limonene. The main

component of the seed essential oils was determined as Carvone and Limonene. The Dillapiole component of the dill flower and seed essential oils was not detected.

REFERENCES

- Amin W.M., Sleem A.A., 2007. Chemical and biological study of aerial parts of dill (*Anethum graveolens* L.). Egyptian Journal of Biomedical Sciences, 23(1):73-90.
- Badoc A., Lamarti A., 1991. A chemotaxonomic evaluation of *Anethum graveolens* L. (dill) of various origins. Journal of Essential Oil Research, 3(4): 269-278.
- Callan N.W., Johnson D.L., Westcott M.P., Welty L.E., 2007. Herb and oil composition of dill (*Anethum graveolens* L.): Effects of crop maturity and plant density. Industrial Crops and Products, 25(3): 282-287.
- Fraşczak B., 2009. Comparison of eight dill cultivars grown in containers in different light conditions. Herba Polonica, 55(3):76-83.
- Hajhashemi V., Abbasi N., 2008. Hypolipidemic activity of *Anethum graveolens* in rats. Phytother Res., 22: 372-375.
- Hälvä S., Craker L.E., Simon J.E., Charles D.J., 1992. Light quality, Growth and Essential Oil in Dill (*Anethum graveolens* L.). Journal of Herb, Spices and Medicinal Plants, 1(1-2): 59-69.
- Hashemzadeh F., Mirshekari B., Khoei F.R., Yarnia M., Tarinejad A., 2013. Effect of bio and chemical fertilizers on seed yield and its components of dill (*Anethum graveolens*). Journal of Medicinal Plants Research, 7(3): 111-117.
- Hosseinzadeh H., Karemi G.R., Ameri M., 2002. Effects of *Anethum graveolens* L. Seed extracts on experimental gastric irritation models in mice. J. BMC Pharmacol., 2(1): 21-30.
- Huopalahti R., Linko R.R., 1983. Composition and Content of Aroma Compounds in Dill, *Anethum graveolens* L., at Three Different Growth Stages. J. Agric. Food Chem., 31(3): 331-333.
- Khamssi N.N., 2014. Influence of water deficit on seed yield and essential oil content of dill (*Anethum graveolens* L.). International Journal of Plant, Animal and Environmental Sciences, 4(2): 297-300.
- Porter N.G., Shaw M.L., Shaw G.J., Ellingham P.J., 1983. Content and composition of dill herb oil in the whole plant and the different plant parts during crop development. New Zealand Journal of Agricultural Research, 26: 119-127.
- Rădulescu V., Popescu M.L., Ilieş D., 2010. Chemical composition of the volatile oil from different plant parts of *Anethum graveolens* L. (*Umbelliferae*) cultivated in Romania. Farmacia, 58(5): 594-600.
- Rana V.S., Blazquez M.A., 2014. Chemical Composition of the Essential Oil of *Anethum graveolens* Aerial Parts. Journal of Essential Oil Bearing Plants, 17(6): 1219-1223.
- Salmasi S.Z., Golezani K.G., Moghbeli S., 2006. Effect of Sowing Date and Limited Irrigation on the Seed Yield and Quality of Dill (*Anethum graveolens* L.). Turk J Agric For., 30:281-286.
- Stavri M., Gibbons S., 2005. The antimycobacterial constituents of Dill (*Anethum graveolens*). Phytother Res., 19: 938-941.
- Valady A., Nasri S., Abbasi N., 2010. Anti-inflammatory and analgesic effects of hydroalcoholic extract from the seed of *Anethum graveolens* L. J Med Plants, 9: 130-124.

AN ALTERNATIVE THERAPY FROM GREEN TEA IN PERIODONTAL DISEASE

Arzu BEKLEN¹

¹Department of Periodontology, Faculty of Dentistry, Eskişehir Osmangazi University, Eskişehir, Turkey

Corresponding author email: arzubeklen@ogu.edu.tr

Abstract

Periodontal disease is initiated by a microbial biofilm called dental plaque, which is a multilayered microbiological ecosystem that adheres to the surface of a tooth. The bacteria in dental plaque produce toxins or enzymes that can irritate the tissue around the teeth. Among these enzymes, matrix metalloproteinases (MMPs) are produced and released by the immune cells to destroy the supporting tissue around teeth. Natural products are one of the promising molecules for diminishing MMPs. In recent years, the health benefits of consuming green tea, like antibacterial effect is under investigation. Green tea polyphenols are mostly catechins, the most predominant being epigallocatechin-3-gallate (EGCG), which presents various beneficial pharmacological properties. In this study the effect of EGCG on the inhibition of MMP-2 on gingival fibroblast cells were evaluated. Gingival fibroblasts were first stimulated with tumor necrosis factor alpha. After washing they were resuspended in serum free medium using EGCG (5 µg/ml) and medium were analyzed by enzyme-linked immunosorbent assay. The results revealed that the cultured gingival fibroblasts significantly released less amount of MMP ($P < 0.05$). It is presented here that EGCG from green tea-acts as an alternative treatment option for periodontal disease.

Keywords: Periodontal disease, green tea, epigallocatechin-3-gallate.

INTRODUCTION

Periodontology deals with the tooth-supporting tissues, the periodontium. The periodontium is consisted of from four different tissue types, namely gingiva, periodontal ligaments, cement and bone (Figure 1). The periodontium is made up of these tissues that surround each tooth and anchor each tooth into the alveolar process (Latin: para = adjacent to; Greek: odus = tooth). Against the invading microorganisms, normal healthy gingival tissues form a protective barrier. Between health and disease there is a fine balanced host response. During the health the host response protects the tissue, whereas in the diseases situation the host response destroys the surrounding tissue. During the normal turn-over situation this balance and destruction situations are balanced. In case, the personal oral health maintenance is not done properly, dental plaque grows on the teeth and this dental plaque biofilms provide a shelter for microorganisms to grow and proliferate. The grown bacteria release bacterial products and consequently individual host response starts to function. If the plaque continues to grow, the surrounding soft tissue deepens and a periodontal pocket occurs around the tooth. In this newly formed space the anaerobic bacteria will have chance to grow and the bacteria population under the subepithelial area will be very much mixed. The host response of the tissue will shift to destructive level. The released bacterial products from this area will expand to epithelium and gingival epithelial cells will produce bioactive mediators and recruit different types of immune cells (Kinane & Lindhe 1997).

The destructive response reaches to underlying connective tissue which is characterized with a mixed inflammatory cells and enlargement. The main cell type of connective tissue is fibroblast. During the inflammation the fibroblasts produce biologically active mediators such as cytokine; TNF- α and matrix metalloproteinases. (Gemmell et al. 1997, Kinane & Lindhe 1997, Mahanonda & Pichyangkul 2007). Bacterial plaque products release an excessive production of inflammatory mediators, such as cytokines. TNF- alpha is a cytokine involved in systemic inflammation. TNF alpha has a strong effect as a immunomodulator.

After TNF- α is released, it causes the cells to produce matrix metalloproteinases (Papadakis & Targan 2000). The fibroblasts of the connective tissue are one of the cell types which also produce TNF- α and MMPs (Baud & Karin 2001).

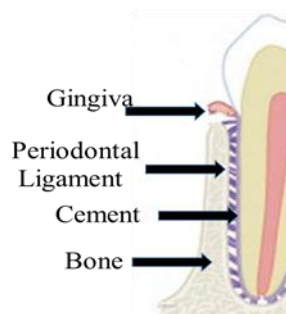


Fig.1. The schematic presentation of periodontal tissues.

Matrix metalloproteinases are a family of structurally related zinc-dependent enzymes. These enzymes play an essential role in physiological states such as tissue remodeling, morphogenesis and wound healing and also in pathological processes. During the destruction of periodontal tissues, the biological roles of the matrix metalloproteinases (MMPs) can be briefly described as to degrade the components of the extracellular matrix (ECM) and collagen (Sternlicht & Werb 2001). MMPs comprise a family of around 25 members that can be categorized into six groups, in which 3 main groups have been under the focus of periodontal research particularly (Figure 2).

Collagenases	Gelatinases	Stromelysins
MMP1	MMP2	MMP3
MMP8	MMP9	MMP10
MMP13		MMP11

Fig.2. Classification of three groups of matrix metalloproteinases.

In recent years, to get benefit from the functional foods as alternative preventive strategies have been under the focus. These studies focus on testing the antimicrobial properties of natural products, such as on periopathogenic bacteria (Ferrazzano et al. 2011). Among these natural compounds, the effect of epigallocatechin-3-gallate (EGCG), a type of tea catechin, received a great interest (Xu, Zhou, & Wu, 2012; Xu, Zhou, & Wu, 2011). EGCG is the most abundant tea catechin in green tea, in which approximately 65% of tea catechins are EGCG (Zaveri, 2006). It has been revealed that rinsing mouth with EGCG solutions presented milder plaque formation in patient groups (Hirasawa, Takada, & Otake, 2006).

Despite the tremendous information in the literature, there is less information about the effect of EGCG on gingival fibroblasts. In this study, I analyzed the effect of EGCG on MMP2 release from gingival fibroblasts.

MATERIAL AND METHODS

Cell Culture and Cytokine Stimulation

I isolated human gingival fibroblasts using an explant culture technique. The gingival fibroblasts were maintained in RPMI-1640 (Gibco BRL, Grand Island, NY, USA) containing 10% BS (BioWhittaker Inc., Walkersville, MD, USA) with 100 U/mL penicillin and 0.1 mg/mL streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA). The cells were incubated at 37° C in a 5% CO₂ atmosphere. After the cells reached to confluent the cells were subcultured at a ratio of 1:3.

Passages 2-4 were used for subsequent experiments. Following that the cells were stimulated with TNF- α (R&D Systems Inc., Minneapolis, MN, USA). The cells were washed three times. Then EGCG (5 μ g/ml) (Sigma-Aldrich, USA) was added to the experiment group. The culture medium was collected after 24 h of incubation. MMP-2 in the supernatants were determined using enzyme-linked immunoabsorbent assay (ELISA) kits.

Matrix Metalloproteinase-2 By Enzyme-Linked Immunosorbent Assay

The level of MMP-2 production was analyzed from gingival fibroblast cell culture supernatants. The ELISA was performed using commercial colorimetric sandwich ELISA kits in accordance with the manufacturer's instructions. Briefly, the standards and samples were pipetted into the wells (Quantikine, R&D Systems Inc., Minneapolis, MN, USA). Then an enzyme-linked biotinylated antibody specific for MMP-2 was added to the wells. After removing any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and the developed color was read at 450 nm.

Statistical Analysis

Statistical calculations were performed using the Prism data analysis program (GraphPad Software Inc., San Diego, CA, USA). One-way analysis of variance (ANOVA) was used to analyze the differences between conditioned cells and the controls. The results are presented as the mean and SEM. $P < 0.05$ was accepted as significant.

RESULTS AND DISCUSSION

The results revealed that TNF- α increased MMP-2 production. However, additional treatment with epigallocatechin-3-gallate significantly decreased MMP-2 concentration in the culture group ($P < 0.05$). (Figure 3).

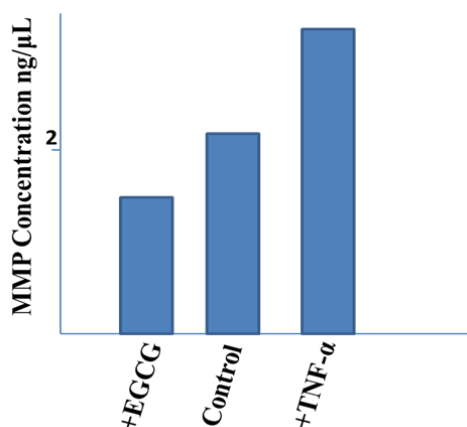


Fig. 3. TNF- α on induction of MMP-2 concentration in gingival fibroblast cultures.

Inflammatory disease of the periodontal tissues result in the destruction of gingival connective tissue, periodontal ligament and finally alveolar bone. Clinically, inflammation can be described as redness, swelling and bleeding upon probing. However, at cellular levels, the inflammatory process is defined by the release of a variety of cytokines (Page & Kornman 1997). Cytokines carry the information from one cell to another. After binding to their specific receptors TNF- α triggers signalling events on human gingival fibroblasts (Vilcek & Lee 1991; Kinane, 1992) and gingival soft tissue cell, namely fibroblasts, which finally mediates the synthesis of MMP-2. From the clinical point of view, after the entrance of bacteria itself, or the bacterial products or even the effect of other surrounding cells; the gingival fibroblasts produce TNF- α to start the molecular activation (Vilcek & Lee 1991).

Earlier studies showed how MMP-mediated mechanisms of periodontal tissue destruction occurs (Kinane, 1992). In my study upon stimulation with TNF-alpha, the fibroblasts reacted with releasing MMP-2, actually to protect itself. However, due to the destructive concentration of proinflammatory cytokine, the destructive enzyme MMP revealed more compared to the control groups. In the control group the meaning of released MMP amount can be defined as to control the inflammation in fibroblasts. However, with the addition of epigallocatechin-3-gallate, the antibacterial activity took the advantage and inflammation was diminished.

CONCLUSION

This study aimed to evaluate the effect of therapy with EGCG on proinflammatory TNF-alpha induced inflammation in gingival fibroblasts compared with non stimulated control groups, focusing on its antibacterial property.

ACKNOWLEDGEMENTS

The data presented in this paper is obtained from the original research work by me.

REFERENCES

- Baud V, Karin M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol* 2001; 11:372-377.
- Ferrazzano GF, Amato I, Ingenito A, Zarrelli A, Pinto G, & Pollio A. Plant polyphenols and their anti-cariogenic properties. *Molecules* 2011 16(2), 1486–1507.
- Hirasawa M, Takada K, & Otake S. Inhibition of acid production in dental plaque bacteria by green tea catechins. *Caries Research* 2006; 40(3), 265–270
- Gemmell E, Marshall RI, Seymour GJ. Cytokines and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. *Periodontol* 2000 1997; 14:112-143.
- Kinane DF, Lindhe J. Pathogenesis of periodontal disease. In: Lindhe J, ed. *Textbook of Periodontology*. Copenhagen, Denmark: Munksgaard; 1997.
- Papadakis KA, Targan SR. Tumor necrosis factor: biology and therapeutic inhibitors. *Gastroenterology* 2000;119:1148-1157.
- Momohara S, Okamoto H, Komiya K, Ikari K, Takeuchi M, Tomatsu T, Kamatani N. Matrix metalloproteinase 28/epilysin expression in cartilage from patients with rheumatoid arthritis and osteoarthritis *Arthritis Rheum* 2004 ; 50: 4074-4075.
- Page RC, Kornman KS. The pathogenesis of human periodontitis: an introduction. *Periodontol* 2000 1997; 14:9-11.
- Sternlicht MD, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; 17:463-516.
- Xu X, Zhou, XD, & Wu, C D. The tea catechin epigallocatechin gallate suppresses cariogenic virulence factors of *Streptococcus mutans*. *Antimicrobial Agents & Chemotherapy*. 2011. 55(3), 1229–1236.
- Xu X, Zhou, XD, & Wu, C D. Tea catechin epigallocatechin gallate inhibits *Streptococcus mutans* biofilm formation by suppressing *gtf* genes. *Archives of Oral Biology*.2012. 57(6), 678–683
- Vilcek J, Lee TH. Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. *J Biol Chem* 1991; 266:7313-7316.
- Zaveri NT. Green tea and its polyphenolic catechins: Medicinal uses in cancer and noncancer applications. *Life Sciences*. 2006 78(18), 2073–2080.

DETERMINATION OF THE TOTAL FLAVONOID SUBSTANCE CONTENT OF ROWANBERRY EXTRACTS

Burhan CEYLAN¹, Yeşim YEŞİLOĞLU²

¹Department of Pharmacognosy and Natural Products Chemistry, Institute of Health Sciences, BezmiAlem Foundation University, İstanbul, Turkey

²Department of Biochemistry, Faculty of Pharmacy, Trakya University, Edirne, Turkey

Corresponding author email: b.ceylan022@gmail.com

Abstract

Sorbus (rowanberry) *L.* is located in the genus *Rosaceae* family. In our country, there are 12 species and taxa which spread naturally. The most important of rowanberry (*Sorbus domestica L.*), maple-leaf rowanberry (*Sorbus torminalis L.*) and bird rowanberry (*Sorbus aucuparia L.*). In this study, total flavonoid content of water and acetone extracts of rowanberry (*Sorbus domestica L.*) was determined in various *in vitro* systems. This standard is used for the gallic acid and quercetin. From the equations of the graphs plotted between the absorbance and the concentration read out with the help of the standards prepared at different concentrations, the total flavonoids substance quantities of the samples were calculated as mg/g extract.

Keywords: Rowanberry, Flavonoid Substance Content, Antioxidant Activity

INTRODUCTION

Flavonoids are an important group of phenolic compounds. In the constructions, it contains the ring and the hydroxyl group. This ring is a phenyl ring and is formed on the C₆-C₃-C₆ flavon skeleton (Fig. 1), which is formed by combining with the propane chain. In addition, it has 15 carbon atoms in its structure [1].

Flavonoids, which appear to be white-yellow pigment in the plants, have been subjected to various classifications. These; flavon, flavonol, flavonane, dihydroflavonol, isoflavone, auron, chalcone and anthocyanidins. Flavanoid skeletons are shown in (Fig. 2).

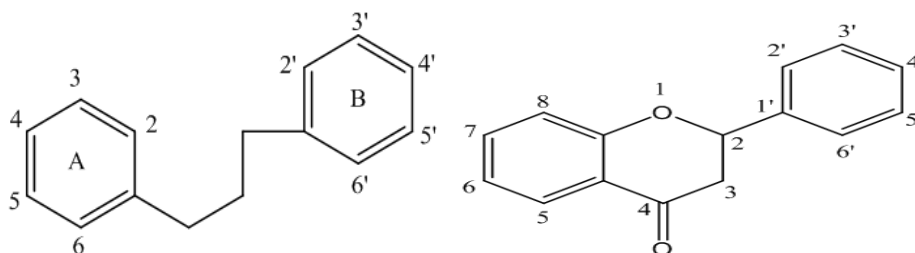


Fig. 1. General structure of C₆-C₃-C₆ system and flavonoids

Flavonoid are the most important agents in polyphenolics and are the chemicals found in the human food chain and in the plants. Flavonoids are much more toxic than plants and alkaloids in terms of their toxicity and diversity. Flavonoids have anti-allergic, anti-inflammatory, anti-microbial, anti-cancer and diarrheal activity [2].

There are also anti-viral effects. The antioxidant activities of flavonoids are higher than those of vitamin C and vitamin E. flavonoids also have effects on cardiovascular system diseases. Natural phenols (flavonoids) inhibit the enzyme topoisomerase and activity similar to chemotherapeutic anti-cancer drug (etoposide, doxorubicin) [3].

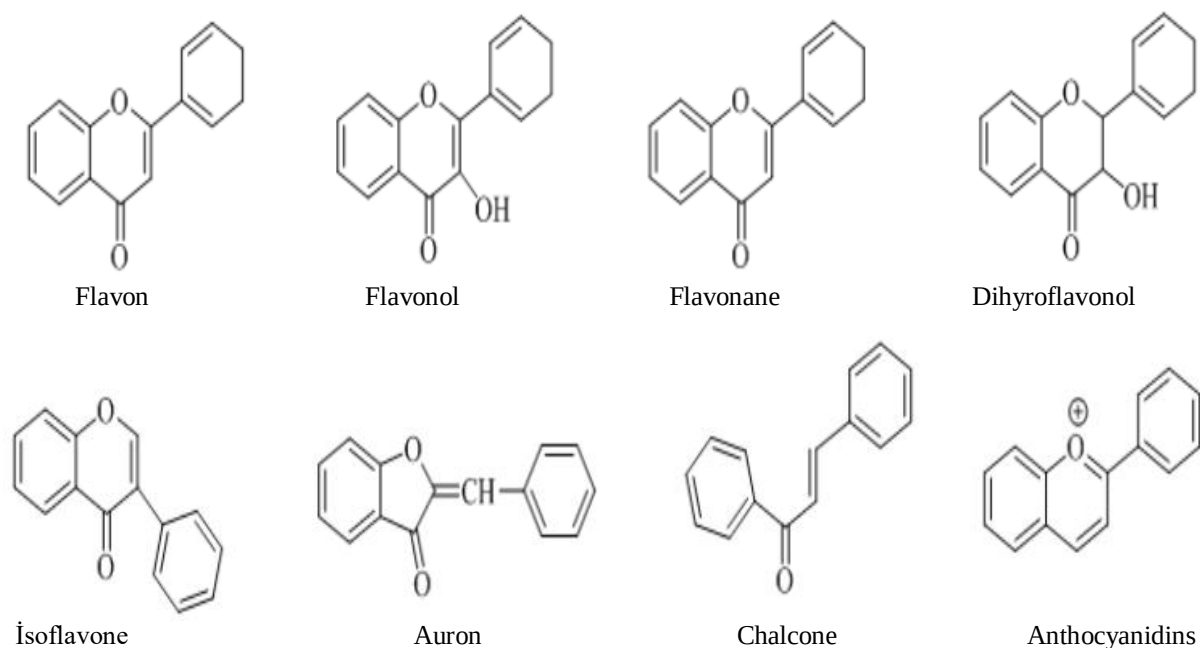


Fig. 2. Flavonoid skeletons.

Flavonoid containing plants; citrus fruits, strawberries, blueberries etc., ginkgo blob, red onion, garlic, red wine and cocoa. Especially grapefruit flavonoids from citrus fruits. Citrus flavonoid; hesperidin, quercetin, routine, tangeritine flavonol and flavon tangeritin [4].

Rowanberry fruit contains tannis, essential oils, organic acids, color pigments, phenolic acids and their derivatives. These are mainly; benzoic acid derivatives, cinnamic acid derivatives, ferulic acid and chlorogenic acid. In addition to these, there are flavonoids such as quercetin and routine [5].

Rowanberry nutritional value is high and is traditionally used as an antidiabetic agent. Fruits and leaves are used against infections (%5) and leaves (%5) due to their constipating effects against diabetes [6].

MATERIAL AND METHODS

The content of total flavonoid substances found in water and acetone extracts of rowanberry fruit and flower was determined according to the method applied by Zhishen et al. [7].

10 mL of water and acetone extracts were prepared the concentrations of 1000 µg/mL of rowanberry fruit and flower. 1 mL of %5 sodium nitrite was added for 6 minutes and 1 mL %10 aluminum nitrate was added to form the flavonoid-aluminum complex. After 6 minutes, 10 mL of %4,3 NaOH was added and the total volume was supplemented with distilled water to 25 mL. After 15 minutes at room temperature, the final solution was thoroughly mixed and the absorbance against the specimen at 510 nm was measured.

The same test was performed in gallic acid and quercetin at concentrations of 50-250 µg/mL used as standars. From equations drawn between absorbance and concentration readings, the total flavonoid sustance quantities of the samples were calculated as mg gallic acid/g extract and mg quercetin/g extract.

RESULTS AND DISCUSSION

Standard graphics were prepared using gallic acid and quercetin (fig. 3 and fig. 4). Using these graphs, the total flavonoid contents of the samples were calculated as the equivalents of mg gallic acid (mg GAE/g extract) and mg quercetin (mg QUE/g extract).

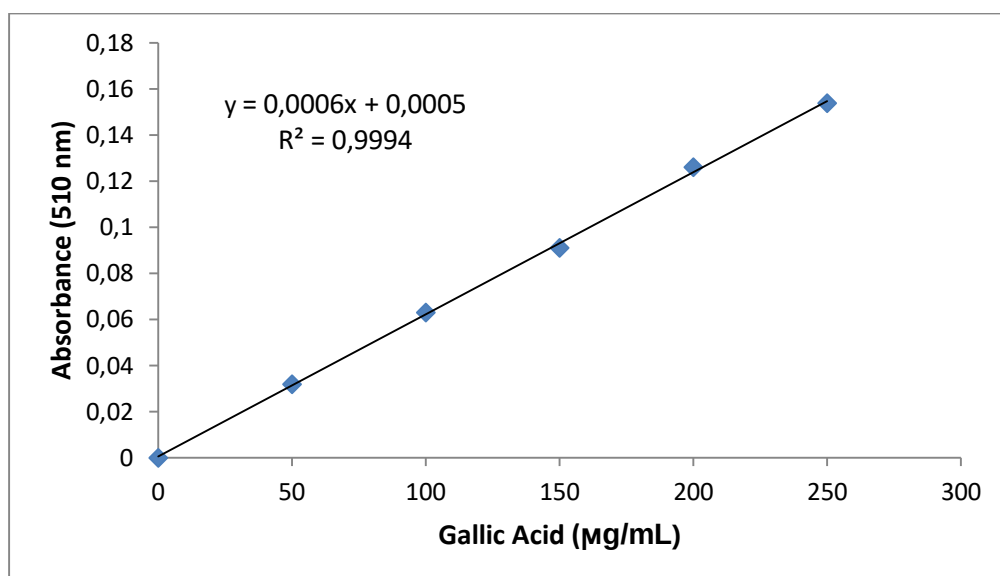


Fig. 3. Gallic acid standard graph.

The total flavonoid content in the fruit-water, flower-acetone and fruit-acetone extracts from the formula obtained from the standard graphic of gallic acid was calculated according to the following formula (R^2 : 0,9994).

$$\text{Absorbance}_{(510 \text{ nm})} = 0,0006[\text{Gallic acid}] + 0,0005$$

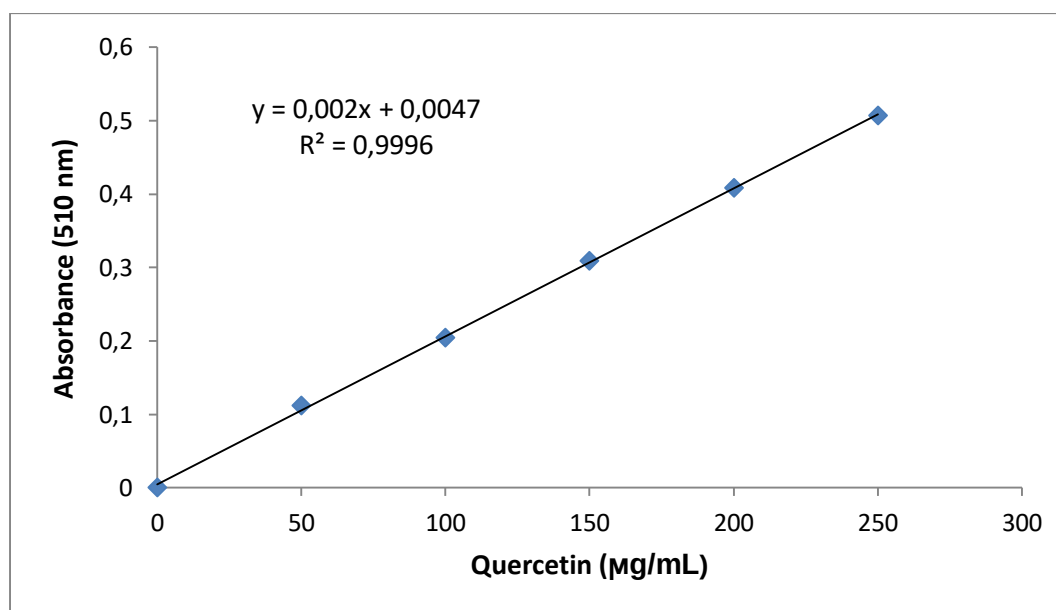


Fig. 4. Quercetin standard graph.

The total flavonoid content in the fruit-water, flower-acetone and fruit-acetone extracts from the quercetin standard from was calculated according to the following formula (R^2 : 0,9996).

$$\text{Absorbance}_{(510 \text{ nm})} = 0,002[\text{Quercetin}] + 0,0047$$

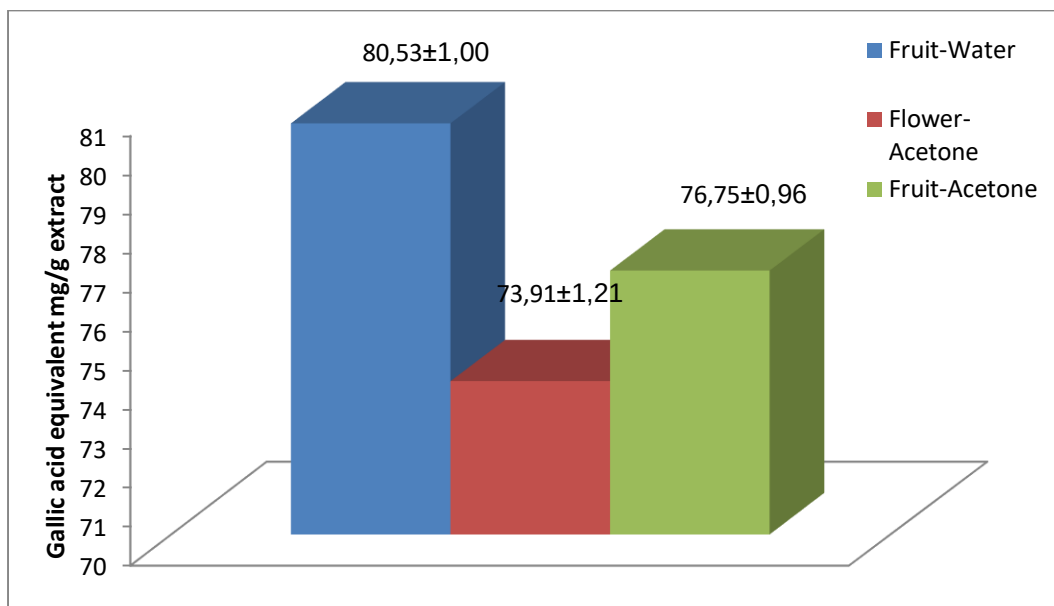


Fig. 5. Flavonoid substance contents of gallic acid equivalent of rowanberry extracts

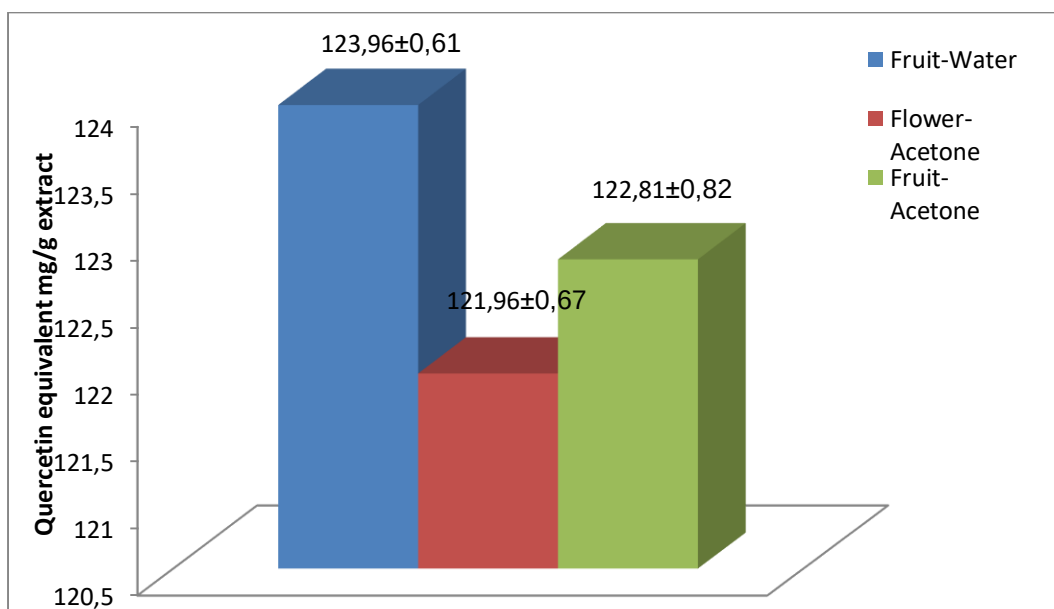


Fig. 6. Flavonoid substance contents of quercetin equivalent of rowanberry extracts

The flavonoid ingredient content (table 1), which is the equivalent of gallic acid and quercetin, calculated from the standard graphical equations are given below.

Table 1. The content of flavonoids, which is the equivalent of gallic acid and quercetin, of rowanberry extracts

Extract	Fruit-Water	Flower-Acetone	Fruit-Acetone
Gallic acid equivalent (mg)	80,53±1,00	73,91±1,21	76,75±0,96
Quercetin equivalent (mg)	123,96±0,61	121,96±0,67	122,81±0,82

CONCLUSION

As a gallic acid equivalent the total flavonoid substance amounts show a decrease in fruit-water>fruit-acetone>flower-acetone, respectively. As a quercetin equivalent, the total flavonoid substance amounts show a decrease in fruit-water>fruit-acetone>flower-acetone, respectively. The highest amount of flavonoids in the extracts was observed in the water extract of the fruit according to both standard compounds.

ACKNOWLEDGEMENTS

This study was supported by Trakya University Scientific Research Projects Unit (TÜBAP) under the project no:2017/74.

REFERENCES

- [1] Podsedek A. Natural antioxidant and antioxidant capacity of Brassica vegetables: A review, LWT-Food Science and Technology 2007; 40(1): 1-11.
- [2] Mujwah AA, Mohammed MA, Ahmed MH. First isolation of a flavonoid from *Juniperus procera* L. using ethyl acetate extract. Arabian Journal of Chemistry 2010; 3: 85-88.
- [3] Sharma V, Janmeda P. Extraction, isolation and identification of flavonoid from *Euphorbia neriifolia* L. leaves. Arabian Journal of Chemistry 2017; 10: 509-514.
- [4] Hossain MA, Rahman SMM. Isolation and characterisation of flavonoids from the leaves of medicinal plant *Orthosiphon stamineus* L. Arabian Journal of Chemistry 2015; 8: 218-221.
- [5] Baytop T. Türkçe Bitki Adları Sözlüğü. Ankara: Türk Dil Kurumu Yayınları, No:578, Türk Tarih Kurumu Basımevi 1994.
- [6] Baytop T. Türkiye’de Bitkiler ile Tedavi (Geçmişte ve Bugün). İstanbul: Nobel Tıp Kitabevleri, LTD, ŞTİ. 1999.
- [7] Zhishan J, Mengcheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chemistry 1999; 64: 555-559.

DETERMINATION OF THE CHLOROPHYLL OF THE ROWANBERRY LEAF

Burhan CEYLAN¹, Yeşim YEŞİLOĞLU²

¹Department of Pharmacognosy and Natural Products Chemistry, Institute of Health Sciences, BezmiAlem Foundation University, İstanbul, Turkey

²Department of Biochemistry, Faculty of Pharmacy, Trakya University, Edirne, Turkey

Corresponding author email: b.ceylan022@gmail.com

Abstract

It is important that the amount of chlorophyll on the earth is determined, because it provides us with a sense of pollution and regeneration on the earth. In food technology, the degradation of nutrients and the long storage of these nutrients without deterioration can be indirectly monitored based on the degradation of chlorophyll. Chlorophyll and bacteriophiles are pigments in the porphyrin structure responsible for photosynthesis in algae, bacteria and plants. These pigments have a fifth (E) isocyclic characteristic ring and phytol group, unlike the other porphyrins, with a magnesium atom at the center and porphyrin structure. In this study chlorophyll a, chlorophyll b and total chlorophyll content of rowanberry (Sorbus domestica L.) leaf were determined spectrophotometrically as mg/kg (fresh) extract.

Keywords: Chlorophyll Content, rowanberry leaf, spectrophotometrically method

INTRODUCTION

Biochemists and biologists trying to explain the mechanism of photosynthesis have shown great interest in this work by studying chlorophyll. Chlorophyll is a mixture of pigments found in green plants. Which is contained in chloroplasts in the cell walls of plants and is necessary for the photosynthesis process. Natural chlorophyll consists of two components; chlorophyll a blue-green color and chlorophyll b yellow-green color [1].

Chlorophyll pigments are used in many areas in the industry; soaps, waxes, inks and resins, in the field of medicine for the preparation of odor eliminating tablets, in medicines against the anemia, as a stain and sweetener in the food industry [2-3].

In addition, their analysis is of great importance as they are used as indicators in the determination of environmental pollution. Since there is such a large working area, it is of great importance to determine the amount and type of pigments present in chlorophyll [2-3].

Chlorophyll consists of two basic components, the porphyrin ring and the phytol group. The porphyrin ring is a ligand in a square plane structure formed by tightly bonding four nitrogen atoms to the Mg center metal atom (Fig. 1) [4].

Early times were thought to consist of a single component of chlorophyll, but then it was determined that a mixture of chlorophyll with spectroscopic methods. Nowadays, chlorophyllene has many known derivations. These; chlorophyll a, chlorophyll b, pyrochlorophyll a, phofoitin a, methylphephorobide and bacterioclorophyll, the most important being chlorophyll a and b.

The different groups in chlorophyll a and b make the absorption spectra a little different. Spectrum absorbs very little into the broad spectral region between 500-600 nm, reflects the light and the plant appears green because the region is the green region of the spectrum (Fig. 2) [4].

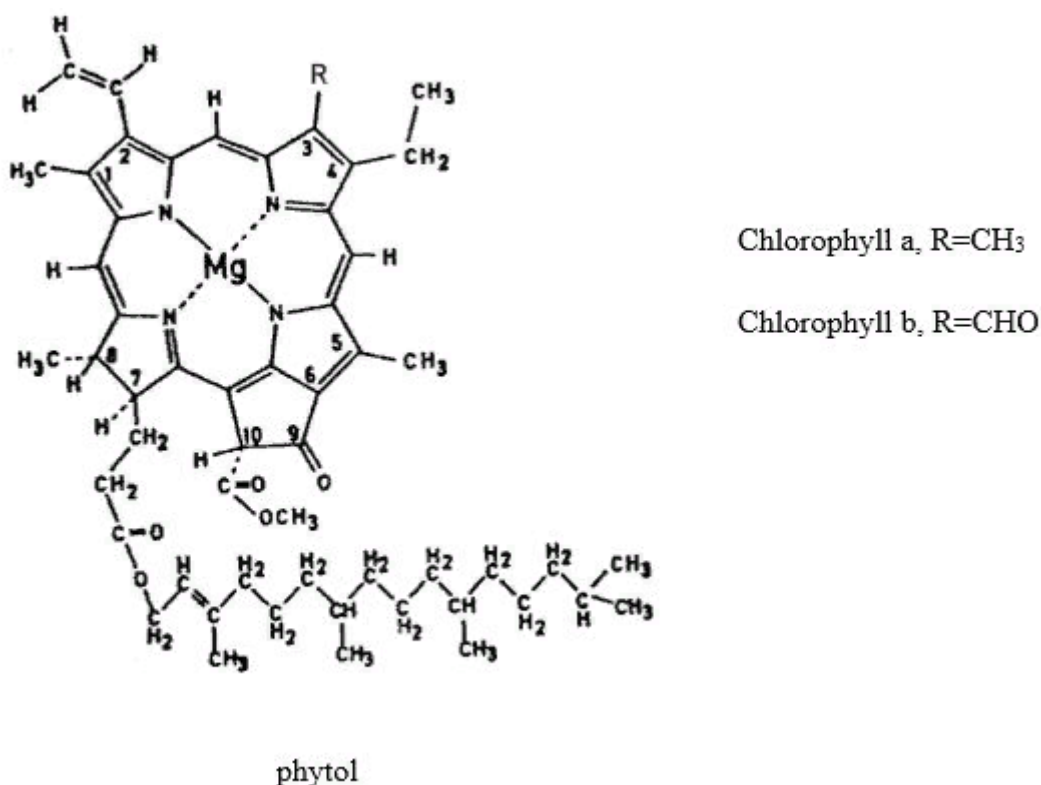


Fig. 1. Chlorophyll a and b

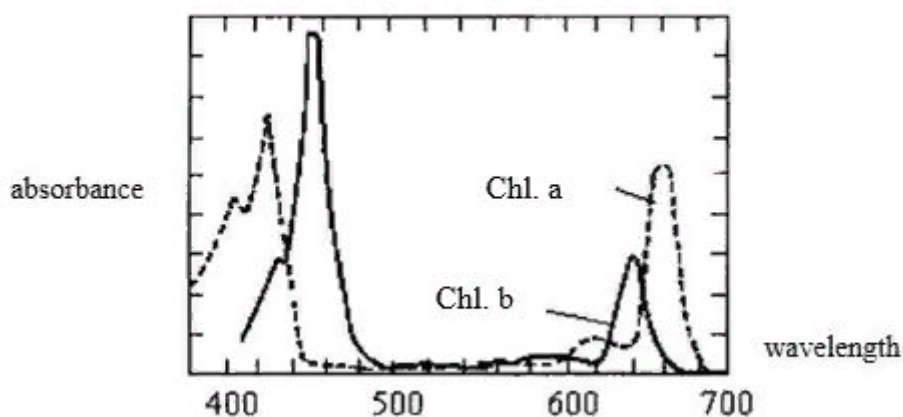


Fig. 2. Absorption spectrum of chlorophyll a and b.

Van Norman's work in 1957 found that chlorophyll a/b ratio affects the absorptivity coefficients, while Vernon was the first to produce equations independent of the chlorophyll a/b ratio in 1960 [5].

The use of fresh extracts is very important to achieve the highest absorbance value in the measurements. Also, the absorbance measurements are affected by the change of the pigments during extraction or by the turbidity in the solvent.

MATERIAL AND METHODS

The chlorophyll content of the rowanberry leaf was determined by the method modified by Arnon [6]

100 mg fresh leaves were homogenized in trace amounts of CaCO_3 and 6 mL of cold acetone (%80). The leaf homogenates were centrifuged at 3500 rpm at 15 °C for 20 minutes. Approximately 4,8-5,8 mL of supernatants were taken. The total chlorophyll content was determined by measuring the absorbance of the supernatants at 645 and 663 nm.

Chlorophyll a, chlorophyll b and total chlorophyll content of plants were calculated in mg/kg according to the formula below.

$$\text{Amount of chlorophyll a (mg/kg)} = 12.7 \times A_{663} - 2.69 \times A_{645}$$

$$\text{Amount of chlorophyll b (mg/kg)} = 22.9 \times A_{645} - 4.68 \times A_{663}$$

$$\text{Total chlorophyll amount (mg/kg)} = 20.2 \times A_{645} + 8.02 \times A_{663}$$

RESULTS AND DISCUSSION

The amount of chlorophyll was calculated as mg/kg wet weight based on wet weight (fig. 3).

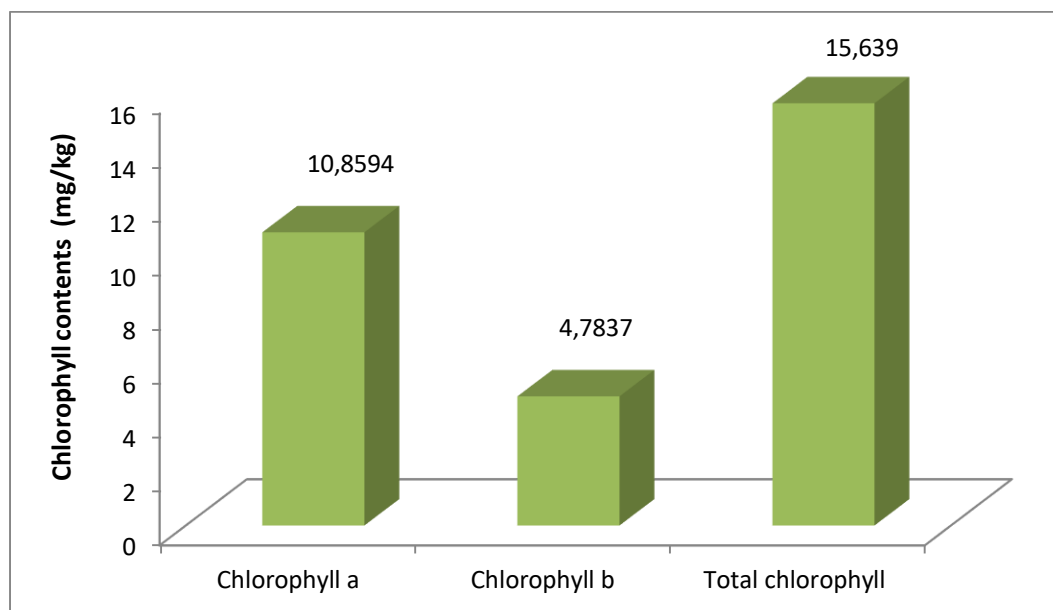


Fig. 3. Chlorophyll content of rowanberry leaf.

The content of chlorophyll a, chlorophyll b and total chlorophyll content of rowanberry leaf was determined as 10,8594 mg/kg (wet), 4,7837 mg/kg (wet), 15,6390 mg/kg (wet) respectively.

ACKNOWLEDGEMENTS

This study was supported by Trakya University Scientific Research Projects Unit (TÜBAP) under the project no:2017/74.

REFERENCES

- [1] Tan YT, Jiang JH, Wu HL. Resolution of kinetic system of simultaneous degradations of chlorophyll a and b by PARAFAC, Analytical Chemistry Acta. 2000; 412: 195-202.
- [2] Walsh K, Jones G, Dunstan H. Effect of irradiance on fatty acid, carotenoid, total protein composition and growth of microcystis aeruginosa, Photochemistry 1997; 44: 817-824.
- [3] Boekel, MAJS. Testing of kinetic models: Usefulness of the multiresponse approach as applied to chlorophyll degradation in foods, Food Research International 1999; 32: 261-269.
- [4] Streitweiser and Heathcock, 1981, Introduction to Organic Chemistry, MacMillan, New York.
- [5] Vernon LP. Spectrophotometric determination of chlorophylls and pheophytins in plant extract, Analytical Chemistry 1960; 54: 1144-1151.
- [6] Arnon DI. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris* L. Plant Physiology 1949; 24: 1-15.

DEVELOPMENTS IN TURKEY'S BAY LAUREL LEAF PRODUCTION AND TRADE

Mustafa ÖZTÜRK¹, Mükremin TEMEL¹, Ahmet Bircan TINMAZ¹, Yasin ÖZDEMİR¹

¹Atatürk Horticultural Central Research Institute Yalova, TURKEY

Corresponding author email: mustafaozturk@tarimorman.gov.tr

Abstract

Bay laurel leaf is one of the most important medicinal and aromatic plants of Turkey. Large sections of the world production and export supply of dry bay laurel leaves is met by Turkey. Turkish bay laurel leaves are regarded as the first quality in the world according to its smell and taste. In the period covering the 2013-2017, which tends to increase Turkey bay laurel leaf production quantities fluctuate over these years. Production increased from 3350 tons in 2003 to 72,629 tons in 2017 with an increase of 726.2%. Bartın, Mersin, Bursa Sakarya, Adana and Muğla are important producers in the production of bay laurel leaf. Turkey's exports of bay laurel leaves increased with 390.3 % from 8.2 million dollars' in 2016 to 40.4 million dollars in the last 15 years (2003-2017). A decrease of 10.7% to 36.1 million dollars was seen in 2017 compared to the previous year. Although it varies according to the countries, the export price of bay laurel leaf unit was realized as 2,71 dollars / kg on 15 years' period covering 2003-2017. Although bay laurel leaf exports have been made to countries close to 75 from Turkey. Amount and value of 52.8% and 38.4% of bay laurel leaf exports were done to Vietnam. Aegean Free Zone, USA, Poland, Japan, Brazil, Germany, Senegal and Romania are the country with the largest number of exports in 2017. 1,18 million dollars' worth of bay laurel leaves have been imported over 1,330 tons in 2017. Georgia is the most important country where we import amount of 78.9% and value of 84.9 % import the was realized. Turkey maintain its leading position of bay laurel leaf trade in world and receive a greater share of the market are inevitable quality production through the foreign markets demand.

Keywords: Turkey, bay laurel leaf, production, export, import

INTRODUCTION

Bay laurel leaf (*Laurus nobilis* L.) is one of our important medicinal and aromatic plants. Its mainland is Minor Asia and Balkans and it is a ever green plant species. Bay laurel leaf, which is considered as a tree of life because it is green in every time, is also referred to as a symbol of immortality in the sources. Bay laurel leaf has a wide place in the culture of Mediterranean countries and has been known for a long time. Our country, especially Mediterranean basin countries such as Greece, Albania, Italy, France, Portugal, Spain, Romania, Morocco, Algeria, Libya and Syria and other countries such as Crimea, Mexico, etc. Georgia and Israel are also cultured (Arslan, 2013). Bay laurel leaf is seen in the form of trees or shrubs in nature. Taking the height of 600-800 meters in Turkey from Hatay until the whole coastline of the Black Sea Northeast, individuals in other species, it shows spread into clusters and groups. Although the soil demand is not enough, the humidity is sufficient, the river beds are preferred (Göker and Acar 1983; Acar, 1987).

MATERIAL AND METHODS

In this study, statistical data and publications related to the production and foreign trade of bay laurel leaf produced by various organizations were used as material.

Percentage calculations, indexes and simple and weighed averages were used, for the analysis of the data compiled from various sources at macro level,

RESULTS AND DISCUSSION

Bay laurel leaf is a source of income for the local population and is one of the most important medicinal-aromatic plants for the country's economy in terms of supporting rural development. They have important functions such as increasing the biodiversity and creating employment in the regions in their spreader area. Turkish bay laurel leaves are considered the first quality in the world as odor and taste. The products with economic value are the leaves and fruits and the oils and essential oils obtained from them. Oils and essential oils are used in perfumery, cosmetics, aromatherapy, physiotherapy and spice production. Dried leaves in the food industry as a aroma and spice, especially in the storage of olives and fish, olives, grapes and dried fruits such as figs are used in packaging to preserve the freshness and flavor (Printer, 2002). Turkey's annual bay laurel leaf production is 22-28 thousand tons in the vicinity, bay leaf production (collection) is made between the plant stopped in July and October of vegetative growth. In the production of bay laurel leaf, it is essential to collect 2 or 3 years of shoots. Bay laurel leaf production is regulated according to the principles of the General Directorate of Forestry numbered 302 and annual production programs, (Anonymous 2018a), and production is permitted according to article 37 of the Forest Law No. 6831. The collection is carried out by the forest villagers and cooperatives who have the right to benefit from the state forests under the supervision and supervision of the General Directorate of Forestry (Yıldırım, 2011). Classification of bay laurel leaves in bay laurel leaf standard (TS 1017) prepared by Turkish Standards Institute; extra, first, row of goods, under the shape of the bottom of the extra class, leaf length of at least 25 mm, up to 100 mm; the width of the leaf is at least 20 mm, maximum 45 mm (Bilgin et al., 2006).

According to Turkey bay laurel leaf production quantities of the year it is given in Table 1. When the chart is analyzed, it is noteworthy that there are fluctuations in the amount of bay laurel leaf production according to years. In 2005, the production, which was around 6400 tons, rose to 27679 tons in 2017 with an increase of 332.5% (Table 1).

Table 1. Bay laurel leaf production quantities of Turkey

Years	Production (ton)
2005	6.436
2006	7.747
2007	11.645
2008	7.042
2009	18.256
2010	15.416
2011	13.928
2012	15.232
2013	15.177
2014	15.578
2015	21.634
2016	21.788
2017	27.679

Source: Anonymous, 2018b.

The information given in the table shows the amount of production realized with the permission of the General Directorate of Forestry. In the inventory studies conducted by the General Directorate of Forestry, the estimated inventory amount of litter has been reported as 310.012 tons (Anonymous 2018b). Turkey's Forest District when according to the Directorate bay laurel leaf amount of production given that schedules two examination Zonguldak Forest District Office providing Turkey 28.6% of bay laurel leaf production quantity while taking attention is the most important region, Sakarya, Bursa, Mersin and Adana are other important bay laurel leaf production Regional Directorates are among the Regional Directorates (Table 2). Turkey is the world's largest high-quality bay laurel leaf manufacturer which is also one of the few countries that export the bay laurel leaf. Buy has a lot uses area such as spices, pharmaceutical, cosmetics and chemical industries, Bay laurel

leaf is one of the major export products of our country, about 70-80% of the world demand is met from Turkey.

According to Turkey's exports of bay laurel leaves his country for years varies. The dried leaves of the bay, produced by the method of collecting from the nature, are exported to many countries of the world after being purified from their branches and foreign substances.

Table 2. Bay laurel leaf production of Turkey as Regional Directorate of Forestry (2017).

Region	Production (kg)	%
Adana	2.589.160	9,4
Sakarya	3.439.519	12,4
Amasya	627.692	2,3
Antalya	1.624.000	5,9
Balıkesir	307.620	1,1
Bolu	38.000	0,1
Bursa	3.189.986	11,5
Çanakkale	10.000	0,0
Isparta	25.000	0,1
İstanbul	809.629	2,9
İzmir	298.100	1,1
Kahramanmaraş	1.679.396	6,1
Kastamonu	1.113.000	4,0
Mersin	2.597.967	9,4
Muğla	1.423.000	5,1
Zonguldak	7.906.910	28,6
Toplam	27.678.979	100,0

Source: Anonymous, 2018b.

Bay laurel leaves between Turkey's export value and quantity of the years 2002-2017 is given in Table 3. 4869 tons and 7.7 million dollars in the bay laurel leaf exports of Turkey in 2002, when the amount and value in 2017 compared to 2002 increased by 161.0% and 369.1%, increased to 12.709 tons in exchange for 36 million dollars.

Table 3. Bay laurel leaf exports of Turkey*

Years	Amount (Ton)	Value (1000 \$)	Export price (\$/Kg)
2002	4.869	7.687	1,58
2003	5.099	8.233	1,61
2004	6.374	12.565	1,97
2005	5.558	11.839	2,13
2006	7.262	17.336	2,39
2007	7.519	20.301	2,70
2008	6.934	20.020	2,89
2009	9.079	24.336	2,68
2010	8.891	25.618	2,88
2011	9.369	26.238	2,80
2012	10.483	29.965	2,86
2013	10.691	32.268	3,02
2014	12.273	35.803	2,92
2015	12.856	36.127	2,81
2016	14.191	40.366	2,84
2017	12.709	36.059	2,84
Average			2,67

Source: Anonymous, 2018c.

*Prepared from records of Turkey Statistical Institute.

Although it varies by country, Turkey in the period covering the years 2002-2017 bay laurel leaf export unit price \$ 1.58/kg 3.02 \$/kg showed changes in the range. In 2013, 1 kg bay laurel leaf was exported at an average of \$ 3.02, while the average unit bay laurel leaf export price for 2002-

2017 was \$ 2.67/kg (table 3). Turkey's bay laurel leaf exports to major countries in 2017 is given in Table 4.

Table 4. Turkey's bay laurel leaf exports to major countries* (2017)

Countries	Amounts (Ton)	%	Value (1000 \$)	%	Unit export price (\$/Kg)
Vietnam	6.712	52,8	14.201	39,4	2,12
Aegean Free Zone	551	4,3	3603	10,0	6,54
Poland	456	3,6	1962	5,4	4,30
USA	753	5,9	2491	6,9	3,31
Brasilia	557	4,4	1420	3,9	2,55
Japan	286	2,3	1449	4,0	5,07
Germany	343	2,7	1263	3,5	3,68
Spain	180	1,4	701	1,9	3,89
Romania	131	1,0	725	2,0	5,53
Senegal	339	2,7	773	2,1	2,28
Total	10.308	81,1	28.588	79,3	2,77
Others	2.400	18,9	7.471	20,7	3,11
Grand total	12.708	100,0	36.059	100,0	2,84

Source: Anonymous, 2018c.

* Prepared from records of Turkey Statistical Institute.

Turkey exports bay laurel leaf to nearly 70 countries. In 2017, the amount of bay leaf exports was 52.8%, the value was 39.4% in Vietnam, the amount was 4.3%, the value was 10.0% in the Aegean Free Zone with an amount of 5.9%, the value was 6.9% bay leaf in exports USA which are noteworthy countries for this perspective (Table 4). Other important export countries are Poland, Brazil, Japan, Germany; Spain, Romania and Senegal. While the bay laurel leaf export price was 2.67 dollars in 2017, the unit price for Ege Free Zone was 6.54, Poland to 4.30, Japan to 5.07, Romania to 5.53, Germany to 4.58, Vietnam to bay 2.12 (Table 4). Exports to Aegean Free Zone should be evaluated separately from exports to other countries. sold goods from Turkey are subject to export free zones regime, the sold goods to Turkey from free zones are subject to the import regime. Free zone users export prices from Turkey (VAT) can purchase goods and services. On the other hand, the provisions of the foreign trade regime do not apply between the free zone and other countries and other free zones. In this context, bay laurel leaves provided by various companies in the Aegean Free Zone are subjected to cleaning and sorting process and are exported to Europe and America, especially to all parts of the world. Turkey's bay laurel leaf imports are given in Table 5. Significant fluctuations are seen in Turkey's bay laurel leaf imports between years.

In 2015, 2302 tons of bay laurel leaf was imported from 1.50 dollar the unit price of and 3 million 455-thousand-dollar total price. In 2017, 1300 tons of bay laurel leaf was imported from the unit price of 1.35 dollar and 1 million 795-thousand-dollar total price. (Table 5).

Important countries for Turkey's bay laurel leaves imports in 2017, according to the quantity and value was given in Table 6.

In 2017 the amount of 78.9% of Turkey's bay laurel leaf imports, 85.0% of the value realized from Georgia and 18.9% of the amount and 6.7% of the value have been realized from Syria. These are most important countries for Turkey's bay laurel leaf imports (Table 6). According to countries, there are significant differences in the import prices of bay laurel leaf. In 2017, bay laurel leaf imports were made 1.45 dollar/kg from Georgia, 2.00 dollar/kg from Poland, 2.17 dollar/kg from Italy, and 0.48/kg from Syria (Table 6).

Table 5. Turkey's bay laurel leaf imports.*

Years	Amount (Ton)	Value (1000 \$)	Import price (\$/Kg)
2002	15	17	1,13
2003	17	103	6,06
2004	20	64	3,20
2005	33	120	3,64
2006	13	44	3,38
2007	12	87	7,25
2008	41	111	2,71
2009	177	339	1,92
2010	510	889	1,74
2011	85	158	1,86
2012	716	1.274	1,78
2013	882	1.538	1,74
2014	1.140	1.770	1,55
2015	2.302	3.455	1,50
2016	1.679	1.872	1,11
2017	1.330	1.795	1,35
Average			1,52

Source: Anonymous, 2018c.

* Prepared from records of Turkey Statistical Institute.

Table 6. Important countries for Turkey's bay laurel leaves imports * (2017)

Country	Amount (Ton)	%	Value (1000 \$)	%	Unit import price (\$/Kg)
Georgia	1049	78,9	1525	85,0	1,45
Austria	10	0,8	85	4,7	8,50
Poland	5	0,4	10	0,6	2,00
Syria	252	18,9	120	6,7	0,48
Italy	6	0,5	13	0,7	2,17
Romania	3	0,2	24	1,3	8,00
Total	1325	99,6	1777	99,0	1,34
Other	5	0,4	18	1,0	3,60
Grand Total	1330	100,0	1795	100,0	1,35

Source: Anonymous, 2018c.

* Prepared from records of Turkey Statistical Institute.

CONCLUSION

Turkey bay laurel leaf production and exports is one of the world's most important countries, its bay laurel leaf exports have increased in amount and value in recent years, has reached 36 million dollars in 2017. In the same year, 1.8-million-dollar bay laurel leaf was imported.

As bay laurel leaf production is done by collecting from nature, it should be ensured that a sustainable plan and operation program and cuts and collections are carried out under supervision to ensure the supply of bay laurel leaf products by adhering to the principle of efficiency and sustainability without causing natural destruction. It is important to increase the value of processed and packaged bay laurel leaf exports with more added value, as each process to be carried out on the plant product will increase the value of the herbal drug and the added value will remain in our country. In order to have a voice in the world markets, it should be ensured that quality bay laurel leaves production is performed in an adequate production scale in accordance with the standards and market demands in terms of the competitive advantage of foreign trade and the image of our country and sustainable export.

REFERENCES

- Anonymous 2018a. Inventory and Planning of Non-Wood Forest Products and Production and Sales Principles, Communiqué No: 302, T.C. Ministry of Forestry and Water Affairs General Directorate of Forestry Non-Wood Products and Services Department, Ankara.
- Anonymous, 2018b. General Directorate of Forestry Non-Wood Products and Services Department, Ankara.
- Anonymous, 2018c. Turkey Statistical Institute Records, Ankara.
- Acar, İ. 1987. Production and Evaluation of Bay (*Laurus nobilis* L.) Leaf and Leaf Etheric Oil, Forestry Research Institute Publications, Technical Bulletin Series No: 186, Ankara,
- Arslan, N. 2013. .com Defne (*Laurus nobilis* L.) Symbol of Peace and Victory us www.gidahatti.com, August 2016.
- Bilgin, F., Parlak, S., Kaymakçı, E. 2006. The Leaf Production Plant (*Laurus nobilis* L.) Plant Tes İzmir-Urla Case,, T.C. Ministry of Environment and Forestry Aegean Forestry Research Institute, Ministry Publication No: 301, Directorate Publication No: 44, Technical Bulletin No: 33, ISSN 1300-9508, Izmir.
- Göker, Y., Acar, İ. 1983. Mediterranean Bay Laurel Leaf from Forest By-Products (*Laurus nobilis* L.), Journal of Istanbul University Faculty of Forestry, Volume: 33, Series: B, Number: 1, Istanbul.
- Yildirim H.T. 2011. Turkey's Non-Wood Forest Products Forest Policy Evaluation in terms of production, the First National Symposium on Mediterranean Forests and Environment, Kahramanmaraş.
- Yazıcı, H. 2002. Investigation of Utilization Possibilities of Bay Leaves and Fruits in Western Black Sea Region, Zonguldak Karaelmas University, Institute of Science and Technology, Ph.D. Thesis, Zonguldak.

DEVELOPMENT OF LIQUID DOSAGE FORM CONTAINING CONJUGATED LINOLEIC ACID AND L-CARNITINE

Veysel Umut ÇELENK¹, Ercüment KARASULU¹

¹Center for Drug Research and Development and Pharmacokinetic Applications, Ege University, 35100, Izmir, Turkey

Corresponding author email: veysel.umut.celenk@mail.ege.edu.tr

Abstract

In this study, in order to accelerate fat burning and increase muscle mass, commonly used conjugated linoleic acid and L-carnitine are prepared as a single liquid dosage form in safflower oil. It was attempted to present an innovative study to produce locally and support such nutritional supplements which are imported into our country. Reliability of quality control studies which is for 9c,11t conjugated linoleic acid, 10t,12c conjugated linoleic acid and L-carnitine supported by analytical validations. The physicochemical stability of active ingredients in the suspension was proven by 6 months short-term stability tests. So, quantification of active ingredients, particle size, zeta potential, viscosity, sedimentation volume and microbiological analyses were performed for the control of the physicochemical stability of the formulation.

Keywords: Conjugated Linoleic Acid, L-Carnitine, safflower oil, suspensions, dietary supplements.

INTRODUCTION

After the discovery of anticarcinogenic and antimutagenic effects of conjugated linoleic acid (CLA), found that it has an inhibitory effect on body fat aggregation, antidiabetic, immunoactive and inhibitory effect on arteriosclerosis risk. So that, CLA has great usage area of functional food production (Çelik, 2006). General diets enable average 200 mg 9c,11t isomer of CLA intake, but physiological effects of CLA occurs in case of consumption much more than 400 mg 9c,11t-CLA. Another active isomer, 10t,12c-CLA is rarely found daily diet (Köknaroğlu, 2007). According to results of toxicity on rats, there is no finding as histopathological harm and hematological anomaly after consumption of 1.5% of diet for 36 weeks. Consumption of CLA more than 3.0 g daily is influenced favourable of body composition. Periodic intake of CLA regulates glucose level and insulin as good as reference drugs (Kelly et al., 2001). Another study shows that CLA intake for sixteen weeks reduce body mass index and total body fat. But, consumption of safflower oil did not provide these effects (Norris et al., 2009).

The most important point of analysis of CLA isomers is resolution and identification of geometrical and positional isomers. Isomerisation must be taken under control during derivatization stage. Gas chromatography combined mass spectrometer (GC-MS) is widely used for CLA analyses. Analysis of CLA by GC-MS instrument needs capillary columns such as CP-Sil 88TM and BPX-70TM which given effective and robust results. Besides, derivatization agents such as 4,4-dimethyloxazoline (DMOX) and 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) are used to volatilize of CLA isomers for identification by GC-MS systems (Christie et al., 2001).

L-Carnitine has a great role on fat metabolism and energy production. In addition, there are important metabolic functions such as transport of long chain fatty acids to mitochondrial matrix, transport of short and medium chain fatty acids from mitochondrial matrix and detoxification of acyl groups. Toxicity of L-Carnitine was reported by EFSA (European Food Safety Authority). According to the results, consumption of 3.0 g L-Carnitine per day for three weeks is tolerable. There is no report for the maximum limit but consumption of 3.0 g L-Carnitine per day is recommended (EFSA, 2012).

There are different analytical methods for determination and quantification of L-Carnitine in various samples. The most important and widely used ones are chromatographic, spectrophotometric and radioenzymatic analyses (Möder et al., 2005).

Carthamus tinctorius L. (Asteraceae) known as safflower is 80-100 cm tall and xerotolerant annually plant. *Carthamus tinctorius L.* has totally 25 species in Turkey. It is cultivated mainly for its seed, which is used as edible oil and as birdseed. Safflower was grown for its flowers, used for foods, dyes, and medicines. (Babaoglu, 2014a; 2014b). Safflower has wide pharmacological and biological activities including cardioprotective, neuroprotective and antitumor activity (Mirhoseini, et al., 2012).

MATERIAL AND METHODS

9c,11t-CLA, 10t,12c-CLA and L-Carnitine were used as active ingredients in formulation and quantification of these compounds were performed by Gas Chromatography Mass Spectrometry (GC-MS) and High Performance Liquid Chromatography Ultraviolet (HPLC-UV) systems *via* validated analysis methods. Linearity, Repeatability, Recovery, Specificity, Selectivity, Limit of Detection (LOD) and Limit of Quantification (LOQ) were applied as validation parameters to prove the validity of methods. 9c,11t-CLA and 10t,12c-CLA were validated between 10.0 µg/mL-250.0 µg/mL concentration levels. Quantification and control ions were selected as *m/z* 81 and *m/z* 95, respectively. Besides, L-Carnitine was validated between 30.0 µg/mL-1500.0 µg/mL concentration levels.

Chromatographic Conditions; Determination of 9c,11t-CLA and 10t,12c-CLA was performed by GC-MS instrument (Shimadzu, Japan) combined with electron impact ionization (EI) and the compounds were separated by fused silica capillary column (Rtx-CLPesticides2-20m*0.18mm*0.14µm). Heat program was adjusted to 120 °C as an initial temperature. Then, the heat program was increased to 200 °C with the rate of 20 °C for the first step. At the second and the third step, heat program was increased to 230 °C with the rate of 15 °C and 300 °C with the rate of 30 °C, respectively. Injection volume was 2 µL and the flow rate was 1.10 mL/min. Determination of L-Carnitine was performed by HPLC instrument (Agilent, USA) combined with ultraviolet (UV) detector at 235 nm and the compounds were separated by C18 column (ACE C18-150*4.6mm). Mobile phase was the mixture of 260 mL acetonitrile and 740 mL sodium perchlorate (0.5 mol/mL) which adjusted pH to 3.8 with perchloric acid solution. After 20 µL injection, isocratic solvent program was performed as 0.5 mL/min flow rate at 25 °C.

Validation of Method; Linearity parameter for 9c,11t-CLA and 10t,12c-CLA were determined by analyzing standard solutions. The linearity range was determined as 10.0 µg/mL to 250.0 µg/mL for both compounds. External calibrations were applied to calculate the concentration of CLA isomers in the formulation. The precision (repeatability) of the reported method was evaluated by repeating the measurements 5 times at 100.0 µg/mL standard concentration in the same day. The recovery experiments were evaluated at three fortification levels (25.0 µg/mL, 50.0 µg/mL and 100.0 µg/mL). The specificity and selectivity of method were performed with standard solutions at 50.0 µg/mL concentration level. Interference and resolution were controlled according to the results. The LOD and LOQ values of both compounds were calculated *via* signal to noise (S/N) ratio. Linearity parameter for L-Carnitine was determined by analyzing standard solutions. The linearity range was determined as 30.0 µg/mL to 1500.0 µg/mL for this compound. External calibration was applied to calculate the concentration of L-Carnitine in the formulation. The precision (repeatability) of the reported method was evaluated by repeating the measurements 5 times at 300.0 µg/mL standard concentration in the same day. The recovery experiments were evaluated at three fortification levels (150.0 µg/mL, 300.0 µg/mL and 750.0 µg/mL). The specificity and selectivity of method were performed with standard solutions at 300.0 µg/mL concentration level. Interference and resolution were controlled according to the results. The LOD and LOQ values of L-Carnitine were calculated *via* signal to noise (S/N) ratio.

Analysis of 9c,11t-CLA and 10t,12c-CLA in the liquid dosage form; 9c,11t-CLA and 10t,12c-CLA included formulation was weighted as 10.0 mg in a 10.0 mL volumetric flask and filled with *n*-hexane to volume. After transfer of 1.0 mL of the formulation solution to another glass tube, 200 µL KOH (2 N) solution was added to the glass tube and shook for 5 minutes. The mixture was put in the incubator at 50 °C for 10 minutes and centrifuged for 5 min at 3000 rpm. Then *n*-hexane phase of the mixture was filtered by 0.45µm PTFE filter and injected to GC-MS instrument. External calibration

was applied to calculate the concentration of 9c,11t-CLA and 10t,12c-CLA in the formulation. The dilution factor was calculated as 1000.

Analysis of L-Carnitine in the liquid dosage form; Formulation which included L-Carnitine was weighted as 100.0 mg in a glass tube. After addition 0.5 mL water and 0.5 mL phenacyl bromide solution (800 mg/mL in ethanol) the mixture was shaken for 10 min and put in the incubator at 65 °C for 30 minutes. Ethyl acetate was added to the mixture at the end of the waiting period and shook for 10 minutes again. When the times out the mixture was centrifuged for 5 min at 3000 rpm. 100 µL of derivatized aqueous phase was separated from bottom of the mixture and diluted to 10 mL by HPLC grade water then injected to HPLC-UV system. External calibration was applied to calculate the concentration of L-Carnitine in the formulation. The dilution factor was accepted as 1000.

Preparing of the liquid dosage form; 1000.0 mg safflower oil, 250.0 mg 9c,11t-CLA, 250.0 mg 10t,12c-CLA and 1000.0 mg L-Carnitine were weighted in a sterilized beaker and mixed by a mixer for 30 minutes. None of excipient or preservative were added to the mixture to have characteristic of natural product.

Stability of liquid dosage form; Formulation was put in an incubator adjusted to 40°C ± 2°C and 75% ± 5% humidity for six months (CDER, 2001; 2003). Quantity of active ingredients, physicochemical properties (particle size, zeta potential, viscosity and sedimentation volume) and microbiological analyses of liquid dosage form were tested at 0th, 3rd and 6th months (Biomerieux TEMPO® TVC, 2009; Biomerieux TEMPO® EC, 2009; Biomerieux TEMPO® YM, 2010; Council of Europe, 2007).

RESULTS AND DISCUSSION

Validation of Method; Linearity levels were adjusted as 10.0 µg/mL, 25.0 µg/mL, 50.0 µg/mL, 100.0 µg/mL and 250.0 µg/mL for 9c,11t-CLA and 10t,12c-CLA compounds. Correlation coefficients for both analytes in the working range were calculated as 0.9996. The linear regression equation for 9c,11t-CLA and 10t,12c-CLA were calculated as, $y=7186.9x-30765$ and $y=6928.1x-3375.8$, respectively. Linearity levels were adjusted as 30.0 µg/mL, 150.0 µg/mL, 300.0 µg/mL, 750.0 µg/mL and 1500.0 µg/mL for L-Carnitine. Correlation coefficients in the working range were calculated as 1.0000. The linear regression equation was calculated as $y=8.8812x+23.742$. Repeatability of 9c,11t-CLA and 10t,12c-CLA were presented as %RSD values which are 0.76 and 0.50, respectively. Besides, repeatability of L-Carnitine was calculated as 0.76. Recovery values of 9c,11t-CLA and 10t,12c-CLA compounds were presented as % recovery. Recovery values of 9c,11t-CLA were calculated as %93.9 for 25.0 µg/g level, % 99.1 for 50.0 µg/g level and % 93.8 for 100.0 µg/g level. Recovery values of 10t,12c-CLA were calculated as %91.4 for 25.0 µg/g level, % 99.7 for 50.0 µg/g level and % 93.3 for 100.0 µg/g level. Besides, recovery values of L-Carnitine were calculated as %96.8 for 150.0 µg/g level, % 97.3 for 300.0 µg/g level and % 101.2 for 750.0 µg/g level. Specificity parameter was controlled by injection of 9c,11t-CLA, 10t,12c-CLA and L-Carnitine standard solutions and blank formulation at the same conditions. No peak or interference was observed at the same retention time. Selectivity parameter was controlled by injection of 9c,11t-CLA and 10t,12c-CLA standard solution at the same conditions. Resolution factors were calculated as 7.88 and 1.43. Besides, resolution factor was calculated as 9.94 for L-Carnitine. LOD and LOQ values of CLA isomers were calculated as 2.4 µg/mL and 8.0 µg/mL, respectively. Besides, LOD and LOQ values for L-Carnitine was calculated as 0.2 µg/mL and 0.5 µg/mL, respectively.

Stability of liquid dosage form; Quantity of 9c,11t-CLA, 10t,12c-CLA and L-Carnitine were calculated at 0th, 3rd and 6th months in liquid dosage form. According to the results, 9c,11t-CLA was calculated as 248.6 mg for 0th month, 246.4 mg for 3rd month and 246.1 mg for 6th month. 10t,12c-CLA was calculated as 249.3 mg for 0th month, 247.8 mg for 3rd month and 246.5 mg for 6th month. L-Carnitine was calculated as 997.1 mg for 0th month, 995.0 mg for 3rd month and 994.1 mg for 6th month (Table 1). Also, physicochemical properties of the formulation were tested at 0th, 3rd and 6th months and the results were given in Table 2. Microbiological analyses were performed at 0th, 3rd and 6th months and the results were presented at Table 3.

Table 1. Quantification of 9c,11t-CLA, 10t,12c-CLA and L-Carnitine in the liquid dosage form.

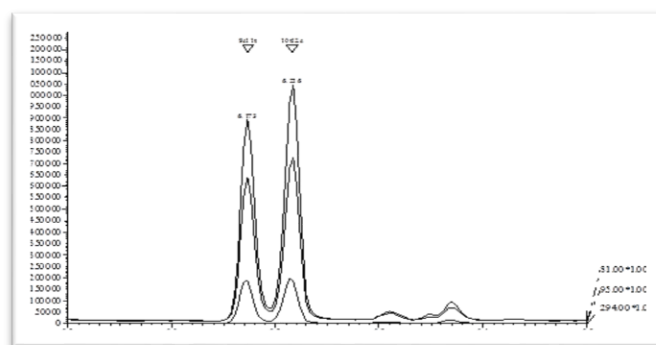
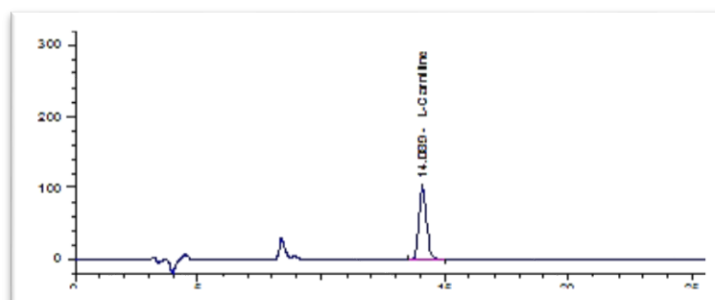
<i>Compounds</i>	<i>0th Month</i>	<i>3rd Month</i>	<i>6th Month</i>
9c,11t-CLA	248.6 mg	246.4 mg	246.1 mg
10t,12c-CLA	249.3 mg	247.8 mg	246.5 mg
L-Carnitine	997.1 mg	995.0 mg	994.1 mg

Table 2. Physicochemical properties of the liquid dosage form.

<i>Physicochemical properties</i>	<i>0th Month</i>	<i>3rd Month</i>	<i>6th Month</i>
Particle size	371 µm	375 µm	373 µm
Zeta potential	0.0499 mV	0.0509 mV	0.0489 mV
Viscosity	6.53 Poise	6.45 Poise	6.40 Poise
Sedimentation volume	0.52	0.53	0.52

Table 3. Microbiological analyses of the liquid dosage form.

<i>Microbiological analyses</i>	<i>0th Month</i>	<i>3rd Month</i>	<i>6th Month</i>
Total Viable Count	< 100 CFU/g	< 100 CFU/g	< 100 CFU/g
<i>Escherichia coli</i>	Not Detected	Not Detected	Not Detected
Yeast-Mold	< 10 CFU/g	< 10 CFU/g	< 10 CFU/g

**Fig 1.** Chromatogram of 9c,11t- and 10t,12c- Conjugated Linoleic Acid ($m/z=81$, $m/z=95$ and $m/z=294$).**Fig 2.** Chromatogram of L-Carnitine at 235 nm.

CONCLUSION

Most of the supplementary products are imported and limited alternative products are generated in Turkey. So, we aimed to unite fat burner effect of CLA and muscle tissue gainer effect of L-Carnitine in the same alternative formulation. A new liquid dosage form of conjugated linoleic acid isomers and L-Carnitine mixture was prepared and quality control analyses were performed. The validity of quality control analysis was proven by validation procedures. Shelf life of the formulation was identified by stability tests. In conclusion, an alternative and innovative product was developed and quality control analyses and stability tests for this liquid dosage form were performed.

ACKNOWLEDGEMENTS

The authors gratefully thank Ege University, Center for Drug Research and Development and Pharmacokinetic Applications (ARGEFAR) and ZADE VITAL[®] for financially supports.

REFERENCES

- Babaoğlu, M., 2014a. Aspir Bitkisi ve Tarımı. Trakya Tarımsal Araştırma Enstitüsü Müdürlüğü İnternet Sitesi: <http://arastirma.tarim.gov.tr/ttae/Sayfalar/Detay.aspx?SayfaId=59>
- Babaoğlu, M., 2014b. Dünya’da ve Türkiye’de Aspir Bitkisinin Tarihi, Kullanım Alanları ve Önemi. Trakya Tarımsal Araştırma Enstitüsü Müdürlüğü İnternet Sitesi: <http://arastirma.tarim.gov.tr/ttae/Sayfalar/Detay.aspx?SayfaId=58>
- Biomerieux TEMPO® TVC, 2009. Automated test for use with TEMPO, for the enumeration of Total viable count in 40-48 hours in food products, , 80 007.
- Biomerieux TEMPO® YM, 2010. Automated test for use with TEMPO, for the enumeration of Yeasts, mold in 72-76 hours in food products, , 80 001.
- Biomerieux TEMPO® EC, 2009. Automated test for use with TEMPO, for the enumeration of Escherichia coli in 22-27 hours in food products, , 80 004.
- CDER, 2003. Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER).
- CDER, 2001. Guidance for Industry Bioanalytical Method Validation, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM).
- Christie WW, Sebedio JL, Juaneda P., 2001. A practical guide to the analysis of conjugated linoleic acid (CLA). INFORM., 12:147-152. Available from: http://lipidlibrary.aocs.org/topics/cla_meth/file.pdf
- Council of Europe, 2007. European Pharmacopoeia 6th Edition, 529-530.
- Çelik, L., 2006. Konjuge Linoleik Asidin Ruminatlarda Biyosentezi, Fizyoloji ve Lipid Metabolizması Üzerine Etkileri. Hayvansal Üretim, 47(1):1-7.
- EFSA, 2012. Scientific Opinion on the safety and efficacy of L-carnitine and L-carnitine L-tartrate as feed additives for all animal species based on a dossier submitted by Lonza Benelux BV. EFSA Journal, 10(5):2676. Available from : www.efsa.europa.eu
- Eryılmaz, T., Cesur, C., Yeşilyurt, M.K., Aydın, E., 2014. Aspir (*Carthamus tinctorius* L.), Tohum Yağı Metil Esteri: Potansiyel Dizel Motor Uygulamaları için Yakıt Özellikleri. Türk Tarım ve Doğa Bilimleri Dergisi, 1(1):85-90.
- Kelly, G.S., 2001. Conjugated Linoleic Acid: A Review. Altern. Med. Rev., 6(4):367-382.
- Köknaroğlu, H., 2007. Beslemenin Sığır Eti Konjuge Linoleik Asit Miktarına Etkisi. Hayvansal Üretim, 48(1):1-7.
- Mirhoseini, M., Mohamadpour, M., Khorsandi, L., 2012. Toxic effects of *Carthamus tinctorius* L. (Safflower) extract on mouse spermatogenesis. J. Assist. Reprod. Genet. 29:457–461.
- Möder, M., Kiessling, A., Löster, H., 2005. Current Methods for Determination of L-Carnitine and Acylcarnitines. Monatshefte für Chemie, 136:1279-1291.
- Norris, L.E., Collene, A.L., Asp, M.L., Hsu, J.C., Liu, L., Richardson, J.R., 2009. Comparison of dietary conjugated linoleic acid with safflower oil on body composition in obese postmenopausal women with type 2 diabetes mellitus. Am. J. Clin. Nutr., 90:468–76.

LIQUID DOSAGE FORM DEVELOPMENT and CHARACTERIZATION WITH β -(1,3 -1,6) GLUCAN

Simge UFACIK ÇELENK^{1,2}, Ercüment KARASULU^{1,2}

¹Ege University Drug Development and Pharmacokinetic Research and Application
Center, 35100 Bornova, İzmir.

²Ege University Faculty of Pharmacy, Department of Biopharmaceutic and
Pharmacokinetic, 35100 Bornova, İzmir.

Corresponding author email: simgeufacik@gmail.com

Abstract

β -glucans are commonly found in yeast (*Saccharomyces cerevisiae*), cereals, water algae (*Laminaria digitata*), bacteria (*Alcaligenes faecalis*) and fungi (*Sclerotium gluconicum*). The β - glucans obtained from these sources have been shown to have therapeutic properties as well as supportive effects on the immune system cells. Black cumin is a natural medicinal plant used to treat a wide variety of diseases such as asthma, hypertension, inflammation, flu, gastrointestinal disorders as well as skin or respiratory disorders. It has also been shown that black cumin seed oil has antimutagenic, antioxidant and antiinflammatory activities as well as antimicrobial activity and stimulating effect on the immune system. In the scope of the study, β - (1,3-1,6) glucan, which is used to support both natural and adaptive immune system, was prepared in the form of suspension in the black cumin seed oil. The reliability of the quality control studies for β - (1,3-1,6) glucan, which is the active ingredient in the prepared product, and black cumin seed oil was supported by analytical method validation and quality control tests such as physical, microbiology analysis. The chemical stability of β - (1,3-1,6) glucan, which is used as an active ingredient, has been demonstrated by the 6-month short-term chemical stability test of the product prepared in suspension form. It is intended that this food supplement consumed worldwide and usually in tablets, hard gelatin capsules in powder form or on the market as syrup is presented with an innovative dosage form complete with local market quality control trials.

Keywords : β -glucans, black cumin oil, stability test, zeta potential

INTRODUCTION

β -Glucans are polysaccharides composed of D-glucose monomers linked together by β -glucosidic bonds. It is commonly found in plants, in grain seeds, in the cell wall of bread yeast, in some fungi and bacteria. Some beta-glucan species are useful as nutrients and some are useful in increasing metabolic resistance.

It has been shown that β - (1,3-1,6) -glucan which we use as active substance can activate leukocytes, stimulate phagocytosis, cytotoxicity, antimicrobial activity of leukocytes. It is also known that it has pro- or anti-inflammatory effects on the immune system cells and affects the natural and adaptive immune system positively.

Another ingredient used in the formulation, the black seeds, can be used in the preparation of cosmetic preparations and nutritional supplement. Black seed oil has been determined to have stimulatory effects on the immune system by antitumor, antioxidant, antiinflammatory and antibacterial activities. It is also used as a natural remedy for treating skin or respiratory disorders as well as asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness, flu, gastrointestinal disorders. It is also known that the seeds are diuretic, emenagog and galactagogue.

Our aim is to develop a liquid dosage form that can be used as nutritional supplement, taking advantage of the immune system effects of β - (1,3-1,6) -glucan and black seed oil. The aim of the improved liquid dosage form is to have a higher bioavailability compared to other β -Glucan products used to support the immune system in the market.

MATERIAL AND METHODS

Stock Standard solution, Blind Standard Solution and Calibration Standard Solutions for Spectrophotometric Quantitative Analysis of β -1,3-1,6-glucan were prepared.

To prepare a master stock such that the concentration was 10000 $\mu\text{g} / \text{mL}$, 111.1 mg of the β -1,3-1,6 glucan standard at 90% purity was weighed to 100 mg. The standard was solved by passing through certain stages with chemicals such as EtOH, NaOH and HCl.

To draw the calibration curve, standard solutions were prepared to 5 different concentrations of the stock standard solution between 125 and 2000 $\mu\text{g} / \text{mL}$. The prepared concentrations were read at a wavelength of 510 nm in an UV-spectrophotometer.

The blind solution was completed to 100 mL by calculating the amounts of chemicals used to prepare the stock standard solution.

In our literature studies for β -glucan we found that 100 mg and over doses were used as therapeutic and under 100 mg doses were used as therapeutic supplements. Taking this into consideration, preparations containing 50 mg / 5 mL of β -1,3-1,6-glucan were prepared to allow the selection of a supplementary or therapeutic dose according to use (5 mL / day-10 mL / day). In a tube, 71.42 mg of β -1,3-1,6-glucan corresponding to 50 mg of 70% purity was weighed and 5.0 g of black seed oil was added. It was suspended in the agitator for 30 minutes with stirring. The prepared mixture was filled into 6-mL oral vials.

1.0 g of the homogenized suspension was weighed and centrifuged at 4000 rpm for 5 min in order to calculate the β -glucan content of the prepared preparation. The upper oil phase was discarded. Then 1 mL of diethyl ether was added to remove the oil between the β -glucan particles. Shaken for 5 minutes on shaker. The agitated mixture was centrifuged at 4000 rpm for 5 min. The ether phase on the sample was discarded. The degreased sample was prepared according to the β -glucan standard preparation procedure and absorbance was read at a wavelength of 510 nm in a UV-spectrophotometer.

Calculations were made for the β -1,3-1,6 glucan in the matrix medium according to the equation obtained from the calibration graph plotted for β -1,3-1,6 glucan.

In order to prove the reliability of β -1,3-1,6 glucan analysis; Method validation was performed by examining the criteria of selectivity, linearity, precision (reproducibility, reproducibility), cascade repeatability and recovery.

Physical analysis of appearance, color, odor and taste of the suspension of β -1,3-1,6 glucan and black seed oil after 6 months of stabilization was performed. In addition, formulation microbiological tests were carried out along with physical tests such as particle size, sedimentation volume, sedimentation velocity, zeta potential, viscosity.

In the case of black seed oil, quality control tests such as determination of fatty acid composition and analysis of tocopherol were carried out.

RESULTS AND DISCUSSION

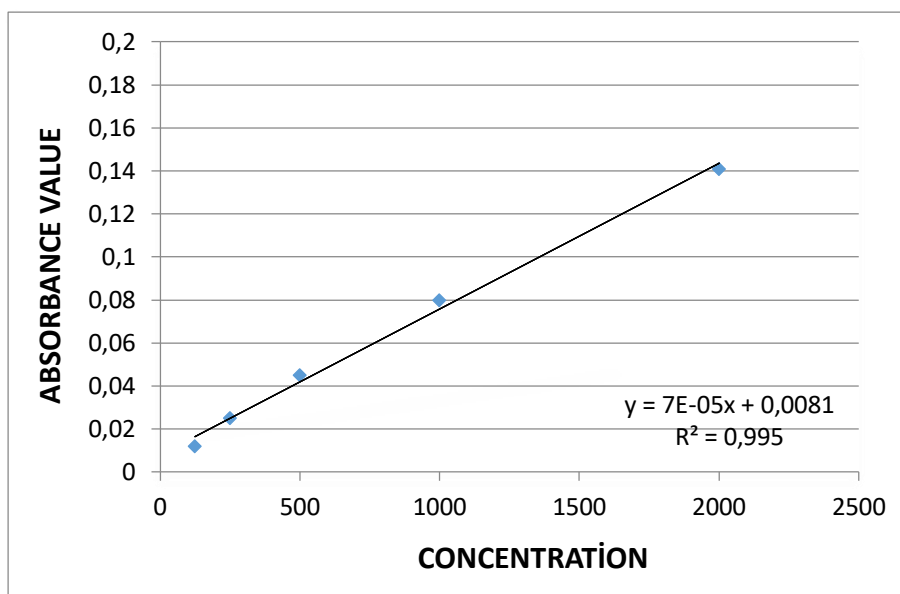
The results obtained by examining the selectivity, linearity, reproducibility, reproducibility, extraction reproducibility and recovery criteria in the analytical method validation optimized for the quality control analysis for the prepared formulation are evaluated in CDER (Center for Drug Evaluation and Research).

As a result of the study for the selectivity criterion, the β - (1,3-1,6) glucan standard was given to the blind sample under the same conditions and the same method and no absorbance was observed at the same wavelength as the active substance.

The regression coefficient calculated for the linearity criterion is $R^2 = 0.995$, which is greater than the minimum limit value of 0.95. The % RSD values corresponding to 125, 250, 500, 1000, 2000 $\mu\text{g} / \text{mL}$ concentrations are 4.9%, 4.0%, 3.8%, 3.3%, 0.8%, respectively.

Table 1: Linearity value of the β -1,3-1,6 glucan.

Sample No	Concentration ($\mu\text{g} / \text{mL}$)	Absorbance Values	Avarage Absorbance Values	% RSD
1	125	0.012	0.012	4.9
		0.012		
		0.011		
2	250	0.025	0.025	4.0
		0.026		
		0.024		
3	500	0.047	0.045	3.8
		0.044		
		0.044		
4	1000	0.077	0.08	3.3
		0.081		
		0.082		
5	2000	0.140	0.141	0.8
		0.140		
		0.142		

**Figure 1:** Linearity graph of the β -1,3-1,6 glucan.

For the reproducibility criterion, average recovery of 500 $\mu\text{g} / \text{mL}$ concentration was found to be 94%, RSD 4.1%.

The % RSD value for the active substances for repeatability is 2.0 %.

Recovery for β - (1,3-1,6) glucan was found to be 96.4%, 94% and 95.6% for 250, 500 and 1000 $\mu\text{g} / \text{g}$ concentrations, respectively, for extraction reproducibility and recovery. It is seen that these values are between the limit values of 80% - 120%.

Table 2: extraction reproducibility and recovery value of β -1,3-1,6 glucan.

Sample No	Concentration ($\mu\text{g} / \text{g}$)	Absorbance	Avarage Absorbance	% Recovery	% RSD
1	250	0.025	0.025	96.4	2.3
2	250	0.024			
3	250	0.025			
4	500	0.040	0.041	94	1.4
5	500	0.041			
6	500	0.041			
7	1000	0.074	0.075	95.6	1.3
8	1000	0.075			
9	1000	0.076			

The results of all these analytical validation criteria are consistent with the CDER values.

When the physical stability test results were taken into consideration, it was determined that there was no change in appearance, color, odor and taste at the end of the 0, 3 and 6 months.

As a result of the suspension tests made for the final product, the formulation showed no significant change in zeta potential, particle size, viscosity, rate of settling and sedimentation volume.

The analysis results at the end of the 0, 3 and 6 month stabilization of the particle size are 137.0 μm , 138.0 μm and 136.0 μm , respectively.

At the end of the stabilization at the 0, 3 and 6 months, the mean values of the sedimentation volume analyzes were 0.023. The F value of a normal suspension should be less than one.

The settling rate equilibrium (stock equilibrium) is used for suspensions with between 0.5 and 2% (g / mL) of substance. At the end of the stabilization at 0, 3, and 6 months, the average results of the analyzes were found to be 116,2 cm / s, 117,2 cm / s and 113,8 cm / s, respectively.

A flocculent and non-caking suspension should be below the zeta potential of 25 mV. The results of the analyzes at the end of the stabilizers of 0., 3. and 6. months for zeta potential were found to be 0.296 mV, 0.294 mV and 0.295 mV, respectively.

The results of the analyzes at the end of the stability at 0, 3 and 6 months for viscosity were 107.3 cP, 108.0 cP and 108.0 cP, respectively.

As a result of the 6-month short-term chemical stability test for the final product, the amount of active substance contained in the formulation prepared has proved to be stable for at least 6 months. The chemical stability was found to be 0.00% and 0.7%, respectively, for β - (1,3-1,6) glucan at the end of the 3rd and 6th months, respectively. In other words, it is below the limit of 5% according to CDER norms.

Table 3: Chemical stability tests results of β -1,3-1,6 glucan.

Period	Contents (mg/g)	Average Contents (mg/g)	% Loss
0.Day	10.1	10.1	0.0
	10.2		
	10.0		
3.Month	10.2	10.0	0.0
	10.0		
	9.8		
6. Month	10.0	9.9	0.7
	10.0		
	9.8		

Microbiological tests performed for the final product showed as ending aerobic mesophilic bacteria count <100 cfu / g, mold-yeast count <10 cfu / and *Escherichia coli* is not found. The results are consistent with the microbiological acceptance criteria for non-sterile dosage forms in the European Pharmacopoeia (aerobic mesophilic bacteria count <105 cfu / g, mold-yeast count <104 cfu / g and *Escherichia coli* = not available).

The RSD values for the fatty acid composition analysis at 0, 3 and 6 months for black seed oil were 0.51 for palmitic acid, 0.30 for stearic acid, 0.10 for oleic acid, 0.02 for linoleic acid and 0.22 for cis-11,14-eicosadienoic acid.

Table 4: Stability test results on the 0th day, 3rd month, 6th month for the composition of fatty acids made in the GC-FID of black seed oil.

	Area% Values			
Fatty Acids	0. Day	3. Month	6. Month	% RSD
Miristic acid (C14:0)	0.16	0.15	0.14	6.67
Pentadecanoic acid (C15:0)	0.03	0.03	0.02	21.65
Palmitic acid (C16:0)	11.86	11.92	11.80	0.51
Palmitoleic acid(C16:1)	0.21	0.21	0.20	2.79
Heptadecanoic acid (C17:0)	0.07	0.06	0.07	8.66
Stearic acid (C18:0)	3.29	3.28	3.30	0.30
Oleic acid (omega-9)(C18:1n9c)	24.32	24.30	24.35	0.10
Linoleic acid (omega-6)(C18:2n6c)	56.51	56.52	56.53	0.02
Gondoic acid (omega-9)(C20:1)	0.36	0.35	0.37	2.78
Linolenic acid (omega-3)(C18:3n3)	0.26	0.24	0.25	4.00
cis-11,14-eicosadienoic acid (omega-6)(C20:2)	2.60	2.59	2.60	0.22
Erucic acid (omega-9)(C22:1n9)	0.07	0.06	0.07	8.66
cis-13,16-docosadenosic acid (omega-6)(C22:2)	0.05	0.05	0.04	12.37
cis-4,7,10,13,16,19-docashexanoic acid (omega-3)(C22:6)	0.03	0.03	0.05	31.49

As a result of analyzes made at 0, 3 and 6 months for tocopherol, the values were 103.0, 102.3 and 100.2, respectively.

The results of the physical tests made for the final product of the prepared formulation are also suitable for the CDER criteria.

CONCLUSION

With the prepared formulation, an alternative to nutritional supplements which is becoming more and more important in everyday life is presented. Considering the positive effect on the immune system, the aim of this sector is to give an innovative product idea to this product prepared at the appropriate dosage.

As a result, it is thought that this suspension form, which is subjected to quality control analyzes and stability tests, may be an alternative to products containing β - (1,3-1,6) glucan, which is imported on the market.

REFERENCES

- Lydia Woods Schindler. Understanding the Immune System(e-book). U.S. Department of Health and Human Services. NIH Publication No. 92-529 Revised October 1991
- Abul K. Abbas, Andrew H. Lichtman, Shiv Pillai. Basic Immunology. Functions and Disorders of the Immune System(e-book). Elsevier Saunders Inc. 2014 Library of Congress Cataloging-in-Publication Data 978-1-4557-0707-2
- Apoorva Sethi, Neeraja Kulkarni, Sandip Sonar, Girdhari Lal. Role of miRNAs in CD4 T cell plasticity during inflammation and tolerance. Front. Genet., 31 January 2013. doi: 10.3389/fgene.2013.00008
- D. K. Meena, Pronob Das, Shailesh Kumar, S. C. Mandal, A. K. Prusty, S. K. Singh, M. S. Akhtar, B. K. Behera, Kundan Kumar, A. K. Pal, S. C. Mukherjee. Beta-glucan: an ideal immunostimulant in aquaculture. Fish Physiol Biochem. Springer Science+Business Media B.V. 2012. DOI 10.1007/s10695-012-9710-5.
- Luca Vannucci, Jiri Krizan, Petr Sima, Dmitry Stakheev, Fabian Caja, Lenka Rajsiglova et al. Immunostimulatory properties and antitumor activities of glucans(Review). International Journal of Oncology. 357-364 DOI: 10.3892/ijo.2013.1974
- Beta Glucan Research web sitesi. Beta Glucan Research – Saccharomyces cerevisiae. 2015 Beta Glucan Research Organization Last modified: 03/25/15 (<http://betaglucan.org/>)
- Helen S. Goodridge, Andrea J. Wolf, David M. Underhill. β -glucan recognition by the innate immune system. Immunological Reviews 2009 Vol. 230: 38–50
- Dalia Akramienė, Anatolijus Kondrotas, Janina Didžiapetrienė, Egidijus Kėvelaitis. Effects of β -glucans on the immune system. Medicina (Kaunas) 2007; 43(8)
- Jiezhong CHEN, Robert SEVIOUR. Medicinal importance of fungal β -(1-3),(1-6)-glucans. Mycological research 111(2007)635–652
- Said Gharby, Hicham Harhar, Dominique Guillaume, Aziza Roudani, Samira Boulbaroud, Mohamed Ibrahim et al. Chemical investigation of Nigella sativa L. seed oil produced in Morocco. Journal of the Saudi Society of Agricultural Sciences, 2014, 1658-077X (<http://dx.doi.org/10.1016/j.jssas.2013.12.001>)
- Mustafa Kiralana, Gülcan Özkan, Ali Bayrak, Mohamed Fawzy Ramadan. Physicochemical properties and stability of black cumin(Nigella sativa) seed oil as affected by different extraction methods. Industrial Crops and Products 57 (2014) 52–58
- Herman Lutterodt, Marla Luther, Margaret Slavin, Jun-Jie Yin, John Parry, Jin-Ming Gao, Liangli (Lucy) Yu. Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. LWT - Food Science and Technology 43(2010)1409-1413
- Salma Cheikh-Rouhou, Souhail Besbes, Basma Hentati, Christophe Blecker, Claude Deroanne, Hamadi Attia. Nigella sativa L.: Chemical composition and physicochemical characteristics of lipid fraction. Science direct Food Chemistry 101 (2007) 673–681
- Helvacızade Gıda İlaç Kimya San. Tic. A.Ş. 2013 Zade Vital web site. (<http://www.zadevital.com/neden-zade-vital/soguk-pres-ile-gelen-saglik>).
- Acartürk F., Ağabeyoğlu I., Çelebi N. ve arkadaşları (2009) “Modern Farmasötik Teknoloji”, Türk Eczacılar Birliği Eczacılık Akademisi Yayınları 2.Baskı.
- Nurşin Gönül. Çok Fazlı Sistemler 2 Süspansiyon ve Emülsiyon Teknolojisi. Ankara Üniversitesi Eczacılık Fakültesi, 2000, Yayın No:82
- Megazyme web sitesi. Megazyme International Co., Ireland. <http://secure.megazyme.com/>
- Guidance for Industry Q1A(R2) Stability Testing of New Drug Substances and Products, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), 2003.
- Automated test for use with TEMPO, for the enumeration of Total viable count in 40-48 hours in food products, Biomerieux TEMPO® TVC, 80 007, 2009.

Automated test for use with TEMPO, for the enumeration of Yeasts, mold in 72-76 hours in food products, Biomerieux TEMPO® YM, 80 001, 2010.

Automated test for use with TEMPO, for the enumeration of Escherichia coli in 22-27 hours in food products, Biomerieux TEMPO® EC, 80 004, 2009.

Guidance for Industry Bioanalytical Method Validation, U.S. Department of Health and Human Services Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), 2001.

European Pharmacopoeia 6th Edition 2007; 529-530.

THE EFFECTS OF *Pinus pinea* L. NEEDLES ESSENTIAL OIL FORTIFIED EGG WHITE PROTEIN POWDER BASED EDIBLE FILMS ON SOME PROPERTIES OF LOR CHEESE

Nazan KAVAS¹, Gökhan KAVAS²

¹Dairy Products Program, Ege Vocational Training School, Ege University, 35100 İzmir, Turkey

²Department of Dairy Technology, Faculty of Agriculture, Ege University, 35100 İzmir, Turkey

Corresponding author email : nazan.kavas@ege.edu.tr

Abstract

The object of this study was to investigate the possibility of *Pinus pinea* L. Needles Essential Oil and Egg White Protein Powder as edible coatings for lor cheese during ripening for 30 days. The effects of edible film (EWPP_{PPN}) produced by the addition (2% v/v) *Pinus pinea* L. needles essential oil (EO_{PPN}) to sorbitol (3% w/v) egg white protein powder (EWPP) based film on the antimicrobial properties of lor cheese were studied. All the cheese samples were contaminated with *E. coli* O157:H7 and *L. monocytogenes* (10⁷cfu⁻⁹) under controlled conditions. Counts of these organisms and yeast-mould were determined after the cheese production. Cheese samples were separately coated with these films and stored at +4°C for 30 days. Analyses were performed on the 1st, 7th, 15th and the 30th days of storage. With the addition of EO_{PPN} to the film, the antimicrobial and antifungal effects on of the studied microorganisms increased. Respectively *E. coli* O157:H7 was the most resistant microorganism to the essential oil while, *L.monocytogenes* was the most sensitive. EWPP showed a bacteriostatic effect on the microorganisms less than EWPP_{PPN}.

Keywords: *Pinus pinea* L needles essential oil, edible films, lor cheese, antimicrobial effect

INTRODUCTION

Nowadays, as it turns into a natural slogan, many researches have been made on the usability of edible films produced from natural biopolymers in the packaging of foods. Edible films and coatings are available with a wide range of properties that can help to alleviate many problems encountered with foods. Edible films can be prepared from protein, polysaccharides, lipids or the combination of these components with the addition of plasticizers and surfactants (Bourtoom, T. 2008). Plasticizers, antimicrobial agents, colors or flavors can be added in manufacturing proses. Antimicrobial active packaging with incorporation of essential oils (EOs) has been studied in the past decades as an alternative to traditional technologies to preserve food products (Hamdy et.al 2017)

Pinus pinea L. a member of the Pinaceae family grows natively in the northern Mediterranean and Aegean coastal regions; southern Europe, north Africa, Spain to Turkey (Demirci et.al. 2015). Little information on the chemical composition of the essential oils obtained from needles and/or branches of some *Pinus* species have been reported (Roussis et al.1995). According to Macchioni et al. (2003), in *P. pinea* the major compound of the essential oils of all the three plant parts is limonene (58.9–62.5%), in *P. halepensis*, α -pinene (18.1–53.6%) and myrcene (13.7–42.1%).

Lor cheese is a low-salt, low-fat form of white cheese with a high protein content. Lor cheese is a kind of local cheese produced by heat treatment of cheese whey which is produced during the production of kashar cheese or tulum cheese. After boiling the whey, which is strained from the curd, the remaining protein is curdled (Kavaz et.al. 2012).

The objective of this study was the production of edible film and coating for lor cheese from a combination of egg white protein powder and essential oil of *Pinus pinea* L. Needles plasticized with sorbitol. As an application, lor cheese will be coated using the formulation to identify the effectiveness of the edible film and coating on the microbiological parameters related to lor cheese shelf life.

MATERIAL METHODS

Production of Lor Cheese

Whey obtained during kashar cheese production was taken into a stainless steel tank and heated to 85 °C. During heating 2 % salt was added. The process was continued until the formation of curd. The product formed on the surface of the tank after the heating process was taken to a filtration tank and filtered in cold temperature (+4 °C) through mesh cloth for 2 days. Pressure was applied to the product to remove the excess whey content in the curd. Later, the cheese was split into three part. The first part control sample (K)(non coated) , the second part was covered with EWPP based film, Other part was covered with 2% EOPPN added films.

Plant Material

Needles of *Pinus pinea* L. were collected from Aydın-Turkey.

Isolation of the Essential Oil

The air dried needles were water distilled for 3 h using a Clevenger-type apparatus.

Analysis of Essential Oils and Volatile Compounds by GC/MS

GC/MS analyses were carried out using Agilent 6890 GC system with Agilent 5973 MS system. The column was used DB-5MS 30 m x 0.25 mm x 0.25 μ m (5% Diphenyl / 95% Dimethylpolysiloxan) Agilent. The oven temperature was initially held at 50°C /3 min, programmed to 160°C at 1.5°C/min, then to 315°C at 3°C/min and the final temperature was held for 30 min. the carrier gas helium with a flow rate of 1.0 ml/min. The split mode 1:20 was used. The injection volume was 1.0 μ l. The mass spectrometer was operated in the electron impact mode (70 eV). The ion source temperature was held at 230°C. The transfer-line was maintained at 280°C. The scanned mass range was from 30 to 500 u.

Preparation of Edible Film and Cheese Samples

Edible coatings were prepared according to the protocols established by Pintado et al.(2010) and McHugh and Krochta (1994), with some modifications. For the preparation of coating material, Alfazol® egg white protein powder (EWPP) was obtained from Kimbiatek Chemical Agents Inc. (İstanbul-Turkey). Egg white protein powder (EWPP) was dissolved in distilled water at concentrations of 3% w/v .The pH (8.0) was adjusted prior to adding plasticizer (3% w/v sorbitol) .All components were homogenized 20,000 rpm/ 1 dakika (3-16 K Type-Model, Sigma, Germany) and heated (90 $\pm$ 2 °C) for the given time (30 min). The film-solution was cooled to room temperature. Coating solution was filtered and divided into two equal parts; the first part was coated edible coating with only EWPP; the second part was coated edible coating with (2% v/v) *Pinus pinea* L. needles essential oil (EOPPN).

The *E. coli* O157:H7 (ATCC 43895) and *Listeria monocytogenes* (ATCC 19118) strains used for the artificial contamination of Lor cheese samples were obtained from Hemakim Corporation (Turkey). For the artificial contamination, 10⁷ cfu/g inocula were used.

Microbiological Analysis

L. monocytogenes was inoculated Palcam Listeria Selective Agar (Base) and incubating at 37°C for 48 hours. Then, colonies were counted (Food and Drug Administration, 2001). *E. coli* O157:H7 was enriched in selective modified EC broth at 35-37 °C'de for 24-48 h. For enumeration

of *E. coli* O157:H7 Sorbitol MacConkey Agar containing Cefixime-Tellurite Supplement was used and incubated at 35-37 °C for 24-48 h. After incubation, sorbitol negative colonies were counted. For yeast and mold enumeration, Yeast-extract-glucose chloramphenicol agar (YGC) (Merck 1.16000) was used with incubation at 25 °C for 3-5 days (IDF Standard 94 b, 1990).

Statistical Analysis

Descriptive statistical values of the findings were determined by using the SPSS Statistical Data Analysis Method.

RESULTS AND DISCUSSION

The components and percentages of the essential oil from *P. pinea* is shown in Table 1. Overall, thirty components were characterized, where limonene (52.8 %), α -pinene (6.4 %), β -caryophyllene (5.1 %) , β -phellandrene (4.7 %), Germacrene D (2.5 %), β -pinene(2.4 %) and myrcene (2.2 %) were identified as major constituents.

Table 1. Chemical composition of *Pinus pinea* L. essential oil

Compounds	%
Limonene	52.8
α -pinene	6.4
β -Caryophyllene	5.1
β -Phellandrene	4.7
Germacrene D	2.5
β -pinene	2.4
myrcene	2.2
Guaiol	1.9
Myrcene	1.7
α -Terpineol	1.2
Terpinolene	1.1

%: percentages were calculated from FID data

Needles of *Pinus pinea* L. essential oil was also screened against human pathogenic microorganisms such as *L.monocytogenes* and *E. coli* O157:H7. *Pinus pinea* L. essential oil showed the strong antimicrobial activity against *Escherichia coli* O157:H7 and *Listeria monocytogenes* in lor cheese samples (Table 2).

The antimicrobial effect of EWPP_{PPN} during storage was higher than that of EWPP and C sample. The antimicrobial effect of PPNs was higher on *L.monocytogenes* than *E. coli* O157: H7. The increase in the antimicrobial activity and the addition of essential oil was statistically significant ($p<0.05$). It was observed that EWPP and EWPP_{PPN} had an antifungal effect against yeasts and moulds on the 30th day. The increase in antifungal activity of PPN essential oil film was statistically significant ($p<0.05$).

Table 2: Microorganism counts and standard deviation values in the samples coated with EWPP and EWPP_{PPN} films and non-coated samples (n=3); (p<0.05)

Microorganisms	Day	Log ₁₀ cfu/g		
		K	EWPP	EWPP _{PPN}
<i>E.coli</i> O157:H7	1	7.05±0.81 ^{aA}	7.04±0.73 ^{aA}	7.03±0.83 ^{aA}
	7	7.45±0.88 ^{aA}	7.35±0.96 ^{aA}	5.98±0.56 ^{bB}
	15	7.89±0.92 ^{aB}	7.52±1.15 ^{aB}	5.70±1.13 ^{bC}
	30	7.99±0.98 ^{aC}	7.65±1.02 ^{aA}	5.20±0.38 ^{bC}
<i>L.monocytogenes</i>	1	7.02±0.30 ^{aA}	7.01±0.53 ^{aA}	7.06±1.24 ^{aA}
	7	7.20±0.52 ^{bA}	7.05±0.64 ^{bA}	5.83±0.42 ^{cB}
	15	7.65±0.23 ^{cB}	7.00±0.70 ^{bA}	4.50±0.86 ^{cC}
	30	7.80±0.50 ^{cB}	6.85±0.85 ^{bB}	4.01±0.23 ^{cD}
<i>yeast and mold</i>	1	6.50±0.92 ^{aA}	6.54±0.04 ^{aA}	6.48±1.28 ^{aA}
	7	7.20±0.50 ^{bA}	6.38±1.12 ^{aB}	3.24±1.13 ^{bC}
	15	7.55±0.63 ^{bA}	6.21±1.03 ^{bB}	2.90±0.43 ^{cC}
	30	7.80±0.90 ^{cA}	5.99±0.73 ^{cB}	2.35±0.81 ^{cC}

^{a,b,c}: The differences between the values in the same column are statistically significant (p < 0.05).

^{A,B,C}: The differences between the values in the same line are statistically significant (p < 0.05).

CONCLUSIONS

In our study, with the addition of EO_{PPN} to the film, the antimicrobial and antifungal effects on of the studied microorganisms increased. Respectively *E. coli* O157:H7 was the most resistant microorganism to the essential oil while, *L.monocytogenes* was the most sensitive. EWPP showed a bacteriostatic effect on the microorganisms less than EWPP_{PPN}. In our study, fortification of Sorbitol , Egg White Protein powder and *Pinus Pineae* L. essential oil may be helpful in the extension of the shelf lives of lor cheese and that further studies on this subject should be carried out.

REFERENCES

- Bourtoom, T. 2008. Edible films and coatings: characteristics and properties. International Food Research Journal 15(3): 237-248
- Demirci, F , Bayramiç, P , Göger, G , Demirci, B , Başer, K . (2015). Characterization and Antimicrobial Evaluation of the Essential Oil of *Pinus pinea* L. from Turkey. Natural Volatiles & Essential Oils, 2 (2), 39-44.
- F. Macchioni, P.L. Cioni, G. Flamini, I. Morelli, S. Maccioni and M. Ansaldi, Chemical composition of essential oils from needles, branches and cones of *Pinus pinea*, *P. halepensis*, *P. pinaster* and *P. nigra* from central Italy. Flav Frag J, 18, 139-143 (2003).
- Food And Drug Administration. 2001. Bacteriological Analytical Manual Online. <http://www.fda.gov> (accessed 2018).
- Hamdy A. Shaaban, Hatem S. Ali, Gamil F. Bareh, Abdel Rahman S. Al-khalifa and May M. Amer, 2017. Antimicrobial Activity of Two Polysaccharide Edible Films Incorporated with Essential Oils against Three Pathogenic Bacteria. Journal of Applied Sciences, 17: 171-183.
- IDF Standard 94 B. 1990. Milk and milk products - enumeration of yeast and moulds — colony count technique at 258°C, Brussels, Belgium
- Kavaz,A.,Arslaner A, Bakirci,İ. 2012. Comparison of quality characteristics of Çökelek and Lor cheeses . African Journal of Biotechnology Vol. 11(26), pp. 6871-6877, 29
- Mchugh, T.H. And Krochta, J.M. 1994. Milk protein based edible films and coatings. Food Technol. 48, 97–103.
- Pintado, C.M.B.S., Ferreira, M.A.S.S. And Sousa, I. 2010. Control of pathogenic and spoilage microorganisms from cheese surface by whey protein films containing malic acid, nisin and natamycin. Food Control 21, 240–246.
- V. Roussis, P.V. Petrakis, A. Ortiz and B.E. Mazomenos, Volatile constituents of needles of five *Pinus* species grown in Greece. Phytochemistry, 39, 2, 357 (1995).

THE INVESTIGATION OF GIBBERELIC ACID EFFECTS ON SEED GERMINATION OF PHACELIA (*Phacelia tanacetifolia* Benthmam) UNDER SALT STRESS

Ruveyde TUNCTURK¹, Barış KOCAMAN¹, Mahdi GHIYASI², Murat TUNCTURK¹

¹Van Yuzuncu Yıl University, Faculty of Agriculture, Department of Field Crops, Van-Turkey

²Urmia University, Faculty of Agriculture, Department of Field Crops, Urmia-Iran

Corresponding author email: ruveydetuncturk@yyu.edu.tr

Abstract

The research was conducted to determine the properties of gibberellic acid pre-treatments that reducing the negative effects of salt stress on the seeds germination of (*Phacelia tanacetifolia* Benthmam) plant under salt stress. In the study, three different gibberellic acid (0, 100, 200 and 300 ppm) were pre-treated; for the control group, no hormone pre-treatment was performed. Seeds planted in petri dishes have been left to germinate after three salt concentrations application 0, 50, and 100 mM (NaCl). Responses of phacelia to priming and salt stress treatments has been observed in terms of some growth and viability (radicle and hypocotyl length, radicle fresh and dry weight, hypocotyl fresh and dry weight, germination power, germination speed, mean germination time, germination and sensitivity index) parameters. As a result of the research; as the salt concentrations increase, the germination and growth parameters of phacelia seeds have been inhibited. It has been determined that the increasing doses of gibberellic acid pre-treatments have significant and positive effects on the germination and growth parameters of phacelia seeds under salt stress conditions. In physiological enhancement of phacelia seeds, the best results were obtained from the 300 ppm GA3+0 Mm (control) salt combination.

Keywords: Gibberellic acid, salt, phacelia (*Phacelia tanacetifolia* Benthmam), germination

INTRODUCTION

Phacelia tanacetifolia Benthmam is an annual herbaceous plant belonging to the family Hydrophyllaceae, native to North America. Phacelian's flowers have rich in terms of nectar and pollen. It is used as bee grassland because of was very long (approximately six or eight week) of flowering time in the country such as USA, Germany, Russia and Serbia. It known as a very good source of nectar all over the world by bee keepers, is among the first 20 plants among the nectar plants in the world (Crane, 1975). Phacelia is also grown plant as green fertilizer, cover plant, green and dry grass, silage, medicinal plant, ornamental plant, soil improver, erosion as well as being grown to increase honey production (Farkas and Zajacz, 2007; Dag, 2013). In addition, it is useful as cut flower due to it has attractive and long vase life (Tiryaki and Keles 2012). Nectar secretion of phacelia was determined as 0.80-0.85 mg/flower/day, honey potency 30-100 kg/da, pollen yield 0.5 mg/flower and pollen potential 13.3 kg/da (Crane et. al.,1984).

There are about 13 species of phacelia in the world, but four species (*Phacelia distans*, *P. ramosissima*, *P. hispida*, *P. tanacetifolia*) are a valuable source of nutrients for honeys (Dag,2013). The most cultivated species in our country is *P. tanacetifolia* Benthmam (Everett, 1963).

It is stated that the germination of phacelia has to in absolute darkness and at low temperature (Chen and Thimann 1964; Chen and Thimann 1966). Germination-inhibiting effects of light and high temperature of *P. tanacetifolia* seeds have been previously described (Schulz and Klein 1965). Reports also indicate that the inhibitory effect of light exposure decreased when seeds were treated with gibberellic acid (GA3) and germinated at temperatures below 8 °C (Macchia et. al. 2001), even though other reports did not recommend any treatment to enhance the germination of phacelia seeds (Emery, 1988). It is known that some salt solutions and plant hormones can be used to remove the physiological dormancy seen in the seeds. For this reason, the most effective plant hormone is GA (Hendricks and Taylorson, 1975).

The purpose of this study is to determine the effects of different concentrations of gibberellic acid hormone under salt stress in removing post-harvest light and high temperature dormancy in phacelia seeds.

MATERIALS AND METHODS

This study, in order to evaluate the effect of seed priming on the germination and seedlings' growth traits of *Phacelia. tanacetifolia* Benthmam under salinity stress, a factorial experiment was conducted in completely randomized design (CRD) with four replications, at the laboratory of Seed Science and Techonology in the Department of Field Crops, Faculty of Agriculture, Van Yuzuncu Yil University, Turkey during the year 2018. Three salt levels (0, 50, 100 NaCl mM) and four gibberellic acid (GA3) levels (0, 100, 200 and 300 ppm) were used in the experiments. Seeds were rinsed with water and distilled water, then surface sterilized in 20% sodium hypochlorite (vol/vol) for 5 minutes, followed by 70% alcohol (vol/vol) for 1 seconds; after, were thoroughly rinsed with sterile deionized water and then seeds were primed.

The seed lots were imbibed with in different doses gibberellic acid (GA3) in priming treatments. Treated seed lots with GA3 were kept in darkness in an incubator at 25 ± 0.5 °C for 24 h. Seeds which were removed from the solutions (GA3) than air dried for 24 hours at 25 °C to reach the original moisture content (12-13%) and proceed for germination test as suggested by ISTA. Unprimed seeds were used as control. Treated seeds were placed in 9 cm diameter sterile petri dishes with filter paper and 20 seeds were placed in each petri dish and 5ml of salt solution was added dish to each petri except for control treatments, to the control treatments was given distilled water (5ml), then petri dishes were placed in a totally dark incubator which stable at temperature (25 ± 0.5 °C). As a final step, after 14 days all parameters were observed, measured and recorded. The root and shoot vigor were calculated as the sum of total root length (cm) and shoot length (cm) of all the seedlings of a replicate divided by the number of seedlings. Fresh root and shoot were then placed in a hot air oven (70 °C for 24 hours) to dry (Schulz and Klein, 1965). Root and shoot dry mass were weighed germination rate, germination power, germination index, mean germination time and sensivity index were calculated with the following formulas. It was considered that number of germinated seeds on day 7 as "germination rate" and number of germinated seeds on 14 st day as "germination power". Germination rate (GR) was calculated using the Equation 1 (Macchia et. al. 2001).

GR = Total seeds germinated after day 14/Total number of planted seeds (Equation 1)

Germination index (GI) was calculated using the Equation 2 (Emery, 1988).

$$GI = \Sigma(G_i/T_t) \text{ (Equation 2)}$$

GI: Germination index; G_i : i. Days germinated seed rate; T_t : count day

Mean germination time (MGT) was calculated using the Equation 3 (Hendricks and T aylorson, 1975).

$$MGT = \Sigma(fx)/\Sigma f \text{ (Equation 3)}$$

f: Number of seeds germinated x: germination day

Sensivity index (SI) was calculated using the Equation 4 (Younesi and Moradi, 2015).

$$SI = \text{MGT in the salt application} / \text{MGT in the control application} \text{ (Equation 4)}$$

Data were analyzed statistically by using analysis of variance with COSTAT (version 6.3) software. Data were analyzed by using both one- or two-way analysis of variance (ANOVA) and comparison of the means was made according to the Duncan test (Singh and Jakhar, 2018).

RESULTS AND DISCUSSION

In this study, salinity and seed priming applications showed that it has a significant effect on all studied characters. Only, the effect of gibberellic acid on the sensitivity index was found insignificant. Furthermore, between factors was found significant interactions (salinity stress x seed priming) for germination power (%), germination index (%), sensitivity index (%), radicle length (cm) and fresh radicle weight (mg). According to this study, while the highest germination power was obtained as 100 % from primed seeds with 100 ppm GA3 and 0 mM (control) NaCl applications, the lowest germination power (73.33 %) was reported from seeds primed with 100 ppm GA3, in salt stress (100 mM NaCl) conditions. Similarly, the best results for germination index were recorded as 85.77 % from 200 ppm GA3 doses with control (0 mM NaCl) application, the lowest value also was obtained as 41.58 % from 200 ppm GA3 with 100 mM NaCl applications.

Table 1. Effect of gibberellic acid application on germination in phacelia plant under salt stress

Applications		Germination power (%)	Germination rate (%)	Mean germination time (day)	Germination index (%)	Sensitivity index (%)
Salt Doses (mM)	GA3					
Kontrol (T0)	GA0 (kontrol)	93.33 a	86.66	2.00	67.16 b	0.36 c
	GA100	100.00 a	90.00	2.56	79.77 a	0.94 b
	GA200	96.66 a	83.33	1.81	85.77 a	1.03 b
	GA300	91.66 ab	88.33	1.71	80.22 a	0.89 b
T0 Ort.		95.41 a	87.08 a	2.02 c	78.23 a	0.80 b
50 mM (T5)	GA0 (kontrol)	80.0 bcd	73.33	2.34	50.37 cd	1.92 a
	GA100	95.00 a	81.66	3.04	45.90 cd	1.28 b
	GA200	90.00 ab	83.33	2.53	65.97 b	1.04 b
	GA300	96.66 a	91.66	2.66	64.01 b	1.23 b
T5 Ort.		90.41 a	82.5 a	2.64 b	56.56 b	1.36 a
100 mM (T10)	GA0 (kontrol)	76.66 cd	70.00	2.67	47.39 cd	1.16 b
	GA100	73.33 d	61.66	3.55	42.12 d	1.14 b
	GA200	86.66 abc	83.33	2.80	41.58 d	1.22 b
	GA300	95.0 a	86.66	2.94	53.49 c	1.15 b
T10 Ort.		82.91 b	75.41 b	2.99 a	46.14 c	1.17 a
GA3 Dozu Ort.	GA0 (kontrol)	83.33 b	76.66 b	2.34 b	54.97 b	1.15
	GA100	89.44 ab	77.77 b	3.05 a	55.93 b	1.12
	GA200	91.11 a	83.33 ab	2.38 b	64.44 a	1.09
	GA300	94.44 a	88.88 a	2.44 b	65.91 a	1.09
CV (%)		7.89	10.23	14.74	9.38	25.76

As for the sensitivity index, increasing salt applications have also increased these values. While the highest value (1.92 %) of sensitivity index was determined from 0 ppm GA3 dose with 50 mM NaCl applications, the lowest value also (0.36 %) was determined from controls of both applications.

In terms of salinity stress, the highest germination rate was determined as 87.08 % from 0 mM (control) salt treatments, also the lowest value was obtained as 75.41 % from 100 mM NaCl applications. In addition, the effect of gibberellic acid on the germination rate was found significant and the best mean (88.88 %) was determined from 300 ppm GA3 dose, lowest germination rate (76.66 %) is due to control of GA3. But, between 0 and 100 ppm GA3 doses of gibberellic acid there isn't any a different as statistically. Based on statistical data, the shortest mean germination time (2.34 day) of seeds primed with 0 ppm GA3. Also, between 0, 200 and 300 ppm GA3 doses of gibberellic acid there isn't any a different as statistically. The longest time for germination (3.05 day) was recorded from 100 ppm GA3 doses. In terms of salinity stress, mean germination time (2.02 day) shortened with control (0 mM NaCl) application. The longest average germination time (2.99 day) recorded from 100 mM NaCl treatments. The results showed that phacelia is highly sensitive to salinity in the germination stage. In carried previous work by Younesi and Moradi¹³ reported that is consistent with the hypothesis that under salinity stress, priming can prepare a suitable metabolic reaction in seeds

and can improve seed germination performance and seedling establishment. These results agree with Singh and Jakar, 2018, in *Vigna mungo*, Al-Thabet et.al. 2004, in rapeseed and Fardus et al. 2018 in wheat, they suggested that salinity may influence germination by decreasing the water uptake. and germination percentage declined with increase of salinity level. However, osmotic stresses reduce germination percentage (Cornelia and Bandici 2008; Maghsoudi and Arvin 2010). Also, the seeds pre-treated with GA3 solutions exhibited higher germination percentage. Koornneef and Veen 1980; Ogava et al. 2003 reported that phytochromes promote seed germination partly by increasing bioactive GA levels in seeds. Sabra et al. 2012 reported that photosynthetic activity in echinacea was affected by salinity and Zollinger et al. 2007 also found a similar reduction in photosynthetic rate and stomatal conductance in *E. purpurea* irrigated with increasing concentrations of salinity. In addition, Sanam et al. 2014 different levels of salinity significantly reduced germination rate, germination vigor and index. The results also showed that by increasing salinity levels, the percentage of germination and normal seedlings significantly decreased and the mean time to germination increased, compared to the control treatment. But the seeds treated with GA3 showed higher viability and better performance under salinity stress condition. Ali et al. 2012; Zadeh et al. 2015 reported that application of GA3 enhanced growth parameters (shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight, leaf area etc.) under saline condition. These results are consistent with those of Jamil and Rha 2007 in sugar beet, Erdemli and Kaya 2015 in sunflower, Yıldız et al. 2017 in sweet william, Singh and Jakhar 2018, in *Vigna mungo* and Zadeh et al. 2015 in echinacea. Promotion in seed germination with GA3 may be due to increased uptake of oxygen and α -amylase activity (Fardus et al. 2018).

Macchia et al. 2001 reported indicate that the inhibitory effect of light exposure decreased when seeds were treated with gibberellic acid (GA3) or potassium nitrate (KNO₃) and germinated at temperatures below 8°C in phacelia. Yildiz et al. 2017; Yalcın, 2010 recorded that the positive and significant effects of GA3 applications on germination power were found. Jamil and Rha 2007 reported that water uptake of primed seeds also increased significantly with increasing concentration of GA3 as compared to control. In previous studies carried in phacelia, reported that the germination of light inhibited seeds partially recovered their germination capacity after treatment for seeds with GA3, supporting the involvement of GA in this phenomenon (Yamaguchi and Kamiya 2002; Chen and Chang, 1972). In study results conducted by Tiryaki and Keles, 2012 showed that melatonin significantly reversed the inhibitory effects of light and high temperature of phacelia seed germination. Erdemli and Kaya 2015 recorded that it was concluded that seed treatment with GA3 can be beneficial for decreasing the effect of abiotic stress conditions on germination. It was seen that as gibberellic acid dose increases germination parameters were improve when compared to control. The effect germination inhibiting in seeds of salt has been revealed in many studies. It has been reported that it was caused to the inhibition of water intake of high salt concentration on the decrease of germination rate, toxicity of salt and the cannot be activated due to salt stress of required enzymes during germination (Mansour, 1994; Essa, 2002; Sadeghian and Yavari, N. 2004). However, Seyedi et al. 2012 reported that, with increasing salinity stress germination characteristics such as germination percentage, germination rate and seedling fresh weight decreased.

All of the early growth parameters has decreased as parallel with salt application. Priming of seeds with different materials particularly GA3 was useful for alleviating salt stress effects and improving germination and seedling establishment under salt stress. Under salinity condition, primed seeds possessed more germination and emergence than control. According to the results of the study; the highest values for investigated characters such as radicula length and fresh radicula weight were determined as 9.90 cm and 89.1 mg from 300 ppm GA3 doses with control applications (0 mM NaCl). The most negative results for radicula length was determined as 4.38 cm from 200 ppm GA3 doses with 100 mM salt stress applications. The lowest fresh radicula weight also was determined as 16.63 mg from 0 ppm GA3 (control) with 100 mM salinity stress applications.

Table 2. Effect of gibberellic acid application on germination in phacelia plant under salt stress.

Applications		Radicula length (cm)	Plumula length (cm)	Fresh radicula weight (mg)	Fresh plumula weight (mg)	Dry radicula weight (mg)	Dry plumula weight (mg)
Salt Doses (mM)	GA3 (ppm)						
Kontrol (T0)	GA0 (kontrol)	6.08 cd	8.98	44.16 cde	125.0	3.03	6.40
	GA100	8.43 b	7.98	50.13 cd	139.73	3.26	7.13
	GA200	8.85 ab	9.61	76.1 b	154.46	4.46	7.63
	GA300	9.90 a	10.5	89.1 a	207.6	4.13	8.56
T0 Ort.		8.31 a	9.27 a	64.97 a	156.7 a	3.72 a	7.43 a
50 mM (T50)	GA0 (kontrol)	6.03 cd	5.56	36.06 ef	88.60	2.40	5.56
	GA100	5.63 cde	5.68	38.43 def	143.70	2.63	6.33
	GA200	7.05 c	5.65	55.06 c	144.0	3.36	7.30
	GA300	6.24 cd	7.21	75.2 b	171.76	2.40	6.4
T50 Ort.		6.24 b	6.02 b	51.19 b	137.01 a	2.70 b	6.40 b
100 mM (T10)	GA0 (kontrol)	4.43 e	4.23	16.63 g	92.96	1.70	4.96
	GA100	4.58 e	4.75	28.4 fg	94.56	2.16	3.90
	GA200	4.38 e	4.53	42.13 cde	122.9	2.66	6.20
	GA300	5.50 de	5.86	31.86 ef	144.33	1.86	5.16
T100 Ort.		4.72 c	4.84 c	29.75 c	113.69 b	2.10 c	5.05 c
GA3 Ort.	GA0 (kontrol)	5.51 c	6.26 b	32.28 c	102.18 c	2.37 b	5.64 b
	GA100	6.21 bc	6.13 b	38.98 c	126.0 b	2.68 b	5.78 b
	GA200	6.76 ab	6.60 b	57.76 b	140.45 b	3.50 a	7.04 a
	GA300	7.21 a	7.86 a	65.38 a	174.56 a	2.80 b	6.71 ab
CV (%)		12.88	11.77	15.07	17.30	19.61	19.19

In terms of gibberellic acid applications, the most positive results for the characters such as plumula length and fresh plumula weight were obtained from 300 ppm GA3 applications as 7.86 cm and 174.56 mg, respectively. The highest dry radicle and plumula weight were obtained as 3.50 and 7.04 mg from 200 ppm GA3 doses. In this experiment, we observed that, growth parameters reduced with increasing salinity (Table 2) which was similar with the report of Sabra et. al. 2012 in echinacea species, Nazarian, 2016 in rapeseed, Salahuddin et al. 2017, in mungbean, Fardus et. al. 2018 in wheat and Singh and Jakhar, 2018 in *Vigna mungo*.

CONCLUSIONS

In terms of some growth and viability parameters (radicle length, shoot length, radicle age and dry weight, shoot age and dry weight, germination strength, germination rate, average germination time, germination and sensitivity index), it was evaluated reactions to priming and salt application of phacelia seeds. According to the results of this research; the germination and growth parameters of phacelia seeds are inhibited as the salt concentration increases. In the salt stress conditions exposed of phacelia seeds have been found to have a significant positive effect on germination and growth parameters of increased doses gibberellic acid pre-treatments. the highest results were obtained from the combination of 300 ppm GA3 and 0 mM (control) salt in the improving as physiological of phacelia seeds. As a result; GA3 treatments before germination to phacelia seeds effect germination inhibitory that caused of salt stress, significantly destroyed and the average germination time was shortened.

REFERENCES

- Al-Thabet, S.S., Leilah, A.A., Al-Hawass, I., 2004, Effect of NaCl and incubation temperature on seed germination of three canola (*Brassica napus* L.) cultivars, Scientific Journal of King Faisal University (Basic and Applied Sciences), 5(1): 1425pp.
- Ali, H. M., Siddiqui, M. H., Basalah, M. O., Al-Whaibi, M. H., Sakran, A. M., Al-Amri, A., 2012. Effects of gibberellic acid on growth and photosynthetic pigments of *Hibiscus sabdariffa* L. under salt stress. African Journal of Biotechnology Vol. 11(4), pp. 800-804.
- Chen S.S.C., Chang J.L.L. 1972. Does gibberellic acid stimulate seed germination via amylase synthesis? Plant Physiol; 49:441–442.
- Chen S.S.C., Thimann K.V. 1964. Studies on the Germination of Light-Inhibited Seeds of *Phacelia tanacetifolia*. Israel Journal of Botany 13:57-73.
- Chen S.S.C., Thimann K.V. 1966. Nature of Seed Dormancy in *Phacelia tanacetifolia*. Science 153:1537-1539.
- Crane, E., 1975. Honey. A Comprehensive Survey. Heinemann in Cooperation with International Bee Research Association. London. UK.608 pp.
- Crane, E., Walker, P., Day, R., 1984. Directory of Important World Honey Sources. International Bee Research Association. London.
- Cornelia, P., Gh. E. Bandici (2008). The effect of the salicylic acid on the peroxidase activity and on the growth of the barley (*Hordeum vulgare*) seedling under salt stress. Bulletin UASVM, Agriculture 65: 208-211.
- Dag, V., 2013. The Effects of Different Nitrogen Doses on The Yield, And Agricultural Characters of Bee (*Phacelia tanacetifolia* Benthany' s. Yuzuncu Yil University, Graduate Thesis, Department of Agronomy.
- Emery D. E., 1988. Seed Propagation of Native California Plants. Santa Barbara Botanic Garden, Santa Barbara, CA; pp. 115.
- Erdemli, H., Kaya, M. D., 2015. The Effects of Gibberellic Acid Doses on Yield and Germination under Abiotic Stress Conditions in Sunflower (*Helianthus annuus* L.). Field Crops Central Research Institute Journal, 24 (1):38-46.
- Essa, T.A. 2002. Effect of salinity stress on growth and nutrient composition of three soybean (*Glycine max* L. Merrill) cultivars. Journal of Agronomy and Crop Sci. 188, 86-93.
- Everett, T. H., 1963. New Illustrated Encyclopedia of Gardening. The Greenstones Press. New York.USA.
- Fardus, J., Matin, M. A., Hasanuzzaman, M., Hossain, M. A., Hasanuzzaman, M., 2018. Salicylic Acid-Induced Improvement in Germination and Growth Parameters of Wheat Under Salinity Stress. The Journal of Animal & Plant Sciences, 28(1): 197-207.
- Farkas, A., Zajacz, E., 2007. Nectar production for the Hungarian honey industry. The European Journal of Plant Science and Biotechnology, 1, 125–151.
- Hendricks S.B., Taylorson R.B. 1975. Breaking of Seed Dormancy by Catalase Inhibition. Proc. Natl. Acad. Sci. U.S.A. 72:306-9.
- Jamil, M., Rha, E. S., 2007. Gibberellic Acid (GA₃) Enhance Seed Water Uptake, Germination and Early Seedling Growth in Sugar Beet under Salt Stress. Pakistan Journal of Biological Sciences 10 (4): 654-658.
- Koornneef M, Veen V. D., 1980. Induction and analysis of giberellin- sensitive mutants of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet; 58:257–263.
- Macchia M, Angelini LG, Ceccarini L. 2001. Study on germination characteristics of *Phacelia tanacetifolia* Benthany seeds obtained from different growing seasons. Ital J Agron. 4:61–66.
- Maghsoudi , K., M.J. Arvin (2010). Salicylic acid and osmotic stress effects on seed germination and seedling growth of wheat (*Triticum aestivum* L.) cultivars. Plant Ecophysiol. 2:7-11.
- Mansour, M.M.F. 1994. Changes in growth, osmotic potential and cell permeability of wheat cultivars under salt stress. Biological Plant, 36: 429-434.
- Nazarian, G. R., 2016. The Effects of Priming By Salicylic Acid Under Salt Stress on Some Morphological and Physiological Characteristics of Canola Plant. PhD Thesis, Department of Field Crops, Ege University Graduate School of Science.
- Ogawa M, Hanada A, Yamauchi Y., 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell; 15:1591–1604.
- Sabra, A., Daayf, F., Renault, S., 2012. Differential physiological and biochemical responses of three Echinacea species to salinity stress. Scientia Horticulturae 135: 23-31.
- Sadeghian, S.Y., Yavari, N. 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. Journal of Agronomy and Crop Science 190,138-144.
- Sanam, S. A., Pirdashti, M. H., Mirjalili, M. H., Hashempour, A., 2014. Effect of exogenous nitric oxide on germination and some of biochemical characteristics of purple coneflower (*Echinacea purpurea* L.) in saline condition. Zist/shinasi Giyahi Iran, 6 (20): 55-74 .
- Salahuddin, M., Nawaz, F., Shahbaz, M., Naeem, M., Zulfikar, B., Shabbir, R. N., Hussain, R. A., 2017. Effect of exogenous nitric oxide (NO) supply on germination and seedling growth of mungbean (cv. Nm-54) under salinity stress. Legume Research, 40(5) : 846-852.
- Schulz MR, Klein RM. 1965. On the mechanisms of light-induced germination inhibition of *Phacelia tanacetifolia*. Am J Bot.; 52:278–281.

- Seyedi, M., Hamzei, J., Fathi, H., Bourbour, A., Dadrasi, V., 2012. Effect of seed priming with zinc sulfate on germination characteristics and seedling growth of Chickpea (*Cicer arietinum* L.) under salinity stress. *International Journal of Agriculture: Research and Review* 2 (3) Electronic Center for International Scientific Information, 108-114.
- Singh, S., Jakhar, S., 2018. 24-Epibrassinolide Mediated Changes on Germination and Early Seedling Parameters of *Vigna Mungo* (L). Hepper Var. Shekhar-2 under Salinity Stress. *Pertanika J. Trop. Agric. Sci.* 41 (1): 485 – 494.
- Tiryaki, I., Keles, H., 2012. Reversal of the Inhibitory Effect of Light and High Temperature on Germination of *Phacelia tanacetifolia* Seeds by Melatonin. *J. Pineal Res.* 52: 332-339.
- Younesi, O., Moradi, A., 2015. Effect of different priming methods on germination and seedling establishment of two medicinal plants under salt stress conditions. *Cercetari Agronomice în Moldova* 48 (3) Iasi: Editura "Ion Ionescu de la Brad", 43-51.
- Yamaguchi S, Kamiya Y., 2002. Gibberellins and light-stimulated seed germination. *J Plant Growth Regul*; 20:369–376.
- Yalcin, O. F., 2010. The Investigation Of Effects Of Gibberellic Acid And IAA (Indol-3-Acetic Acid) On Germination Of Seed Of *Silene Salsuginea* Hub.-Mor. Under Salt Stress. Selcuk University, Institute of Science, Ms. Thesis.
- Yıldız, S., Karagoz, F. P., Dursun, A., 2017. Germination in Salt Stress of Sweet William (*Dianthus barbatus* L.) Seeds Applied Pretreatment of Gibberellic Acid. *Atatürk Univ., J. Of The Agricultural Faculty*, 48 (1): 1-7.
- Zollinger, N., Koenig, R., Cerny-Koenig, T., Kjelgren, R., 2007. Relative salinity tolerance of intermountain western United States native herbaceous perennials. *HortScience* 42, 529–534.
- Zadeh, S. Y., Ramin, A. A., Baninasab, B., 2015. Effect of gibberellic acid, stratification and salinity on seed germination of *Echinacea purpurea* cv. Magnus. *Herba Polonica*; 61(3): 13-22.

EVALUATION THE PERFORMANCE OF SOME SAFFLOWER (*Carthamus tinctorius* L.) GENOTYPES UNDER MEDITERRANEAN CLIMATE CONDITIONS BASED ON SEED YIELD AND OIL YIELD.

Mouloud BOUHOUHOU¹, Samy Mostafa MOHAMED², Elsayed AbouElfotowh OMER² and Mourad BENSARI³.

¹ENS Assia Djébar, Constantine, Algeria.

²Medicinal and Aromatic Plants Research Department, National Research Centre, Cairo, Egypt.

³Genetics, Biochemistry and Plant Biotechnologies Laboratory – University Mentouri, Constantine, Algeria.

Corresponding author email: bouhouhoumouloud@gmail.com

Abstract

Since Safflower is a drought tolerant crop, the objective of this research was the investigation of seed and oil yields of safflower varieties under semi-arid conditions.

The research revealed that the most suitable safflower variety, under semi-arid conditions of eastern Algeria was CYPRUS which's providing from ICARDA, it gave the highest seeds yield (420.53 g/m²) and oil yield (114,56 g/m²). The American variety 80/482/3S come in second with 358,46 g/m² and 80,29 g/m² for the two yields respectively, while the least variety is OT-455 (Indian origin) with 144,68 g/m² for seeds yield and 40,63 g/m² for oil yield. Considering the seeds fixed oil percentage (%OY), we note the advantage of American varieties, where GILA produced the highest percentage (38.47 %), and KUSUMBA (Pakistani origin) had the lowest with 23,30 %.

Keywords: *Carthamus tinctorius* L., seed yield, oil yield, semi-arid areas.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.), which is a member of the family Asteraceae (Compositae), is a multi-purpose plant cultivated since ancient times not only for the dye contained in its flowers, the oil in its achenes, and its medicinal properties, but also for the ornamental value of its colorful inflorescences. In ancient Egypt, safflower wreaths or garlands were used at funerals (Uher, 2005).

Most countries in West Asia and North Africa (WANA) have a critical and growing shortage of edible vegetable oils, which are healthier and cheaper than animal fats. The demand for vegetable oils, both from the shift in consumer behavior, and from population growth has been met nearly in total by imports, which drains the countries money reserves (Yau, 2003).

In 2007, world safflower acreage and production were around 725.000 ha and 536.000 tones respectively. The world safflower acreage constitutes less than 0.5% of total area planted with oilseed crops (FAO, 2008).

The objective of this research was the investigation of the seed yield and oil content of different safflower varieties under semi arid conditions (Mediterranean climate) in eastern Algeria.

MATERIAL AND METHODS

Nine safflower (*Carthamus tinctorius* L.) varieties ('SYPRUS' from ICARDA and the others from USDA germplasm of Maryland, USA) (Table 1) were cultivated in a randomized complete block design (RCBD) experiment with three replicates. Each plot had 3 rows of 2m length with inter and into row spacing of 50 and 10cm, respectively. The investigation was conducted during the 2007-2008 season (sowing in the last week of December) in the experimental area of the regional station of INPV (National Institute of Plant Protection) in Constantine (Elevation: 555.96m, Latitude: 36°20' N, Longitude: 06°38' E).

Table 1: Origins and names of varieties used in the study.

Nº	Varieties	Origin	Source
1	80/482/3S	USA	USDA germplasm, Maryland, USA
2	FINCH		
3	GILA		
4	KUSUMBA	Pakistan	
5	OLE	USA	
6	OT-455	India	
7	RIO	USA	
8	ROYAL		
9	SYPRUS	Syria	ICARDA, Aleppo, Syria

All agronomic practices were applied on time according to the recommendations except the fertilization which was not applied. Where, weed control was done mechanically by hoeing the row space in the first week of March.

The climatic data of the region are representing in Table 2 and was obtained from the site: <http://www.wunderground.com/>.

Table 2: Climatic data of experimental area in 2007-2008 season (in growth period).

Months	Precipitation (mm)	Temperature (°C)			Relative humidity (%)
		Low	Average	High	
September	54,34	14,6	21,3	28,9	64,3
October	39,12	11,8	17	23,2	79
November	24,13	04,4	10	16,2	79,1
December	103,20	3,25	6,50	9,37	92,00
January	14,50	1,77	7,38	13,00	80,87
February	11,40	2,28	8,14	14,00	76,33
March	114,00	2,94	9,03	15,13	78,74
April	29,60	5,80	12,98	20,17	68,67
May	71,60	11,32	18,22	25,13	67,23
June	15,00	13,40	21,13	28,87	61,17
July	7,20	18,58	26,63	34,68	51,58

Samples were harvested in the first week of August, where the plant population was 13.3 plants/m².

Seeds number per plant, hundred (100) seeds weight and yield of seeds were determined on ten randomized plants/plot. Fixed oil percent in the seeds of the different varieties was determined separately using 5.0g of the seeds with Soxhlet apparatus with petroleum ether (40 – 60 °C).

Finally, the data of yield components and oil content were subjected to statistical analyses using MINITAB for evaluation of variance and correlation between varieties and yield components.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) revealed significant differences between the nine safflower genotypes for all yield components and oil content (Tables 3 & 4).

Table 3: Yield of seeds and oil content of safflower varieties.

Number	Varieties	Yield of seeds (YS) (g/m ²)	Yield of Oil (PO) (% of seeds)	Yield of Oil (YO) (g/m ²)
1	80/482/3S	306,47	26,53	80,29
2	FINCH	150,63	36,13	54,57
3	GILA	187,92	38,47	72,11
4	KUSUMBA	181,67	23,30	42,33
5	OLE	224,31	34,60	77,40
6	OT-455	144,68	28,87	40,63
7	RIO	186,02	35,00	65,93
8	ROYAL	235,97	36,60	86,53
9	SYPRUS	420,29	27,27	114,56

Table 4: ANOVA for Yield of seeds (YS) and oil content (PO, YO) for safflower varieties tested.

Source	Sum of the Squares (SS)	Mean Square (MS)	F	P
V1 (YS)	184898	23112	6.56	0.000***
V2 (PO)	700.35	87.54	6.75	0.000***
V3 (YO)	12913.8	1614.2	3.70	0.010*

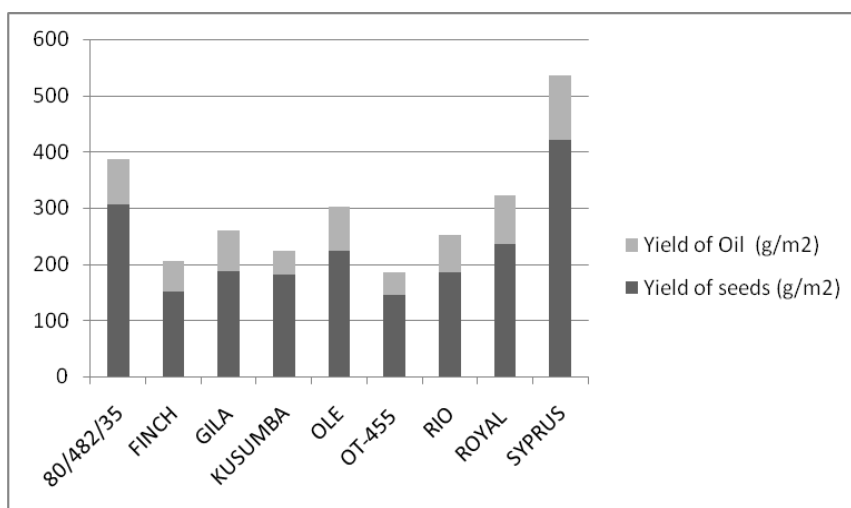
*, **, ***: significant at 0.05, 0.01 and 0.001 level of probability using the F test.

Gencer *et al.* (1987) stated that seed yield and oil content are the primarily selection criteria for safflower breeding. The correlation among the all pairs of variables (YS, PO and YO) are shown in Table 5. The highest positive correlations were obtained between YS with YO ($r = 0.866^{***}$) (see also Figure 1).

Table 5: Correlation coefficients between variables (YS, PO and YO) measured.

	V1 (YS)	V2 (PO)
V2 (PO)	-0,296	
P	0,134 ^{ns}	
V3 (YO)	0,866	0,196
P	0,000***	0,328 ^{ns}

***: significant at 0.001, ns: non significant level of probability using the F test.

**Figure 1:** Yield of seeds and oil contents of safflower varieties.

PO is negative non-significantly correlated with YS ($r = -0.296^{ns}$). Similar results were observed by Pahlavani (2005), who reported that improvement of seed yield in safflower could be decline through oil content and 100-seed weight due to negative association between these traits with seed yield.

It can concluded, that selection for increasing seed yield in safflower may have considerable effect on oil content (Pahlavani, 2005).

CONCLUSION

The results obtained in this study indicate that oil seed production can be increased by growing drought tolerant safflower in regions where annual precipitation is not adequate (Kolsarici and Güney, 2002; Kaya *et al.*, 2004), and the introduction of safflower in the Mediterranean type climate will, to a small extent, help in increasing local production of edible oil (Akhtar *et al.*, 2006).

From all varieties tested, 'SYPRUS' may be recommended as elite genotype for cultivation in semi-arid area of Mediterranean climate as it showed high relative yield performance and oil content.

Since the highest seed yields are expected when all components of seed yield are maximized (Mahasi *et al.*, 2005), we concluded that hybridization program is recommended between 'SYPRUS' (the variety which's presented the highest yield of seeds) and 'GILA' (the variety which's presented the highest fixed oil percentage of seeds).

ACKNOWLEDGEMENTS

This research was supported by Algerian Ministry of Higher Education and Scientific Research. The authors thank USDA and ICARDA gemplasms for providing safflower varieties. Our thanks are due to Dr Mezedjri L. for his help concerning the statistical analysis.

The technical support of Constantine INPV Director and staff is acknowledged with gratitude.

REFERENCES

- Akhtar, B., Pourdad, S.S., Mustafa, P., Theib, O., 2006. Influence of supplementary irrigation and variety on yield and some agronomic characters of safflower under rainfed conditions in Northern Syria", *Sesame and Safflower Newsletter* 21.
- FAO (2008). Available: <http://www.faostat.fao.org>.
- Gencer *et al.* (1987) in Burhan, A., 2007. The path analysis of yield and its components in safflower (*Carthamus tinctorius* L.)", *Journal of Biological Sciences*, 7(4), pp. 668-672.
- <http://www.wunderground.com>
- Kaya, M.D., İpek, A., Öztürk, A., Kolsarici, Ö., 2004. Variation of some morphological traits of safflower (*Carthamus tinctorius* L.) grown different soil salinity levels in the first development stage", *Turkish Journal of Field Crops*, 9 (1), pp. 53-61.
- Kolsarici, O., Güney, E., 2002. Effects of Different Row Distances and Various Nitrogen Doses on the Yield Components of a Safflower Variety, *Sesame and Safflower Newsletter*, 17, pp. 108-111.
- Mahasi, M.J., Pathak, R.S., Wachira, F.N., Riungu, T.C., Kamundia, J.W., 2005. Development and evaluation of safflower (*Carthamus tinctorius* L.) cultivars for the marginal rainfall areas of Kenya: morphological characterization, genetic diversity and adaptation studies, *Sesame and Safflower Newsletter*, 20.
- Pahlavani, M.H., 2005. Some technological and morphological characters of safflower (*Carthamus tinctorius* L.) from Iran", *Asian Journal of Plant Sciences*, 4, pp. 234-237.
- Uher, J., 2003. Safflower in world floriculture: a review, *Sesame and Safflower Newsletter*, 20.
- Yau, S.K., 2003. Optimal sowing date for rainfed safflower in the high elevation Bekaa valley of Lebanon", *Sesame and Safflower Newsletter*, 18.

CONSUMER HABITS TOWARDS MEDICINAL AND AROMATIC PLANTS IN ERZURUM PROVINCE

BANU KADIOĞLU¹, Sibel KADIOĞLU¹, Gökhan TAŞĞIN¹

¹Eastern Anatolia Agricultural Research Institute, Erzurum, TURKEY

Corresponding author email: banu250@hotmail.com

Abstract

Turkey is a country where there are around 12.000 plant species whose 1/3 is endemic and 30% of this rate is aromatic. Among rich variety of plants, medicinal and aromatic herbs have a significant place and it is known that in Turkey about 500 plant species are used as medicine by the general public. Today, although the aromatic herbs and spices are confused with each other and considered to be the same, there are critical differences between those. In the literature scans, there were different studies that did not consume spice consumption but there were no studies that directly examined medicinal and aromatic plant consumption. 384 questionnaires were conducted with the aim of determining the habits of the consumers on the information and consumption of medicinal and aromatic plants. The main material of the study was the data obtained by the questionnaire survey made with the consumers and the obtained data were analyzed by using the appropriate statistics. Consumers data obtained from the survey have been statistics analyzed with descriptive statics, likert scale and likert scale.

Keywords: Medicinal and aromatic plants, costumer, habits, biological diversity, Erzurum

INTRODUCTION

Since it is located in three phytogeographic zones, Turkey has an important economic potential in terms of medicinal and aromatic plants collected from nature and cultured with its unique climate, ecological conditions, rich flora and fauna. The Northeastern Anatolia Region and the Erzurum Province in this region with their different climate, geography and topographic features, are also very rich in medicinal and aromatic plants which have an important role in biodiversity and for the local people. In addition to many natural medicinal and aromatic plants, coriander, basil, tarragon, mint, parsley and dill are also cultivated albeit in small quantities.

Medical and aromatic plants, which are consumed intensively in developed countries and whose consumption has increased in recent years especially in large cities, increase their share in the country's economy with each passing day. Although Turkey is the gene source of many medicinal plants, these plants are not sufficiently involved in agricultural production activities. In addition, there is no healthy statistical data on the sale and use of medicinal plants. In addition, in the domestic medicinal and aromatic plant sector, consumption and consumer preferences and socio-economic characteristics of the consumers are not sufficiently known. Examining and revealing factors affecting the markets such as the rapidly growing medicinal and aromatic plants use and consumers' preferences, attitudes and habits related to this is one of the priority issues. In this study, it was tried to determine the factors that shape the habits of consumers and the plants consumed and their consumption.

MATERIAL AND METHODS

The main material of the study was the primary data obtained by the face to face survey method with MAP (Medical And Aromatic Plants) consumers residing in Palandöken, Yakutiye and Aziziye settlements, which constitute the center of Erzurum province. A survey was conducted with 384 consumers. In evaluating the statistics of the data, methods, frequencies, percentages and likert scale appropriate for the question type were used.

RESULTS AND DISCUSSION

Demographic Structure of Consumers

Socioeconomic and demographic structures of consumers affect the consumption of medicinal and aromatic plants, like in other food products. 49.7% of the consumers surveyed were male and 50.3% were female. Approximately 46.1% of the participants were “aged between 31-46” and 75.5% were “married”. When the educational status of the participants is examined, it is noteworthy that 34.9% of them have a “bachelor's degree” from a faculty. As the individuals' level of education increases, the level of awareness of the person about himself/herself and his environment is also expected to increase. Learning provides an advantage to the individual in accessing and using information. When the employment status is examined, it is seen that 19.6% of the participants are “civil servants”, 18% are “shopkeepers and housewives” 46.6% have 4-6 family members and their monthly income ranges between 2501-4000 TL, monthly food expenditures are in the range of 100-500 TL and monthly health and MAP expenditures are between 10-50 TL”. It is also noted that 79.2% of consumers were born in Erzurum province and districts, 20.3% in other provinces and 0.5% were born abroad.

MAPs Used By Consumers and Reasons for Use

For the general purpose of use, 83.3% of consumers use medicinal aromatic plants (Arya et al., 2012), 54.4% consume medicinal aromatic plants generally against cold (Tuzlacı and Erol 1999, Yücel and Tülükoğlu 2000), (Table 1). Consumers also stated that they consume linden (40%) the most as medicinal and aromatic plants (Çubuk et al., 2008; Akbulut and Özkan, 2016). Because the winter months are quite cold in the region, the consumption of plants that strengthens the immune system can be said to be preferred by consumers.

Table 1. MAPs used by consumers and their intended use

<i>Intends use of MAP</i>	<i>n</i>	<i>%</i>	<i>General disease</i>	<i>n</i>	<i>%</i>
Health	320	83,3	Blood pressure	16	5,0
Aroma	38	9,9	Diabet	23	7,2
Other	26	6,8	Heart	14	4,4
			Kidney	10	3,1
<i>Most used MAP</i>	<i>n</i>	<i>%</i>	Influenza	174	54,4
Linden	127	40	Stres	33	10,3
Greentea	8	3	Slimming	9	2,8
Rosehip	32	10	Strengthening the Immune	18	5,6
Daisy	15	5	Women's diseases	23	7,2
Garlic	13	4			
Herbaltea	5	2			
Ginger	20	6			
Mint	20	6			
Cinnemon	18	6			
Linden+Ginger	30	9			
Ferula	32	10			

The rate of using medicinal and aromatic plants by collecting from nature is 72.7%. In general, plants collected from nature are collected from mountains (30,2%) and meadow (17,8%). The collected plants were determined to be 6.8% great plantain, 5.6% rosehip, 4.9% rumex and 4.5% ferula. They also stated that they prefer to use MAPs “always “(73.5%) and” when necessary (58.5%)” as the frequency of use. According to the results of the survey, 38.8% of the consumers stated that they prefer to consume medicinal and aromatic plants in the form of tea as infusions (25%) and decoctions (75%). In response to the question 'which part of the medicinal and aromatic plants do you consume in general, 35.6% stated that they consume their leaves (Table 2).

Table 2. Usage information of MAPs.

<i>Collection from nature</i>	<i>n</i>	<i>%</i>	<i>MAPs use forms</i>	<i>n</i>	<i>%</i>	<i>MAPs used parts</i>	<i>n</i>	<i>%</i>
Yes	104	52,6	Herb	52	16,3	Onion	17	5,3
No	210	27,3	Oil	15	4,7	Tuber	5	1,6
Sametimes	70	20,1	Leaf	31	9,7	Flower	34	10,6
			Herbaltea	124	38,8	Leaf	114	35,6
			Cream	15	4,7	Root	42	13,1
			Solution	2	0,6	Branch	8	2,5
			All	81	25,3	All	100	31,3

In addition, 76.3% of the respondents stated that they prefer using medicinal and aromatic plants to chemical drugs, and that they use them to maintain the most healthy lifestyle and to make them feel better psychologically. This can be interpreted as the fact that chemical drugs can threaten life with different effects.

Consumers who knew (87,5%) the MAPs usage period stated that they obtained this information from the place of purchase (50,6%). 43.1% of the consumers stated that they used MAPs in recommended dosing and they learned this, that is, the dosage and for how long to use them from the herborists (33%). It was found that 58% of the consumers were influenced by their friends and gained the habit of using MAPs. All consumers who use TAB for health stated that they knew the instructions for use and 40.6% said that their use was traditional and that they learned it from their parents, elders etc.

Again, a large number of consumers (42.2%) prepare certain mixtures themselves, and therefore prefer products with instructions for use. Those who consume the products with instructions in accordance with the instructions are 95.3%.

CONCLUSION

As a result; The demographic profile of those who participated in this research to determine the medicinal and aromatic plant consumption habits of people living in Erzurum province are the consumers who are between 31-46 years of age, married, completed their undergraduate education and have an income between 2501-4000 TL and whose food expenditure is between 100-500 TL and medicinal and aromatic plant expenditure is between 10-50 TL. The majority of consumers are from Erzurum, consisting of civil servants, artisans and housewives.

It was determined that consumers consumed medicinal and aromatic plants continuously when necessary and purchased them from herborists; they collect medicinal and aromatic plants from nature, collect great plantain, rosehip, rumex and ferula. They have the habit of using medicinal and aromatic plants from their friends, buy them with the recommendation of their friends, learned the instructions of use traditionally, prefer to consume their leaves in the form of tea.

At the end of the study, the determination of consumers' knowledge of medicinal and aromatic plants and whether they are conscious about this matter were assessed. The data may be useful if co-operated with those who are concerned with this matter. In this context, the widespread impact of the study can be seen if the data obtained from social feedback studies are used.

ACKNOWLEDGEMENTS

The data presented in this paper is obtained from the project financially supported by general directorate of agricultural research and policy (TAGEM, Project number: TAGEM/TEAD/A15/P02/003).

REFERENCES

- Akbulut, S. ve Özkan, Z. C., 2016. Herbalist-customer profile in medicinal and aromatic herbs trade: A Case Study of Kahramanmaraş, Turkey. *Kastamonu University Journal of Forestry Faculty*, 16 (1), 246- 252.
- Arya, V., Thakur, R. and Kumar, S., 2012. Consumer buying behaviour towards Ayurvedic medicines/products in Joginder nagar-A survey. *Ayurpharm-International Journal of Ayurveda and Allied Sciences*, 1(3), 60-64.
- Çubuk, S., Nakiboğlu, B. ve Keleş C., 2008. Tüketicilerin yeşil (ürün) satın alma davranışlarının sosyo-demografik değişkenler açısından incelenmesi. *Ç.Ü. Sosyal Bilimler Enstitüsü Dergisi*.
- Demircioğlu, Y., Yaman, M. ve Işıl, Ş., 2007. Kadınların baharat kullanım alışkanlıkları üzerine bir araştırma. *TSKY Koruyucu Hekimlik Bülteni*, 6(3).
- Dicle, M., 2010. İzmir ili bornova ilçesinde tıbbi bitkilere ilişkin tüketici davranışlarının belirlenmesi üzerine bir araştırma. *Ege Üniversitesi Fen Bilimleri Enstitüsü (Yüksek Lisans Tezi)*
- Kılıçhan R., ve Çalhan,H., 2015. "Mutfakların sihri baharat: Kayseri ilinde baharat tüketim alışkanlıklarının belirlenmesine yönelik bir çalışma (Spices the magic of cuisines: determination of spice consumption habits in kayseri province)", *Journal of Tourism and Gastronomy Studies*, Cilt.3, No.2, ss.40-47.
- Özkan, E. ve Yıldız, S., 2015. *International journal of economic and administrative studies*. Year:8 Number 15, Summer 2015/ ISSN 1307-9832.
- Sayılı, M., 2012. Tokat ilinde tüketicilerin ari ürünleri tüketim durumları ve alışkanlıkları, *U. Arı Drg. Şubat 2013 / 13* (1): 16-22.
- Tarakçı, Z., Selçuk, Ş., K. ve Çoşkun, H., 2000. Üniversite öğrencilerinin içme sütü tüketim alışkanlıkları üzerine bir araştırma. *Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi*, 13(1):15-21
- Tuzlacı, E. ve Erol, M.K., 1999. Turkish folk medicinal plants, Part II: Eğirdir (Isparta). *Fitoterapia*, 70: 593-610.
- Yaldız, G. ve Kiliç, E., 2010. Rize ili kentsel alanda tüketicilerin baharat tüketim alışkanlıklarının belirlenmesi. *Rize Üniversitesi, Pazar Meslek Yüksekokulu, Tıbbi ve Aromatik Bitkiler Programı, Rize*.
- Yücel, E. ve Tülükoğlu, A., 2000. Gediz (kütahya) çevresinde halk ilacı olarak kullanılan bitkiler. *Çev Kor. Cilt: 9 Sayı: 36*, 12-14.

A TRADITIONAL HEALING “TIRMIS OR TERMIYE” LUPIN (*Lupinus albus* L.)

Sibel KADIOĞLU¹, Banu KADIOĞLU¹

¹East Anatolian Researches Institute, Erzurum/Turkey

Corresponding author email: sibel.kadioglu@hotmail.com

Abstract

Lupin, motherland of which is the Mediterranean basin, is a herbaceous and annual plant derived from the legumes class, *Lupinus albus* L. is known in various regions of Turkey by various names such as termiye, bitter fava bean, Jewish fava bean, delice fava bean, giaour fava bean, wolf fava bean, corn fava bean. It is grown as an ornamental plant or consumed as a traditional flavor- snack in Antalya, Konya and Isparta provinces.

It is a traditional flavor that is listed in the cars of the peddlers as cure for many ailments. Lupin, which is beneficial for goitre, rheumatism, haemorrhoid, also increases milk production and urine output. Lupinus is diuretic and cleanses the urinary tract, resolves kidney inflammation, helps with kidney stones and sands, It relieves headaches, cuts lumbago and sciatica pain, reduces the amount of albumin, throws the salt which accumulated in the body, strengthening and reducing of intestinal parasites are known, and it is claimed to reduce blood sugar in diabetics.

Lupin, which contains alkaloids such as lupanin, sparteine and anagyrene, is very important in the pharmaceutical industry. It is also a remarkable plant with high protein (33%) and rich mineral content. But unfortunately, lupin production and its cultivated area has declined considerably over the last 25 years. Lupin production, which is cultivated on 3700 acres, is around 400 tons. Traditional lupin, which is useful in many aspects, should be sustained by supporting consumption and research studies.

Keywords: Tirmis, termiye, bitter fava bean, cookies, cure

INTRODUCTION

Lupin is an annual herbaceous plant from the legume family. Its roots are strong and spread over the top layers of the soil with a thick primary root and lateral roots. There are non-regular nodosities on the primary and lateral roots. White lupin is a medium-sized plant and can grow up to 1.20-1.50 cm in favorable conditions.

The leaves are broad and large, leaflets are hairy, stipules are narrow, pointed towards the tip and the surface is covered with fine hairs. The flowers are clustered and located at the ends of the stems. Its sepals are green, petals are white in white lupin, and it varies according to species and variety in other lupin species, blue in blue lupin and yellow in yellow lupin. From the legume class, the Latin name is derived from the seeds of a plant *Lupinus albus* L. (or *Lupinus termis*). The motherland is the Mediterranean basin.

Lupinus genus contains more than 300 species, but only 4 of them have agricultural importance. These are *Lupinus albus* (white lupin), *Lupinus angustifolius* (blue or narrow-leaved lupin) and *Lupinus luteus* (yellow lupin), and the only new world species *Lupinus mutabilis*. White lupine (*Lupinus albus* L.) crops were important to many Mediterranean civilizations and was domesticated in the Old and New World (Putnam et al., 1993). The first three originate from South America while *L. mutabilis* originates from the Mediterranean region (Hondelmann, 1984). Tirmis is similar to corn and actually it is a sort of bitter bean. It was consumed as food in Ancient Egypt and Ancient Rome. Today it is still used in mediterranean countries. According to 2016 data, lupin is cultivated in an area of approximately 987.208 ha in the world and 534.084 ha of this area is in Australia. World production of lupin is 1.284.843 tons (FAOSTAT 2018).

Lupin in Turkey

Turkey made both species and their wild forms of culture, it is rich in both culture lupin species made limited purposes. Subspecies belonging to these species are quite common in natural vegetation. Lupin in our country is a plant known by the names such as white lupin, lüpen, ak lüpen, beyaz lüpen, delicebakla, gavurbaklası, yahudibaklası, kurtbaklası, mısırbaklası, termis, lüpen (tirmis/termiye), pırtlancı, ak acıbakla, beyaz acıbakla and termiye. Bitter bean, wild bean (*Lupinus termis*) (MŞ 73b). It was made in Arabic, characterized by the title "Turkish" bitter ("açığ" bitter, sur EDPT 20). In the same period texts are also available with "termiye" and "turmuş" (Önler, 2004).

The three important species are blue bitter faba, yellow bitter faba and white bitter faba. The lupin species that are cultivated in Turkey is an annual local population. Because lupin is a sensitive to limy soils, it is usually cultivated in low-lime Göller (Lakes) region in Turkey.

Eating culture in our country varies from region to region. Street vendors' snacks can be quite surprising for foreigners. Among these, traditional foods such as maraş tarhanası, şirden, midye, ayçiçeği, mısır, nohut, buzlu badem and tirmis which are consumed as street food and snacks are also quite remarkable. It is grown as an ornamental plant or consumed as a traditional flavor- snack in Antalya, Konya, Afyonkarahisar, Burdur and Isparta provinces (Anonymous 2018).

When examined with respect to the years, it is observed that the production of lupin in our country has decreased in the last 25 years; In the last 10 years, the overall trend has been in the direction of a slight increase until 2012, but remained stable after 2012. In 2017, 402 tons of lupin were harvested from 3714 decares (TURKSTAT 2018; Table 1).

Table 1. Lupin Production of Turkey.

Year	Sown area - Decares	Production - Tone
2008	3610	289
2009	3710	369
2010	3810	381
2011	3820	417
2012	4681	423
2013	3767	411
2014	3767	411
2015	3742	409
2016	3761	411
2017	3714	402

Lupin Usage Areas

- ✓ In Turkey, lupin, which contains high protein and fat, is consumed as a snack and its alkaloids are used (Mulayim and Acar 2008). It can be used in pasta, bulgur and bread making and many commercial products such as breakfast, salad and soup; even some species can be consumed as coffee.
- ✓ Sweet lupins are used in grazing and feeding animals as green herbs with abundant leaves, large amounts and quality proteins (Baytop 1994).
- ✓ It is used in floriculture and garden adornment. Blue and yellow flowered lupins are preferred as ornamental plants.
- ✓ The bitter water drained during the sweetening process of the seeds has the potential to be used in the biological control.
- ✓ Lupin is planted as sole crops or mixtures for soil improvement purposes and green fertilization. Thanks to its deep roots and the nodosity bacteria found in its roots, they transform the free nitrogen in the air into beneficial form for plants and soil. In addition, since its organic matter yield is very high, it is used both in green fertilization and as a pioneer plant, which increases the importance of this plant. Lupin is actually the plant of poor, light,

especially low-lime soils and therefore not able to feed other fodder crops of the legume family. It can grow in poor and sandy soil.

- ✓ They are also important in health, they are used as a medicinal plant. Some species are said to be used in medicine and cosmetics industry. Since it contains alkaloids such as lupanin, sparteine and anagryne, lupin has an important place in the pharmaceutical industry (Kayserilioğlu 1990).
- ✓ It is also stated that Lupin was used in the past as a dewormer, diuretic, booster and in agro-spraying by the people. It is also reported that in the past it was used against boils, wounds and in intestinal and stomach diseases, and it was also used for washing sheep against scabies. Lupin contains alkaloids such as sparteine and anagryne, as well as lupanine and albine alkaloids, which are the most commonly found elements in its structure. These alkaloids increase the importance of the plant in the pharmaceutical industry.

Nutritional Value of Lupin

Lupin, which contains 2-3 times more protein than grains, is also a rich source of vitamins, minerals, calcium and iron. Although soy is at the top in terms of vegetable protein production, lupin with high protein content (28-47.6%) can compete with soy provided its production and yield is increased (Williams 1979; Sator 1983). The protein content in the grain of white lupin is 40.9% (37% of digestible protein) and the fat content is 8-12%.

The 100 g lupin bean (dry weight) consumed by humans contains 561 mg phosphorus, 23 mg potassium, 379 mg calcium, 82 mg magnesium, 67 mg sodium, 3.7 mg boron, 0.8 mg copper, 4.5 mg iron, 111 mg manganese and 6 mg zinc. The moisture content of the ready-to-consume lupin is 70% and the shell ratio in the grain is around 20%. According to this, when a person consumes approximately 400 g of boiled fresh lupin per day, this can meet about 22-60% of the mineral requirement (Table 2). However, with the same amount of consumption, daily required manganese intake will be exceeded (Yorgancılar et al., 2009).

Table 2. Nutrient content of lupin.

Nutrient Content	Fats	Vitamins	Minerals
Water 117.99 g	Total fats 4.85 g	Vitamin A (IU) 12 IU	Calcium (Ca) 85 mg
Energy 198 kcal	Total saturated fatty acids 0.574 g	Vitamin B1 or Thiamine 0.222 mg	Iron (Fe) 1.99 mg
Proteins 25.85 g	Total monounsaturated fatty acids 1.959 g	Vitamin B2 or Riboflavin 0.088 mg	Magnesium (Mg) 90 mg
Total carbohydrates 16.40 g	Total polyunsaturated fatty acids 1.212 g	Vitamin B3 or Niasin 0.822 mg	Phosphorus (P) 212 mg
Total dietary fiber 4.6 g		B6 vitamin or pyridoxine 0.015 mg	Potassium (K) 407 mg
		Vitamin B9 or folic acid (DFE) 98 µg	Sodium (Na) 7 mg
		Vitamin B12 or Cyanocobalamin 0 µg	Zinc (Zn) 2.29 mg
		Vitamin C or Ascorbic acid 1.8 mg	

Traditional and Daily Use

Tirmis or termiye normally grows in the summer. It can be consumed for 12 months but it is usually sold in winter months because it stalls quickly in the heat. Tirmis is made from mature seeds of the lupin. Its grains are bitter. So, it needs to be sweetened. For this reason, it is boiled and soaked in water to be sweetened. In sweetening, lupin is first soaked in water for one day, and then lightly boiled. The color of the boiled lupin becomes yellow, then it is put in a container filled with water; the water in the cap is changed every day, and after about ten days lupin's bitterness and smell go away.

It has a thick transparent shell that can be easily peeled off. This crust is squeezed between the thumb and forefinger. Or, in the mouth, remove the skin and eat the yellow, fleshy portion inside. The lupin is consumed after being salted or soaked in salty water.

Due to their health benefits, it is recommended that 10-20 debittered lupin beans be eaten daily. As well as being prepared and consumed in the house, the lupin is also sweetened by the experts called "tirmişçi" and sold at the bazaar (Figure1). Tirmis is one of the common flavors of the Mediterranean countries. Turmus or termis in Egypt, altramuces in Spain, tremeços in Portugal are called. Tirmis is served beside the beer in bars. Italy, Greece, Syria, Lebanon, Jordan and Israel. It is served on the same plate with olives as a traditional Christmas snack in Italy. Most of these countries sell tirmis on mobile benches on the streets.



Figure 1. The street vendors (lupin sellers).

Tirmis is also produced and sold in markets by profession named “tirmisci”. Tirmisci is a person who makes and sells tirmis. In Antalya there is a very common expression: "without invitation only the tirmisci goes wedding ceremony", this indicates that tirmis has a very important place in folk culture. As a traditional profession tirmiscilik is passed down from ages in Antalya, Konya and Isparta. “Tirmisci “ has peddled their tirmis both in the past and today.

Therapeutic Properties of Lupin

When it comes to medicinal and aromatic plants, both in terms of the active substances they contain and in terms of consumption areas, they cover a very large area and their world trade volume is increasing every day. It is stated that the medicinal plant market's trade volume, which was about 60 billion dollars annually in 2000, will rise to 107 billion dollars in 2017. It is known that our country has a great economic potential for medicinal and aromatic plants collected from nature and cultivated in terms of having different climate and ecological conditions, having a large number of plant species and varieties of flora and being the gene center of many plants.

Compared to many cultivated plants, studies carried out on medicinal and aromatic plants are still limited. In this respect, it is clear that scientific studies carried out on medicinal and aromatic plants will provide valuable contributions to this field and will be a resource for the country's economy. In addition, it is necessary to transfer the results obtained from these studies to implementation and to ensure their widespread use by the sector.

- ✓ Seeds of lupins or tirmis are healing. Leaves or branches are not used for disease treatment.
- ✓ Lupin can also be used by boiling, as the seeds can be consumed in powder form.

Jewish legumes, known to have a positive effect on skin wounds, can also be used to protect the body against the bites of harmful insects. In a study conducted at Beni-Sueif, it was stated that it was used by people as dust for skin disorders (AbouZid and Mohamed 2011)

- ✓ lupin is a diuretic and cleans the urinary tract,
- ✓ reduces kidney inflammation, helps getting rid of kidney stones and sands,
- ✓ relieves headaches,
- ✓ relieves the pain of rheumatism, lumbago and sciatica,
- ✓ reduces the amount of albumin, helps excreting excess salt that accumulates in the body,

- ✓ The lupin, rich in carbohydrates, is a nutritious plant. It protects against diseases through resistance enhancing feature,
- ✓ Its immun booster effects and effectiveness in parasite removal is known ((Çömlekçioğlu and Karaman, 2008),
- ✓ and it is claimed to lower the blood sugar levels in diabetic (Çömlekçioğlu and Karaman, 2008; Durmuşkahya and Öztürk, 2013).

For these reasons in our country; traditional lupin, which is useful in many aspects, should be sustained by supporting consumption and research studies.

REFERENCES

- AbouZid S. F. Mohamed A. A. 2011. Survey on medicinal plants and spices used in Beni-Sueif, Upper Egypt. Journal of Ethnobiology and Ethnomedicine 2011, 7:18, <http://www.ethnobiomed.com/content/7/1/18>.
- Anonymus 2018. http://www.toroslargazetesi.com.tr/seydisehir-in-sanslarindan-biri-de-termiye-bitkisi_d6076.html
- Cömlekçioğlu, N., Karaman, Ş., 2008. The medicinal plants found in the local herbal markets in the city of Kahramanmaraş in Turkey. KSU Journal of Science and Engineering, 11: 23-32.
- Durmuşkahya, C. and Öztürk, M., 2013. Ethnobotanical Survey of Medicinal Plants Used for the Treatment of Diabetes in Manisa, Turkey (Tinjauan Etnobotani bagi Tumbuhan Ubatan yang Digunakan dalam Rawatan Kencing Manis di Manisa, Turki) Sains Malaysiana 42(10)(2013): 1431–1438.
- FAOSTAT 2018. Food and Agriculture Organization of the United Nations, Statistics (<http://www.fao.org/faostat/en/#data/QC>).
- Hondelmann W (1984). The lupin-ancient and modern crop. Theoretical and Applied Genetics 68: 1-8.
- Kayserilioğlu, R., 1990. Konya Yöresinde Lüpen (Acıbakla-Termiye) Üretimi. T.C. Bayındırlık ve İskân Müdürlüğü, Devlet Su İşleri Genel Müdürlüğü, IV. Bölge Müdürlüğü, Etüd ve Plan Şubesi Notları, Sayfa: 1-13, Konya. Mülayim, M., Acar, R., 2008. Konya'nın Yöresel Değeri Ak Acıbakla (Lüpen= Termiye) Bitkisi ve Kullanımı, Konya Ticaret Borsası Dergisi, 11(30): 44-49.
- Putnam, D. H., S. R. Simmons, and L. L. Hardman.1993. Vernalization and seeding date effect on yield and yield components of white lupin. Crop Sci. 33: 1076- 1083.
- Önler, Z., 2004. XIV-XV. Yüzyıl Tıp Metinlerinde Türkçe Bitki Adları. Kebikeç, S. 18, 273-301.
- Sator, C., 1983. In vitro Breeding of Lupins. Perspectives for Peas and Lupins as Protein Crops. (R Thomson and R Casey, eds.) In Proc. Int. Symp. Protein Production from Legumes in Europe, Sorrento, Italy pp. 79-87.
- TURKSTAT, 2018. Turkish Statistical Institute, Turkey.
- Williams, W. 1979. Studies on the development of lupins for oil and protein, Euphytica 28: 481-488.
- Yorgancılar M. Atalay E. and Babaoğlu M. Acılığı giderilmiş termiye tohumlarının (lüpen= lupinus albus l.) Mineral içeriği Selçuk Üniversitesi, Selçuk Tarım ve Gıda Bilimleri Dergisi 23 (50): (2009) 10-15 ISSN:1309-0550.

TIBBİ VE AROMATİK BİTKİLER ENDÜSTRİSİ'NDE TEKNOLOJİ TRANSFERİ VE İNOVASYONUN FİRMALARIN ÜRETİM SÜREÇLERİNDEKİ STANDARDİZASYON, AKREDİTASYON VE PATENT HAKLARINA ETKİLERİ

Armando ALIU¹, Mehmet MELEMEN², Dorian ALIU³

¹İstanbul Ticaret Üniversitesi, Örnektepe Mah. İmrahor Cad. No: 88/2 Beyoğlu, İstanbul, Türkiye

²Marmara Üniversitesi, Cuma Yolu Cad. Anadoluhisarı, Beykoz, İstanbul, Türkiye

³Çanakkale Onsekiz Mart Üniversitesi, Terzioğlu Yerleşkesi, Çanakkale, Türkiye

Sorumlu yazar e-posta: aaliu@ticaret.edu.tr

Özet

Bu çalışmanın amacı, Tıbbi ve Aromatik Bitkiler Endüstrisi'nde (TABE) faaliyet gösteren firma ve tedarikçilerin üretim ve dağıtım süreçlerinin incelemesini yapmak ve bu sürecin teknoloji transferi gerçekleştirilirken standardizasyona, akreditasyona, fikri ve sınai mülkiyet haklarına etkisini araştırmaktır. Bu kapsamda, farklı ülkelerdeki birçok uygulamanın Balkanlar ve Türkiye'de TABE'deki işleyişler ve eylemler ile kıyaslanması yapılmıştır. Araştırmanın ana hedeflerinden birisi "Tarihi Baharat Yolu"nın çağın ileri teknoloji ve inovasyon dikkate alınarak küresel piyasalarda önemli bir paya sahip olan TABE'de gerçekleşen dönüşümü mercek altına almaktır. Dolayısıyla, Hindistan'dan Balkanlara uzanan bu rota dikkate alınmıştır. Orta ve Doğu Avrupa Ülkeleri ile Güney-Doğu Avrupa Ülkeleri'nin bu rotadaki stratejik önemine sistematik bir şekilde vurgu yapılmıştır. Modern Baharat Yolu rotasında faaliyet gösteren firmalar arasındaki teknoloji transferi ve ticaret hacimlerine çalışmada yer verilmiştir. Ayrıca, sosyal ve çevresel sorumluluk çerçevesinde irdelenen konular şunlardır: organik ve ekili ürünler, biyoçeşitlilik, ekosistem yönetimi, gıda sağlığı ve güvenliği, çevreci ürünlerin gelişimi ve standardizasyonu, çevre ile ilişkili kalite yönetimi ve belgelendirme, patent hakları. Bu konular tıbbi ve aromatik bitkilerin aşırı üretilmesinin önüne nasıl geçilebileceğini daha iyi anlamak amacıyla ele alınmıştır. Buna ilaveten, TABE'de faaliyet gösteren Küçük ve Orta Büyüklükteki İşletmeler (KOBİ) ölçeğindeki firmaların Çok Uluslu Şirketler ile daha çok efektif rekabet edebilmesinin yollarını ortaya çıkarmak için araştırma kapsamında inovatif stratejiler, yalın üretim süreci, sektörel başarı faktörleri, yeni nesil girişimcilerin TABE'deki rolleri tartışılmıştır. Çalışmada, bilginin ticarileştirilmesi bağlamında KOBİ'lere sunulan bilimsel ve profesyonel desteğin doğrudan KOBİ'lerin dış ticaret performansına anlamlı bir katkısının olduğu iddia edilmiştir. Araştırmanın yöntemi kapsamında örnek olay araştırması, piyasa araştırması, sektörel gözlemler ve belge analizleri gerçekleştirilmiştir. Araştırmada öncelikle mikro ve mezo düzeyde analizler gerçekleştirilmiş ve daha sonra makro düzeyde kıyaslamalı araştırmalar TABE'deki argümanlar dikkate alınarak yapılmıştır.

Anahtar Kelimeler: Tıbbi ve Aromatik Bitkiler Endüstrisi (TABE), Teknoloji Transferi, Fikri ve Sınai Mülkiyet Hakları, İşbirliği, Sürdürülebilirlik, Paydaşlar

THE INFLUENCES OF TECHNOLOGY TRANSFER AND INNOVATION ON STANDARDISATION, ACCREDITATION AND PATENT RIGHTS OF COORPORATES' MANUFACTURING PROCESSES IN MEDICINAL AND AROMATIC PLANTS INDUSTRY

Abstract

The purpose of this study is to examine the production and distribution processes of the corporations and suppliers operating in the Medicinal and Aromatic Plants (MAPs) industry. The study highlights the effects of these processes on the standardisation, accreditation, intellectual and industrial property rights while technology transfer is being carried out. In this context, many applications in various countries were compared with operations and actions in MAPs industry in the Balkans and Turkey. One of the main objectives of this investigation is to take a look at the transformation that occurred in the MAPs industry which has a significant share in the global markets by taking into consideration the advanced technology and innovation of the "Historical Spice Road." Thus, the route that is extending from India to the Balkans was taken into account. The strategic importance of Central and Eastern European Countries and South-Eastern European Countries in this route was emphasised in a systematic manner. Technology transfer and trade volumes amongst corporations operating in the "Modern Spice Route" were included in the study. Furthermore, organic and cultivated products, biodiversity, ecosystem management, food health and safety, the development and standardisation of environmental products, environmental quality management and certification and patent rights are the issues that were

taken up to better conceive how to overcome the excessive production of MAPs products. Likewise, innovative strategies, lean manufacturing processes, industrial success factors, the roles of new generation entrepreneurs in the MAPs industry were argued within the scope of investigation to reveal the ways in which the Small and Medium-sized Enterprises (SMEs) can compete more effectively with Multinational Corporations (MNCs). In this study, it was claimed that scientific and professional support presented to SMEs in frame of commercialisation of knowledge has directly a substantial contribution to SMEs' foreign trade performance. Case study research, market research, sectoral observations and document analyses were conducted within the scope of research methodology. Primarily, micro and meso-level comparative analyses were performed and then macro-level comparative analyses were made by taking into consideration the arguments in MAPs industry.

Keywords: Medicinal and Aromatic Plants (MAPs) Industry, Technology Transfer, Intellectual and Property Rights, Collaboration, Sustainability, Stakeholders

GİRİŞ

Dünya çapında geleneksel ve modern tıp sistemlerinde 50.000 ile 70.000 arasında bitki türünün kullanıldığı bilinmektedir. Sayılmamış olan ek türler ise hızla büyüyen kozmetik ve botanik endüstrilerinde kullanılmaktadır. Bu malzemelerin büyük bir kısmı doğada var olan yabancı bitki türlerinin toplanmasıyla sağlanmaktadır (Leaman, 2006: 97). Aromatik bitkilerin kullanım alanı, ev-içi kullanımda basit şekliyle başlayarak, eczacılık alanı, gıda sanayi, tütün-sigarcılık sanayi, alkollü ve alkolsüz içecekler, vernik ve boya sanayi ile kimya sanayisini kapsayacak şekilde oldukça geniştir. Bunların sadece aromatik yapısını ön planda tutan özleri (esansları) kullanılmamakta, ayrıca kendine özgü bileşenleri de kullanılabilir (Asllani, 2004: 20; Máthé, 2015).

Bazı tıbbi ve aromatik bitkilerin insanlarda fizyolojik işleyişi değiştirme gücüne sahip olduğu, tıpta dönüşüm yarattığı, bu bitkileri yetiştiren, işleyen ve kullananlara servet kazandırdığı otoritelerce ifade edilmektedir (Máthé, 2015; Negi vd., 2018; Roosta, Moghaddasi, ve Hosseini, 2017). Gelişen teknoloji ve inovasyon her alanda olduğu gibi TABE'yi de etkisi altına almıştır. Gün geçtikçe önemi artan TABE'nin temel ihtiyacı haline dönüşen teknoloji transferi ve buna bağlı olarak inovasyon, standardizasyon, akreditasyon ve fikri mülkiyet hakları bağlamında tecrübeli firmaların aynı sektör içerisinde faaliyet gösteren ortak firmalar ile işbirliğinin sağlanması ve uluslararası finansal araçlar (örneğin; hibeler, fonlar vb.) ile desteklenmesi için gerekli dayanışma ortamının oluşturulması elzemdir. Piyasa araştırması kapsamında firmalar birbirleri ile temas kurmakta ve gelecek geri bildirimleri optimum düzeyde değerlendirmek için pazarda faaliyet gösteren büyük ölçekli şirketlerin talep ettikleri standartlara uygun ürünlerde ve hizmetlerde inovatif perspektifin yaygınlaştırılması üzerinde çaba göstermektedir. Bu bağlamda, ihtiyaç analizleri ile TABE'de işbirlikleri ölçeğinde teknoloji transferi ile sektörde ticarileştirilebilecek ürün yelpazesinin geliştirilmesine stratejik açıdan önem verilmektedir. TABE'nin bütünsel (*holistic*) anlamda büyümesi ve gelişmesini sağlamak için bu sektörde yer alan çiftçilerin ve toplayıcıların tedarik kanallarının uluslararası standardizasyon çalışmaları bağlamında yeniden yapılanması ve ekolojik / çevre dostu stratejilerin uygulanması gerekmektedir. Ayrıca, «İş Dünyası – Akademik Sektör – Sivil Toplum – Yöre Halkı» mekanizmasındaki etkileşimlere yoğunlaşarak TABE'de faal olan tüm çiftçi ve tedarikçilerin kümeleme analizinin (*cluster analysis*) yapılması ve ağ veritabanlarının (*network databases*) oluşturulması gerekmektedir. Yerel paydaşların katılımı ile yerel düzeylerde çiftçilerin ve tedarikçilerin yoksullukla mücadele (He vd., 2018), refah düzeylerinin artırılması, daha temiz çevre ortamının sağlanması ve sürdürülebilir kalkınmanın yerel düzeyde yerleşik yapıya sahip olması ve evrensel iş ahlâkı ölçüsünde karşılıklı stratejik çıkarların güçlendirilmesi sağlanabilir. Buna ilaveten, yerel düzeyde gençlerin TABE'nin hem teorik hem de uygulama alanlarına yönlendirilmesi ve teşvik edilmesi bu endüstrinin daha dinamik ve sağlıklı bir şekilde gelişmesine olanak sağlayabilir. Bu kapsamda, genç araştırmacılara ve lisansüstü öğrencilere araştırma destekleri ve çeşitli burslar verilebilir. Bu şekilde, genç araştırmacılar TABE'de teknoloji transferi, inovasyon, üretim süreçleri, standardizasyon, patentler, bilginin ticarileştirilmesi, sosyal ve çevresel sorumluluk gibi alanlara yönlendirilmesi gerçekleştirilebilir.

ARAŞTIRMANIN METODOLOJİSİ

Araştırmanın yöntemi kapsamında örnek olay araştırması, piyasa araştırması, sektörel gözlemler ve belge analizleri gerçekleştirilmiştir. Araştırmada öncelikle mikro ve mezo düzeyde analizler gerçekleştirilmiş ve daha sonra makro düzeyde kıyaslamalı analizler TABE'deki argümanlar dikkate alınarak yapılmıştır. Araştırmada örnek olay kapsamında incelenen ülkeler Orta ve Doğu Avrupa Ülkeleri (ODAÜ), Güney-Doğu Avrupa Ülkeleri (GDAÜ) ve Batı Balkan Ülkeleri (BBÜ)'dir.



Şekil 1: ODAÜ, BBÜ ve GDAÜ Kesişim Kümesi

Şekil 1'de ODAÜ, BBÜ ve GDAÜ kesişim kümesi gösterilmektedir. Bu ülkeler ile ilgili tarım alanındaki veriler 2013 – 2017 tarih aralıkları dikkate alınarak Comtrade (UN Statistics Division) veritabanı üzerinden “tıbbi ve aromatik bitkiler (medicinal and pharmaceutical plants)” başlığı altında derlenmiştir.¹ Tıbbi ve aromatik bitkiler Birleşmiş Milletler İstatistik Birimi'nin Comtrade veritabanının ‘HS 1211’ başlıklı emtia grubunun ‘SITC.3 292.4’ kodlu farmasötik bitkiler kapsamında tasnif edilmiştir (Lange 2006: 155).

Örnek olay araştırmasının tasarım ve uygulamasına yönelik üç aşaması şu şekildedir: (1) araştırmanın hedefleri, tasarımı ve yapısı; (2) her örnek olayın tasarıma uygun olarak gerçekleştirilmesi (3) araştırmacının örnek olayı bulgular doğrultusunda düzenlemesi ve çalışmanın hedefe ulaşması için bilimsel katkıyı değerlendirmesi (Aliu vd., 2018; George ve Bennett 2005: 73). TABE'de piyasa araştırması, sektörel gözlemler ve belge analizleri gerçekleştirilmiştir. Bu kapsamda, öncelikle dünya genelinde TABE ithalat ve ihracat verileri incelenmiştir. Bir sonraki aşamada ise seçilen üç bölge içerisinde bulunan ülkelerin özellikle olarak TABE verileri kıyaslamalı olarak araştırılmıştır. Dolayısıyla, seçilen bölgelerdeki ülkelerin TABE ölçeğinde ticaret akışları, ticaret yapısı, ticaret hacmi ve ticaret değerlerine yer verilmiştir. Ayrıca, tıbbi ve aromatik bitkilerin çok sayıda sektör içerisinde yaygın kullanımı ticari anlamda sektörler arası mukayese yapılmasını gerektirebilmektedir. Bu açıdan değerlendirildiğinde, TABE'nin sadece firma düzeyinde (mikro) veya ülkeler düzeyinde (makro) değil, yanı sıra sektörler arası (mezo) düzeyde de anlamlı ilişkilendirmeler gözlemlenebilmektedir.

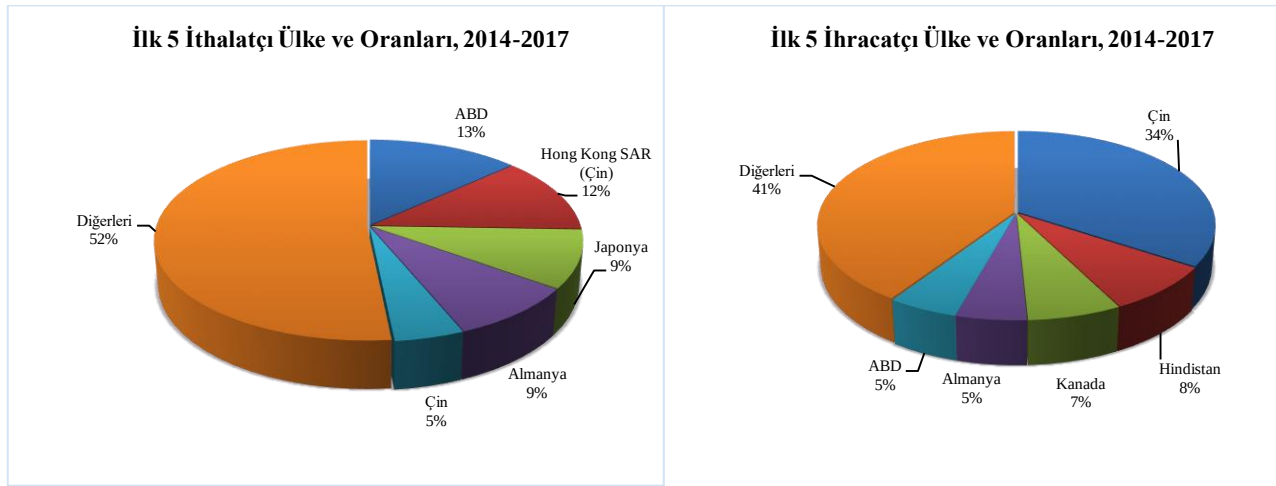
BULGULAR VE TARTIŞMA

Çalışmada, ‘Tarihi Baharat Yolu’nun çağın ileri teknolojisi ve inovasyon dikkate alınarak küresel piyasalarda önemli bir paya sahip olan TABE’de gerçekleşen dönüşüm mercek altına

¹ (1) ODAÜ; Bulgaristan, Hırvatistan, Çekya, Estonya, Macaristan, Letonya, Litvanya, Polonya, Romanya, Slovakya, Slovenya (11 AB Üyesi Ülke); ve Arnavutluk, Bosna & Hersek, Makedonya, Karadağ, ve Sırbistan; (2) BBÜ; Arnavutluk, Bosna & Hersek, Makedonya, Kosova, Karadağ, ve Sırbistan; (3) GDAÜ; Arnavutluk, Bosna & Hersek, Makedonya, Kosova, Karadağ, Sırbistan, Bulgaristan, Hırvatistan ve Yunanistan. Comtrade veritabanında tıbbi ve farmasötik bitkiler başlığında Kosova için veriler bulunmadığından dolayı bu ülke araştırma kapsamı dışında tutulmuştur.

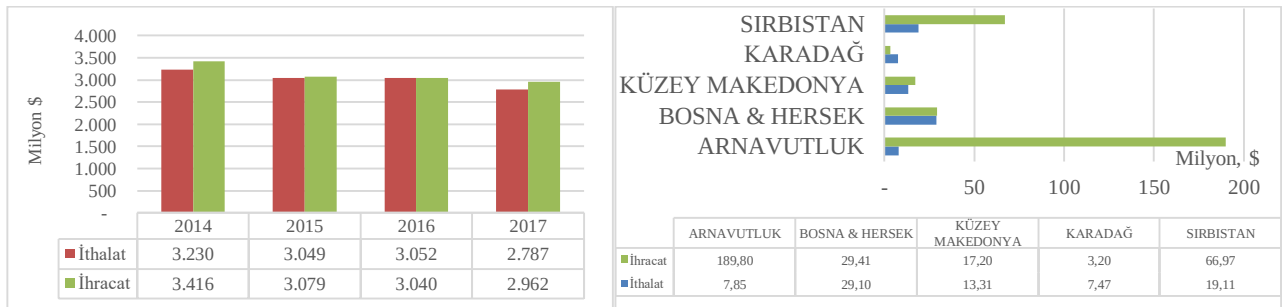
alınmıştır. Dolayısıyla, Hindistan'dan Balkanlara uzanan bu rota içerisinde ODAÜ, BBÜ ve GDAÜ analiz edilmiştir. Günümüz modern ekonomik sistem içerisinde 'Baharat Yolu' rotasındaki firmalar arasındaki teknoloji transferleri ve ticaret hacimleri artan bir eğilim göstermektedir.

ODAÜ ile GDAÜ'nün kesişiminde bulunan BBÜ stratejik olarak TABE'de 2013-2017 yılları arasında anlamlı bir gelişim göstermiştir. Hexa Research'ın yayınladığı bir sektörel rapora göre TABE'nin küresel ölçekte 2016 yılında ulaştığı toplam piyasa hacmi 71,19 milyar USD civarındadır. Bu oranın 2024 yılında 117,02 milyar USD düzeyine ulaşacağı tahmin edilmektedir. Bitkisel ekstraktların 2016 yılında ulaştığı toplam piyasa hacmi 27.1 milyar USD'dir. Bu oranın 2024 yılında 44.6 milyar USD düzeyine erişeceği tahmin edilmektedir (Hexa Research, 2017a, 2017b). Uluslararası Ticaret Merkezi'ne göre az gelişmiş ülkelerdeki ihracatçılar özellikle ekstraktların ihracatı ile sürdürülebilir üretim ve dış ticaret gelişimini sağlamayı hedeflemektedirler (International Trade Centre, 2018). Ancak, bu oranlar sadece TABE'yi değil, yanı sıra bu endüstrinin kapsadığı ve etkilediği diğer sektörleri de içermektedir.



Şekil 2: Küresel Ölçekte TABE'de İlk 5 İthalatçı ve İhracatçı Ülke ve Oranları, 2014-2017.

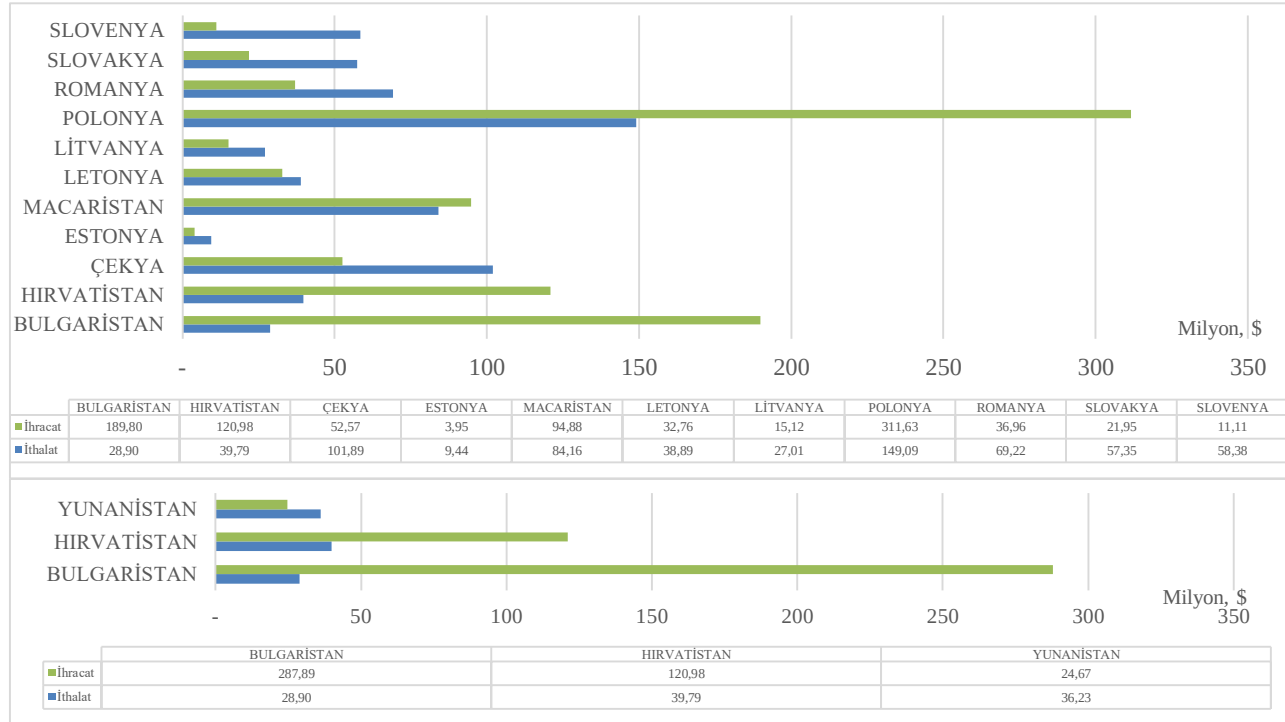
Bu çalışmada, BM İstatistik Birimi'nin hazırlamış olduğu Comtrade veritabanında yayınlanan veriler esas alınmıştır. Küresel ölçekte 2014-2017 yılları arasında toplam ithalat hacmi 12.11 milyar USD'dir. İlk 5 ithalatçı; ABD (1.60 milyar USD), Hong Kong, SAR – Çin (1.48 milyar USD), Japonya (1.13 milyar USD), Almanya (1.06 milyar USD) ve Çin'dir (562 milyon USD). Bu 5 ithalatçı küresel piyasadaki toplam ithalat hacminin yaklaşık yarısını kapsamaktadır. Küresel ölçekte 2014-2017 yılları arasında toplam ihracat hacmi 12.49 milyar USD'dir. İlk 5 ihracatçı; Çin (4.27 milyar USD), Hindistan (1.02 milyar USD), Kanada (847 milyon USD), Almanya (639 milyon USD) ve ABD'dir (633 milyon USD) (Aliu ve Aliu, 2018). Bu 5 ihracatçı küresel piyasadaki toplam ihracat hacminin yaklaşık %60'ını kapsamaktadır.



Şekil 3: Küresel Ölçekte (2014-2017) ve BBÜ'de (2013-2017) TABE'de İthalat ve İhracat Oranları.

Küresel ölçekte 2014-2017 yılları arasında hem ithalat hem de ihracat oranlarında belirli düzeyde bir azalış trendi görülmektedir. Genel olarak, son 4 yıllık zaman dilimi dikkate alındığında TABE'de ihracatın ithalata oranla daha yüksek olduğu görülmektedir. BBÜ'de 2013-2017 yılları arasında TABE'de ihracatta Arnavutluk 189,80 milyon USD ile lider görünümündedir. Bu bölgede,

TABE’de ithalat oranının en yüksek olduğu ülke ise Bosna Hersek’tir (Aliu ve Aliu, 2018). Ayrıca, bu bölgede TABE’de ülkeler arası ticaret hacmi de yükselmektedir. Bunun nedenlerinden birisi de bu ülkelerde faaliyet gösteren firmalarda noksan olan veya daha fazla gereksinim duyulan tıbbi ve aromatik bitkileri firmalar aralarında anlaşarak tedarik etmekte ve sürekli dayanışma halinde bulunmaktadır.



Şekil 4: ODAÜ ve GDAÜ’de Tıbbi ve Aromatik Bitkiler İthalatı ve İhracatı, 2013-2017.

ODAÜ ve GDAÜ, BBÜ’yü kapsamaktadır. Ancak, iki bölge arasındaki fark şudur ki; her iki bölgede de Bulgaristan ve Hırvatistan bulunmasına rağmen, Yunanistan sadece GDAÜ kategorisi içerisinde yer almaktadır. ODAÜ’de en yüksek ihracat ve ithalat oranına sahip ülke Polonya’dır. GDAÜ’de ise en yüksek ihracat oranı Bulgaristan’a, en yüksek ithalat oranı ise Hırvatistan’a aittir.

Türkiye’nin TABE’de en çok ithalat gerçekleştirdiği ülke ODAÜ ile GDAÜ’nün kesişimi olan BBÜ kategorisi içerisinde bulunan ve ihracatta liderliği diğer ülkelere bırakmayan Arnavutluk’tur. Türkiye, 2013-2017 yılları arasında Arnavutluk’tan toplamda 19.83 milyon USD civarında ithalat gerçekleştirmiştir. Geleceğe yönelik beklentiler dikkate alındığında, Arnavutluk Türkiye’nin bu sektörde 1 numaralı ithalatçısı olma trendini koruyacağı varsayılmaktadır.

TABE’de üretim teknolojileri, teknoloji transferi, inovasyon gibi başlıklarda şu konular ilgi çekici hale gelmiştir: uçucu yağların ekstraksiyonu için tıbbi ve aromatik bitkilerin damıtılması (Munir vd., 2014), tıbbi ve aromatik bitkilerin güneş damıtma sistemi ile çiftlikte üretilmesi (Munir ve Hensel, 2010), hasattan paketlenmeye kadarki süreçte teknolojik işlemenin ve aşılama ların tıbbi ve aromatik bitkilerin antioksidan kapasitelerine etkisi (Giao vd., 2013), kirli çevre şartlarında ağır metallerin tıbbi ve aromatik bitkilerde ikincil metabolit biyosentezi, oksidatif statüsü ve fito-ekstraksiyon’a etkisi (Lajayer, Ghorbanpour ve Nikabadi, 2017), TABE’de yıkıcı olmayan bir teknik olarak hiperspektral görüntüleme (HSI) (Kiani, van Ruth ve Minaei, 2018), sıvı-sıvı mikroekstraksiyon (LLME) ve katı fazlı mikroekstraksiyonun (HS-SPME) gaz kromatografisi-kuadrupol kütle spektrometresi (GC-qMS) ile birleştirilmesi ile tıbbi ve aromatik bitkilerin zeytin yağı çeşitleri ile birlikte üretilmesi (Perestrelo vd., 2017), organik tarım kapsamında tıbbi ve aromatik bitkilerin ekilmesi sürecinde kaynak kullanım verimliliği (Sujatha vd., 2011), tıbbi ve aromatik bitkilerde epigenetik ve epigenomik düzenlemeler (Hao ve Xiao, 2018), ekili ürün yetiştirmede sulama, gübreleme ve bitkinin korunması; hasat sonrası işlemede yıkama, soğutma, kesme, damıtma, buhara tabi tutma, ışınlama, kurutma, fümigasyon, ayıklama, paketlenme ve depolama (Maikhuri vd.,

2017; Novak, 2014; Sharma ve Kala, 2018), uçucu organik bileşenler (VOCs), gaz kromatografisi ve kütle spektroskopisi, sensörler ile sinyal işleme ve gelişmiş veri işleme algoritmaları (Kiani vd., 2016), tıbbi ve aromatik bitkilerin korunmasında organofosfatlar, karbamatlar, piretroidler ve neonikotinoidler gibi sentetik insektisitlerin kullanımı (Gahukar, 2018), tıbbi ve aromatik bitkilerin antioksidan ve antienflamatuvar özellikleri üzerindeki polifenolik kompozite olan mevsimsel etkisi (Piccolella vd., 2018) gibi.

Tıbbi ve aromatik bitkilerde patent koruması hakkı; yenilik standartları, yaratıcı adım ve endüstriyel korumayı kapsayan genetiği değiştirilmiş tıbbi ve aromatik bitkiler, bitki hücreleri, kimyasal bileşenler ve tohumlar için mevcuttur (Overwalle, 2006: 127). Dolayısıyla TABE’de ticari ilişkilerin geliştirilmesinde bu çerçevede bazı düzenlemelere gidilmiştir (European Commission and TRAFFIC, 2013). TABE’de bilgi üreticileri ve tüketicileri stratejik teknolojik entegrasyonda katma değer oluşturabilmektedir. Bu bağlamda, akademik teknoloji transferinin bilginin ticarileştirilmesi kanalıyla sektörel teknoloji transferi ile bütünleşmesi inovasyonun TABE’de yerleşik bir konum kazanmasına neden olabilmektedir (Carayannis vd., 2018; McNett, 2017).

SONUÇ

TABE’de sosyal ve çevresel sorumluluk çerçevesinde organik ve ekili (*cultivated*) ürünler (Lubbe ve Verpoorte, 2011) dahil olmak üzere tıbbi ve aromatik bitkilerin aşırı üretilmesinin önüne geçebilmek amacıyla biyoçeşitlilik, ekosistem yönetimi, gıda sağlığı ve güvenliği, çevreci ürünlerin gelişimi ve standardizasyonu, çevre ile ilişkili kalite yönetimi ve belgelendirme, patent hakları ile ilişkili konularda firmalara ve tedarikçilere eğitimlerin verilmesi ihtiyacı günümüzde daha da belirginleşmektedir (Miho, 2011). TABE piyasasının gelecek yıllarda daha fazla büyümesi ve gelişme göstermesi bu alan ile ilgili daha çok bilimsel araştırma ve Ar-Ge çalışmalarının yapılması, finansal desteklerin verilmesi ve uygulamalı projelerin hayata geçirilmesi ile hızlandırılabilir.

TABE’de faaliyet gösteren KOBİ’ler ölçeğindeki firmaların Çok Uluslu Şirketler ile daha fazla rekabet edebilmesi için inovatif stratejilerin geliştirilmesi, yalın üretim sürecinin tanıtılması, sektörel başarı faktörlerinin aktarımı, yeni nesil girişimcilerin TABE’ye kazandırılması gerekmektedir. Bilginin ticarileştirilmesi bağlamında KOBİ’lere sunulacak olan bilimsel desteğin doğrudan dış ticarete anlamlı bir etkisi vardır (Aliu ve Aliu, 2017).

ODAÜ, BBÜ ve GDAÜ gibi gelişmekte olan ülkelerde sıkça görülen orta-gelir tuzağından çıkılması için geliştirilmiş olan üretimde yüksek teknolojinin kullanılması yoluyla daha yüksek katma değer taşıyan ürünlerin üretilmesi TABE için de geçerlidir. Bu çerçevede, firmaların kullanmakta olduğu üretim teknolojilerinin durum analizi yapılmalı ve yüksek teknolojinin kullanımının sağlanmasıyla TABE’de daha fazla katma değer ve yüksek standartlar taşıyan ürünlerin küresel piyasada müşteri bulması hedeflenmelidir. Dolayısıyla, TABE’de faaliyet gösteren firmaların “kazan-kazan prensibi” ile yüksek teknolojiye sahip firmanın ortaklıktan doğan müşteri portföyünden kazanç sağlaması, diğer yandan yüksek teknoloji ihtiyacı duyan firmanın ortak girişimden uluslararası standartlarda üretim sürecine katkı sağlaması mümkün hale gelecektir.

Gün geçtikçe önemi artan TABE’nin temel ihtiyacı haline dönüşen teknoloji transferi ve buna bağlı olarak inovasyon, standardizasyon, akreditasyon ve fikri mülkiyet hakları bağlamında tecrübeli firmaların aynı sektör içerisinde faaliyet gösteren ortak firmalar ile işbirliğinin sağlanması ve gerekli dayanışma ortamının oluşturulması genel anlamda bir verimlilik artışının sağlanmasına neden olabilmektedir. TABE’nin bir bütün olarak sürdürülebilir kalkınma için, bu alanda faaliyet gösteren işletmelerin sürdürülebilirliğini göz önünde bulundurarak, yüksek talep ve tehdit altında olan türlerin yetiştirilmesine ihtiyaç duyulmaktadır. Bu bağlamda, uygun teknolojinin endüstride faaliyet gösteren firmalara transferi ve belirli uluslararası standartlar (Chandra ve Sharma, 2018; Kala, 2015; Roy vd., 2018; Silvius vd., 2017; The Rainforest Standard, 2017) çerçevesinde işlevselliği konusuna dikkat çekmekte fayda vardır. Ayrıca, artan rekabet ortamı, piyasada daha etkin olmak için firmaların, üniversite ve kamu kuruluşları ile işbirliği yapmalarını teşvik etmektedir. Bu tür işbirliklerinin hem ekonomik sorunlara hem de çevre sorunlarına çözüm bulmak adına faydalı oldukları görülmektedir. Bu işbirlikleri TABE’de faal olan firmaları (KOBİ’ler, Startup’lar, Spin-off şirketler vb.); TABE’ye

ilgi duyan girişimcileri; yatırım ve ortaklık gibi iş potansiyeli arayışında bulunan ilgili firmaları vb. gibi geniş bir paydaş kesimini içermektedir.

Birçok doğal tıbbi ve aromatik bitki türünün aşırı düzeyde sömürüldüğü gerçeğinin artması ile bu sektörde faal olan bazı kurumlar, ekili ürünler yerine organik bitki türlerinin yetiştirilmesi için birtakım işlevsel sistemlerin geliştirilmesi gerektiğine vurgu yapmaktadır. Bu bağlamda, sürdürülebilir hasatın (Barata vd., 2016) yerel ekonomilere katkıları ve uzun vadede toplayıcılara daha fazla kazanç ve değer sağladığı dikkate alındığında çoğu hasat edilen organik türlerin korunması stratejisi sektörde daha sürdürülebilir bir kalkınma modeli ve üretim sisteminin geliştirilmesi gereksinimi belirgin bir hâl almaktadır. Dolayısıyla, gelecekte yapılacak çalışmaların sürdürülebilir hasat oranları ve uygulamaları ile ilgili bilgi eksikliğine, tanımlanmamış arazi kullanım haklarına, hukuk ve politika rehberliğinin noksanlığına, inovasyon ve ileri teknolojinin yalın üretim sürecini ne ölçüde etkilediğine odaklanabilir (Schippmann, Leaman ve Cunningham, 2006: 75). Bu şekilde, ekili bitkilere artan ilgiye rağmen doğal yabani bitkileri toplamanın biyolojik, ekonomik ve sosyal faktörlere dayanan birçok tıbbi ve aromatik bitki türünün çeşitliliğinin korunmasına sağladığı olumlu katkı vurgulanabilir (Leaman, 2006: 97).

TEŞEKKÜR

Bu çalışma, İstanbul Ticaret Üniversitesi'nde yürütülen "Tıbbi ve Aromatik Bitkiler Endüstrisi'nde Teknoloji Transferi ve İnovasyonun Firmaların Üretim Süreçlerindeki Standardizasyon, Akreditasyon ve Patent Haklarına Etkileri" başlıklı ve "ICU-TTO_KOSGEB-EUREKA/EU-2018-0002" kodlu büyük ölçekli uygulamalı bilimsel araştırma projesi kapsamında hazırlanmıştır. Bu kapsamda, İstanbul Ticaret Üniversitesi Teknoloji Transfer Ofisi (TTO) Koordinatörü Sayın Prof. Dr. Abdül Halim ZAIM'e, Sayın Dr. Öğretim Üyesi Umut EROĞLU'na (ÇÖMÜ) ve İstanbul Ticaret Üniversitesi Bursiyer Araştırmacı Sayın Ardita BYLO'ya yardımlarından ve desteklerinden ötürü teşekkürlerimizi sunarız. Ayrıca, Antalya Organize Sanayi Bölge Müdürlüğü'ne projemizi 29-30 Mart 2018 tarihleri arasında Antalya OSB Ar-Ge ve İnovasyon Proje Pazarı Etkinliği'nde sergilenmeye hak kazanan proje olarak seçmesinden dolayı teşekkürlerimizi sunarız.

REFERENCES

- Aliu, A., Aliu, D., 2017. Collaborations and sustainability in medicinal and aromatic plants industry: Examining the business communication amongst stakeholders in MAPs industry. International Congress on Political, Economic and Social Studies, 2: 14-34.
- Aliu, A., Aliu, D., 2018. Comtrade medicinal and aromatic plants (MAPs) statistics of western balkan countries (WBCs), central and eastern european countries (CEECs) and south-eastern european countries (SEECs) in a comparative perspective. Scholars Portal Dataverse, V1, <https://doi.org/10.5683/SP2/Z3CAKE>
- Aliu, A., İsmail, A., Aliu, D., Bylo, A., 2018. Küresel ekonomik yönetim ve çokmerkezli yönetimde devlet dışı aktörlerin etkileşimleri: Güneydoğu Avrupa ülkeleri ve Çin'de küresel işbirliği. (Yayınlanmamış Kitap Bölümü)
- Asllani, U. (2004). *Esencat e Bimeve Aromatike e Mjekesorete Tretave Shqiptare*. Stepia Botuese 8 Nentori, Tirane, Albania.
- Barata, A.M., Rocha, F., Lopes, V., Carvalho, A.M., 2016. Conservation and sustainable uses of medicinal and aromatic plants genetic resources on the worldwide for human welfare. Industrial Crops and Products, 88: 8-11.
- Carayannis, E.G., Rozakis, S., Grigoroudis, E., 2018. Agri-science to agri-business: The technology transfer dimension. Journal of Technology Transfer, 43(4): 837-843.
- Chandra, P., Sharma, V., 2018. Strategic marketing prospects for developing sustainable medicinal and aromatic plants businesses in the Indian Himalayan region. Small-scale Forestry, DOI: <https://doi.org/10.1007/s11842-018-9396-3>
- European Commission and TRAFFIC, 2013. Reference Guide to the European Union Wildlife Trade Regulations. Brussels, Belgium.
- Gahukar, R.T., 2018. Management of pests and diseases of important tropical/subtropical medicinal and aromatic plants: A review. Journal of Applied Research on Medicinal and Aromatic Plants, 9: 1-18.
- George, A.L., Bennett, A., 2005. *Case Studies and Theory Development in the Social Sciences*. Cambridge, MA: MIT Press.
- Giao, M.S., Pereira, C.I., Pintado, M.E., Malcata, F.X., 2013. Effect of technological processing upon the antioxidant capacity of aromatic and medicinal plant infusions: From harvest to packaging. LWT – Food Science and Technology, 50: 320-325.
- Hao, D.C., Xiao, P.G., 2018. Deep in shadows: Epigenetic and epigenomic regulations of medicinal plants. Chinese Herbal Medicines, p. 1-10.
- He, J., Yang, B., Dong, M., Wang, Y., 2018. Crossing the roof of the world: Trade in medicinal plants from Nepal to China. Journal of Ethnopharmacology, 224: 100-110.

- Hexa Research, 2017a. Herbal Medicine Market Size and Forecast, By Product (Tablets & Capsules, Powders, Extracts), By Indication (Digestive Disorders, Respiratory Disorders, Blood Disorders), And Trend Analysis, 2014 – 2024. September 2017 <https://www.hexaresearch.com/research-report/global-herbal-medicine-market>
- Hexa Research, 2017b. Herbal Medicine Market Size To Reach USD 117.02 Billion By 2024. September 2017 <https://www.hexaresearch.com/press-release/herbal-medicine-market>
- International Trade Centre, 2018. Medicinal and Aromatic Plants and Extracts. August 2018 <http://www.intracen.org/itc/sectors/medicinal-plants/>
- Kala, C.P., 2015. Medicinal and aromatic plants: Boon for enterprise development. *Journal of Applied Research on Medicinal and Aromatic Plants*, 2: 134-139.
- Kiani, S., Minaei, S., Ghasemi-Varnamkhasti, M., 2016. Application of electronic nose systems for assessing quality of medicinal and aromatic plant products: A review. *Journal of Applied Research on Medicinal and Aromatic Plants*, 3: 1-9.
- Kiani, S., van Ruth, S.M., Minaei, S., 2018. Hyperspectral imaging, a non-destructive technique in medicinal and aromatic plant products industry: Current status and potential future applications. *Computers and Electronics in Agriculture*, 152: 9-18.
- Lajayer, B.A., Ghorbanpour, M., Nikabadi, S., 2017. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicology and Environmental Safety*, 145: 377-390.
- Lange, D., 2006. International trade in medicinal and aromatic plants. In: Bogers, R.J., Craker, L.E., Lange, D. (eds.). *Medicinal and Aromatic Plants*, p. 155-170, Springer, the Netherlands.
- Leaman, D.J., 2006. Sustainable wild collection of medicinal and aromatic plants. In: Bogers, R.J., Craker, L.E., Lange, D. (eds.). *Medicinal and Aromatic Plants*, p. 97-107, Springer, the Netherlands.
- Lubbe, A., Verpoorte, R., 2011. Cultivation of medicinal and aromatic plants for speciality industrial materials. *Industrial Crops and Products*, 34: 785-801.
- Maikhuri, R.K., Phondani, P.C., Rawat, L.S., Jha, N.K., Maletha, A., Bahuguna, Y.M., Kandari, L.S., (eds.) (2017). Conservation and management strategies of medicinal plant resources through action research approaches in Indian Himalaya. *Iranian Journal of Science and Technology Transactions of Electrical Engineering*, 41: 771-777.
- Máthé, Á., (ed.). 2015. *Medicinal and Aromatic Plants of the World: Scientific, Production, Commercial and Utilization Aspects – Vol.1*. Springer, Dordrecht, the Netherlands.
- McNett, J., 2017. Barriers and bonds to communication and knowledge transfer. In: Cooper, C.L., (ed.) 2017. *The Blackwell Encyclopedia of Management*. Blackwell Publishing. Blackwell Reference Online. 24 February 2017 http://www.blackwellreference.com/subscriber/tocnode.html?id=g9780631233176_chunk_g97806312349375_ss1-17
- Miho, A., 2011. *Monitorimi Biologjik Mjedisor*. Julvin 2, Tirana, Albania.
- Munir, A., Hensel, O., 2010. On-farm processing of medicinal and aromatic plants by solar distillation system. *Biosystems Engineering*, 106: 268-277.
- Munir, A., Hensel, O., Scheffler, W., Hoedt, H., Amjad, W., Ghafoor, A., 2014. Design, development and experimental results of a solar distillery for the essential oils extraction from medicinal and aromatic plants. *Solar Energy*, 108: 548-559.
- Negi, V.S., Kewlani, P., Pathak, R., Bhatt, D., Bhatt, I.D., Rawal, R.S., Sundriyal, R.C., Nandi, S.K., 2018. Criteria and indicators for promoting cultivation and conservation of medicinal and aromatic plants in Western Himalaya, India. *Ecological Indicators*, 93: 434-446.
- Novak, J., 2014. EUROPAM statement on requirements for a batch certification of medicinal and aromatic plants (MAPs). *Journal of Applied Research on Medicinal and Aromatic Plants*, 1: 70-71.
- Overwalle, G., 2006. Intellectual property protection for medicinal and aromatic plants. In: Bogers, R.J., Craker, L.E., Lange, D. (eds.). *Medicinal and Aromatic Plants*, p. 121-128, Springer, the Netherlands.
- Perestrelo, R., Silva, C., Silva, P., Câmara, J.S., 2017. Global volatile profile of virgin olive oils flavoured by aromatic/medicinal plants. *Food Chemistry*, 227: 111-121.
- Piccolella, S., Crescente, G., Pacifico, F., Pacifico, S., 2018. Wild aromatic plants bioactivity: A function of their (poly)phenol seasonality? A case study from Mediterranean area. *Phytochemistry Reviews*, DOI: <https://doi.org/10.1007/s11101-018-9558-0>
- Roosta, R.A., Moghaddasi, R., Hosseini, S.S., 2017. Export target markets of medicinal and aromatic plants. *Journal of Applied Research on Medicinal and Aromatic Plants*, 7: 84-88.
- Roy, S., Mir, R.A., Gangwar, A.K., Gangwar, R., (2018). Herbs for health: Communicating for conservation, cultivation and sustainable utilisation of medicinal and aromatic plants. In: Payyappallimana, U., Fadeeva, Z. (eds.). *Ensure Healthy Lives and Promote Well-Being For All: Experiences of Community Health, Hygiene, Sanitation and Nutrition. Learning Contributions of the Regional Centres of Expertise on Education for Sustainable Development*, p. 62-71, The United Nations University, Tokyo, Japan.
- Schippmann, U., Leaman, D., Cunningham, A.B., 2006. A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In: Bogers, R.J., Craker, L.E., Lange, D. (eds.). *Medicinal and Aromatic Plants*, p. 75-95, Springer, the Netherlands.
- Sharma, N., Kala, C.P., (2018). Harvesting and management of medicinal and aromatic plants in the Himalaya. *Journal of Applied Research on Medicinal and Aromatic Plants*, 8: 1-9.
- Silvius, G., Schipper, R., Planko, J., Brink, J. van der, Köhler, A., 2017. *Sustainability in Project Management*. Farnham: Taylor and Francis.
- Sujatha, S., Bhat, R., Kannan, C., Balasimha, D., 2011. Impact of intercropping of medicinal and aromatic plants with organic farming approach on resource use efficiency in arecanut (areca catechu L.) plantation in India. *Industrial Crops and Products*, 33: 78-83.
- The Rainforest Standard, 2017. RFS executive summary. 24 May 2017 <http://cees.columbia.edu/therainforeststandard>

USE OF LINSEED AS A MEDICAL AND AROMATIC PLANT IN THE HEALTH SECTOR

Orhan KURT¹, Merve GÖRE¹

¹University of Ondokuz Mayıs, Faculty of Agriculture, Department of Agronomy, Atakum/SAMSUN

Corresponding author email: merve.gore@omu.edu.tr

Abstract

As a consequence of the increase of healthy nutrition knowledge of consumers, the functional food orientation which is beneficial for health as well as nutrition is increased. Functional foods are known as food or food ingredients that provide benefits to physiological and metabolic activities as well as to meet basic nutrient needs. Functional foods play a key role in preventing disease and maintaining a healthier and better quality of life.

Linseed flavonoids, lignans, phenolic compounds and many other phytochemicals are naturally derived plant sources. In this respect, linseed products enter the functional food category. Linseed is used in the health sector as a medicinal and aromatic plant. In this article, information on the importance and composition of linseed will be presented and then the usage areas and uses of linseed products in the health sector will be discussed.

Keywords: Linseed, Medicinal and aromatic plant, Healthy lifestyle

INTRODUCTION

Linseed; in the Linaceae family, the *Linum* genus is one of the few plants in the world where agriculture is first constructed. There is no consensus on the origin center of linseed. However, common belief is; the origin of the oil type of linseed is the Mediterranean region, and the origin of the fiber type of linseed is the North Europe. However, both linseed groups are grown in many parts of the world today (Kurt, 1995).

Linseed has been used for many 10,000 years as a human being and animal food. In terms of healthy and quality living, especially the seed oil of the linseed and the oil extracted from the seed are used. Linseed seed is a source of Alfa Linolenic Acid (ALA: Omega-3), Linoleic Acid (LA: Omega-6), Oleic Acid (OA: Omega-9), Lignan (SDG), Mucilage and Vitamin A (Beta-carotene) (İşleroğlu et. al., 2005). The linseed seed also contains high-fiber. In addition, linseed seeds contain plenty of potassium, with small amounts of magnesium, iron, copper, zinc and various vitamins. Due to this rich content, linseed is used as a functional food, and many areas are used to preserve and maintain healthy life.

Composition of Linseed

Oil content and fatty acid composition

Linseed is rich in oil, protein and fiber. Linseed contains an average of 40-45% oil, 18-20% protein, 25-30% total food fiber, 7-8% moisture and 3-4% ash (Anon., 2001). Linseed oil contains 5-6% palmitic acid (16: 0), 3-4% stearic acid (18: 0), 16-18% oleic acid (18: 1n-9), 15-16% linoleic acid (18: 2n-6) and 55-60% linolenic acid (18: 3n-3). Maximum variability in fatty acid composition; oleic acid (14-60%), linoleic acid (3-21%) and linolenic acid (31-72%) were observed (Mazza, 1998). Linseed oil is one of the lowest proportions of vegetable oils in terms of saturated fatty acids that are not desirable for nutrition. It is also moderate in terms of the desired monounsaturated fatty acids (Anon., 1994).

Proteins

Linseed seed contains two main storage protein groups, high molecular weight soluble in salt and low molecular weight soluble in water. The protein of the linseed seed can affect blood glucose in two different ways; (a) induce insulin secretion, leading to a decrease in the glycemic index, (b) interactions with polysaccharides, and may be effective in the glycemic index of foods. It has also been determined that linseed seed proteins have antifungal properties. For this reason, these proteins have the potential to be used to inhibit mold growth in some food systems (Friedman and Levin, 1989).

The amino acid composition of the linseed protein is similar to the soybean protein. However, they have structurally more lipophilic and lower trypsin inhibitor activity than soybean proteins (Bhatty and Cherdkiatgumchai, 1990). It has also been found that linseed seeds provide a twofold reduction in triglyceride concentration compared to soybean protein and have much more hypotriglyceridemic activity than soybean protein of linseed seed (Oomah and Mazza, 1993).

Carbohydrates

Linseed carbohydrate (sugar and starch) is poor in content. There is only 1 gr of carbohydrate in 100 gram linseed seeds. For this reason, Linseed does not make much contribution to carbohydrate supplement maintenance.

Food fibers

Fiber is formed as structural material on cell walls of plants. The total fiber consists of the sum of the food fibers and the functional fibers. Functional fiber; carbohydrates that are extracted from plants, are indigestible and have beneficial effects on humans. Food fibers and functional fibers; it cannot be indigestible and cannot be absorbed by the human small intestine. For this reason it goes directly to the large intestine (Institute of Medicine, 2002).

The total fiber content of Linseed seeds is 28%. The largest fiber fraction in the linseed is cellulose. Mucilage is a heterogeneous polysaccharide and forms a large part of the soluble fiber fraction of linseed seed and has hypoglycemic effect in humans (Green and Dribnenki, 1994). Lignins, a branched fiber type found in the cell walls of woody plants, contribute to the durability and stiffness of the cell walls.

Lignans

Lignans are phenolic compounds and have two basic sources, vegetable and animal. When plant lignans are consumed, they are converted into mammal lignans in the colon (Anon, 2001; Bloedon and Szapary, 2004). Linseed and Linseed products contain so much lignans that they cannot be compared with other plants and products. (Milder et al., 2005) (Table 1). This lignan is the richest source (0.2-3.7 mg/g seed) in terms of the mammalian lignan precursor secoisolacetic acid diglucoside (SDG) (Mazza, 1998). Linseed seed also contains minor lignans, including isolarikiresinol, pinoresinol and matairesinol (Kris-Etherton et al., 2002).

Table 1. Lignan content ($\mu\text{g}/100$ g fresh edible weight) of solid foods (modified Milder et al., 2005).

Source	Amount	Source	Amount	Source	Amount	Source	Amount
Flaxseed	301129	wheat	210	Bean	236	White cabbage	787
Sesame seed	39348	Rice	40	Apricot	450	Tomato paste	187
Sunflower	891	Broccoli	1325	Strawberry	334	Sweet pepper	172
Cashew	629	Garlic	536	Peach	293	Curly kale	2321
Peanut	94	Onion	36	Orange	78	Flaxseed bread	12474
Poppy seed	10	Kiwi	129	Olive oil	248	Multi-grain bread	6744

Phenolic acids

In oilseed crops, phenolic acids are formed as hydroxylated derivatives of benzoic and cinnamic acids. Linseed seeds contain a total of 8-10 g/kg phenolic acid, 5 g/kg esterified phenolic acid and 3-5 g/kg etherified phenolic acid. The main phenolic acids found in these products are; trans-ferulic (46%), trans-sinapic (36%), p-kumaric (7.5%) and trans-caffeic (6.5%) acids (Oomah and Mazza, 1998).

Phenolic acids and analogs such as naturally occurring caffeic and gallic acid are known to exhibit a wide range of biological functions associated with carcinogen modulation. Cinnamic acid esters, such as caffeic acid phenethyl and benzyl esters, have an anti-proliferative effect against some types of cancer cells. The antioxidant, antimicrobial and anti-cancer effects of the linseed seed bioactive functions are due to phenolic acids in its structure (Yarım and Kurt, 2001; İşleroğlu et al., 2005).

Flavonoids

Flavonoids are a group of natural benzo- γ -pyran derivatives and are involved in photosynthesizing cells (Cook and Saman, 1996; Anon., 1997). Flavonoids have biological activity against allergies, inflammation, free radicals, hepatotoxins, microbes, viruses, ulcers and platelet aggregation. Flavonoids inhibit the activity of enzyme systems, including cyclooxygenase and lipoxygenase, despite lipid peroxidation, capillary permeability and brittleness reduction (Daun and Przybylski, 2000). In addition, flavonoids protect cells against carcinogens by increasing the pumping of certain carcinogens from cells or by stimulating detoxification enzymes (Hasler, 2002).

The flavonoid content of the linseed seed ranges from 35 to 71 mg/100 g (Daun and Przybylski, 2000) and the main flavonoids are flavan C- and O-glycosides (Freeman, 1995). Up to 30 mg of flavonoid intake per day provides approximately 50% reduction in coronary heart disease compared to less than 19 mg/day (Connon, 1999; Kris-Etherton et al., 2002).

Tocopherols

Tocopherols are the most potent natural antioxidants dissolved in oil. Linseed oil contains α , β , γ and δ tocopherols and the total tocopherol content is about 40-50 mg/100 g (Freeman, 1995; Mazza, 1998). 26% of total tocopherols are found in seed coat. Tocopherol protects polyunsaturated fatty acids in the cell membrane from oxidation and contributes to antioxidant capacity by keeping selenium in a reducing form (Mazza, 1998).

Linseed Use for Treatment and Protection

Breast cancer

Breast cancer is known to be affected by sex hormones, especially the estrogen **hormone**. Located in the linseed, both ALA and lignans affect cancer development. In women with the highest ALA ratio in adipose tissue, the risk of developing breast cancer is so low. There is an inverse relationship between ALA consumption and breast cancer risk. When a group of women is fed with ground linseed cake and the other group is fed with whole grain wheat cakes; it has been found that the women in the group fed with cakes have a significant decrease in the spread of breast cancer cells and in tumor development (Yarım and Kurt, 2001).

When different vegetable oils are added for 6 weeks to the diet of female mice that are administered with chemicals that promote breast tumors, among the oils tested, linseed oil was found to have the most inhibitoriest effect and the lowest number of breast tumors. In addition, in mice fed with linseed oil for 7 weeks, it was determined that the size of tumors in the breast was reduced by 50%. It has been determined that mice fed milled linseed into their diet are significantly less likely to

develop breast tumors than those fed control feeds and that metastases to other organs and lymph nodes decrease significantly as well. In addition, it has been found that tumor growth in human breast tumor transplanted mice decreases with the application of 10% linseed seed and decreases the amount of dimethylbenzothiazine causing tumor formation in mammalian living beings by adding 5% linseed seeds (Aubrecht et al., 1998).

Colon cancer

When plant lignans are consumed, they are transformed into mammalian lignans such as enteriol and enterolactone in the colon. Lignans also affect the cholesterol homeostatic by reducing the enzyme that plays a role in the formation of bile acid from cholesterol, thereby reducing the risk of colon cancer. When mice treated with a cancerous substance are fed with 5% or 10% milled linseed or lean linseed flour for 4 weeks; a significantly lower number of abnormal cells and fewer cell proliferation were detected in the colon than in control mice (Yarım and Kurt, 2001). Abnormal cells are early warning signs of colon cancer risk (Thompson et al., 1996).

Prostate cancer

Linseed seed reduces total testosterone levels and free androgen index values in prostate cancer (Bloedon and Szapary, 2004). When a group of patients with prostate cancer added 30 g of milled linseed to their daily diet for one month, the spread of cancer cells decreased (Demark-Wahnefried et al., 2001). It has been observed that prostate cancer progression is blocked when milled linseed is added to the diet of mice with advanced cancerous cells. In addition, mice with linseed milled to their diet were found to have increased cancer cell death and decreased cell proliferation (Halim and Kurt, 2001).

Skin cancer

Linseed seed extracts, and particularly purified lignans, may exhibit antioxidant activity in the form of protection against skin cancer and inhibition of the activation of promutagens and procarcinogens (Yarım ve Kurt, 2001).

Cardiovascular Diseases

CVD and atherosclerosis

Atherosclerosis develops in childhood CVD. When atherosclerosis develops, deposits of cholesterol and other blood lipids accumulate on the walls of blood vessels. These diseases are affected by low density lipoproteins (LDL), eicosanoids, cytokinins and other factors that are oxidized. Plaques can form on the walls of blood vessels. These plaques can grow so large that they block blood flow to the heart and brain (Yarım and Kurt, 2001). The clotting of blood platelets may result in thrombosis. Such a structure can cause a myocardial infarction or heart attack when it occurs in the heart and the blood flow is blocked. If this mass stops the flow of blood in the brain, it causes a catastrophic event (Yarım and Kurt).

Fatty acids taken with food are all effective in this process (McDonald, 1994). In foods, linseed is one of the richest sources of alpha-linolenic acid. Some of the alpha-linolenic acid is converted into long chain N-3 fatty acids EPA and DHA in the body. These; elevates good cholesterol, reduces high blood pressure, reduces blood clotting tendency, reduces plasma triglyceride level, decreases arrhythmia risk. Linseed seed significantly reduces the concentration of lipoprotein, which is a strong marker of total cardiovascular disease. Linseed seed fiber reduces serum cholesterol, a risk factor for coronary heart disease (Yarım and Kurt, 2001).

Arrhythmia

This disease is caused by blood clotting due to various reasons in the heart. Animals are protected against Arrhythmias; pure eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to be effective (Kang and Leaf, 1996). In laboratory studies with heart cells taken from mice; ALA, EPA and DHA have similar effects in preventing Arrhythmias disease (Kang and Leaf, 1996). Omega 3 fatty acids protect the Arrhythmia by first releasing it from the phospholipids and then making the physical condition of the membrane less permeable.

Coronary heart diseases

Reducing the consumption of saturated fatty acids and trans fatty acids and increasing the consumption of polyunsaturated fats, monounsaturated fats and food fibers helps reduce total cholesterol and LDL cholesterol levels. Fatty acid-rich foods such as ALA in Linseed play a role in preventing events such as heart attacks and stroke (Anon, 2002).

Paralysis

Serum phospholipids have been found to have a lower risk of stroke in men with higher ALA (Simon et al., 1995). Therefore, it is not wrong to say that the addition of dietary linseed seed or flour reduces paralysis (Yarim and Kurt, 2001).

Diabetes

Linseed contains fiber, lignan SDG and ALA, which protect against diabetes. When linseed bread and white bread are added to the diet of healthy adult young people, It was determined that the change in blood sugar in the group with linseed sowing was 28% lower (Prasad et. al., 2000).

Functions of the immune system

The immune system is capable of successfully defending itself against foreign substances such as bacteria and viruses. Components such as ALA and lignans found in Linseed seeds affect immune cells and eicosanoids and cytokines that control immune reactions. In addition, ALA and lignans found in Linseed can play a positive role in the clinical treatment of autoimmune diseases such as arthritis and systemic lupus erythematosus (Ingram et al., 1995).

Kidney diseases

Systemic lupus erythematosus (SLE) is an inflammatory disease that has major effects on human health, including kidney failure, arthritis, clotting and epileptic seizures. When milled linseed is added to the diet of SLE rats for 4 weeks (15 g, 30 g or 45 g daily); decreased renal failure, increased renal function, and reduced inflammation (Cooper et al., 1998).

Digestion (Laxation)

Linseed is a functional food with potency to increase laxation. Chronic lactation problems generally arise in the elderly due to inactivity, low fiber intake, or drug use (Yarim and Kurt, 2001). Soluble fiber; reduces glycemic index by increasing the viscosity of the small intestinal content and delaying absorption and digestion of carbohydrates (Anon., 1994). In addition, the fibers delay gastric emptying, increase transit time, and increase secretion of bile acid. As a matter of fact, it has been determined that when linseed ground 50 gr into daily diet of 10 healthy young group is added, it increases 30% in intestinal motility (Hamadeh et al., 1992).

Osteoporosis

Phytoestrogens play a role in preventing bone loss. Lignans and ALA in linseed are effective in preventing bone loss and osteoporosis by blocking the production of prostaglandins and reducing the rate of bone loss. Indeed, linseed has been reported to have a positive effect on bones by increasing antioxidant activity in menopausal women (Arjandi, 2001).

Climacteric

The lignans found in the linseed reduce the estrogen hormone and have a repressive effect on the menopause process and thus play a role in making the menopausal process more comfortable. In a study conducted with menopausal women for two months; a group of women in a group were added to the daily diet of 40 gr milled linseed. One group of estrogen-progesterone hormone was administered orally by women in the other group. The result of the evaluation was that linseed was as effective as hormone therapy in terms of increasing menopausal symptoms in the middle. It has also been reported that linseed seed reduces estrogen lipoprotein levels in postmenopausal women (Lemay et al., 2002).

General Purpose Use

Linseed, apart from the above areas; a) to strengthen memory by the contribution it makes to the renewal of cells, b) to regulate hormone balance, to reduce the distractibility due to concentration and aging, and c) to prevent mental disorders. Linseed flour is used for faster healing of wounds, for treatment of calluses, eczema and psoriasis. Linseed soup is used for treatment of respiratory diseases and stomach disorders. In addition, linseed reduces the need to eat because it gives a feeling of satiety when you eat seeds or flour. Therefore, it helps to gain a healthy body by preventing weight gain (Yarım ve Kurt, 2001).

CONCLUSION

Linseed is one of the plants that are used to maintain a healthy and quality life as a functional food. Linseed, when both content and functions are considered; primarily for prevention of many diseases, for the treatment of illness. Clinical trials on functional foods such as Linseed can play a key role in achieving a healthy, quality and sustainable life. It can also contribute to the creation of healthy generations and society.

RERERENCES

- Anonymous, 1997. Contractual analyses. Flax Council of Canada, Winnipeg, MB.
- Anonymous, 1994. Fatty acid analyses. POS Pilot Plant Corporation, Saskatoon, SK.
- Anonymous, 2002. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids. National Academies Press, Washington, DC, pp. 7-1-7-69 (dietary fiber), 8-1-8-97 (fat and fatty acids).
- Arjmandi, B.H., 2001. The role of phytoestrogens in the prevention and treatment of steoporosis in ovarian hormone deficiency. *J. Am. Coll. Nutr.* 20: 398S-402S.
- Aubrecht, E., Horacsek, M., Gelencser, E., Dworschak, E., 1998. Investigation of prolamin content of cereals and different plant seeds. *Acta Alimentaria* 27: 119-125.
- Bhatty, R.S., Cherdkiatgumchai, P., 1990. Compositional analysis of laboratory prepared and commercial samples of linseed meal and of hull isolated from flax. *J. Am. Oil Chem. Soc.* 67: 79-84.
- Bloedon, L.T., Szapary, O.P., 2004. Flaxseed and cardiovascular risk. *Nutrition, Reviews*, 62: 18-27.
- Cook, N.C., Saman, S., 1996. Flavonoids-chemistry, metabolism, cardio protective effects and dietary sources. *Journal of Nutrition Biochemistry*, 7: 66-76.
- Connon, J.J., 1999. Celiac disease. In: *Modern Nutrition in Health and Disease*, eds. Shils ME, Olson JA, Shike M and Ross AC, Lippincott Williams & Wilkins, Philadelphia, pp.1163-1168.
- Cooper, G.S., Dooley, M.A., Treadwell, E.L., 1998. Hormonal, environmental, and infectious risk factors for developing systemic lupus erythematosus. *Arthritis Rheum.* 41: 1714-1724.

- Daun, J.K., Przybylski, R., 2000. Environmental effects on the composition of four Canadian flax cultivars. *Proc. Flax Inst.* 58: 80-91.
- Demark-Wahnefried, W., Price, D.T., Polascik, T.J., 2001. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: Exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. *Urology* 58: 47-52.
- Freeman, T.P., 1995. Structure of flaxseed. In: *Flaxseed in human nutrition*, eds. Cunnane SC and Thompson LU, AOCS Press, Champaign, IL, pp11-21.
- Friedman, M., Levin, C.E., 1989. Composition of jimson weed (*Datura stramonium*) seeds. *J. Agric. Food Chem.* 37: 998-1005.
- Green, A.G., Dribnenki, J.C.P., 1994. Linola — A new premium polyunsaturated oil. *Lipid Tech.* 6: 29
- Hamadeh, M.J., Liede, A.C., Ganguli, S., 1992. Nutritional aspects of flaxseed in the human diet. *Proc. Flax Inst.* 4: 48-53.
- Hasler, C.M., 2002. Functional foods: Benefits, concerns and challenges. A position paper from the American Council on Science and Health. *J. Nutr.* 132: 3772-3781.
- İşleroglu, H., Yıldırım, Z., Yıldırım, M., 2005. Fonksiyonel bir gıda olarak keten tohumu. *GOÜ. Ziraat Fakültesi Dergisi*, 22 (2): 23-30
- Ingram, A.J., Parbtani, A., Clark, W.F., 1995. Effects of flaxseed and flax oil diets in a rat-5/6 renal ablation model. *Am. J. Kidney Dis.* 25: 320-329.
- Kang, J.X., Leaf, A., 1996. Antiarrhythmic effects of polyunsaturated fatty acids: Recent studies. *Circulation* 94: 1774-1780.
- Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, M.S., Binkoski, A.E., Hilpert, K.F., Griel, A.E., Etherton, T.D., 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine*, 113: 71-88.
- Kurt, O., 1995. Genetic and agronomic assessment of cultivars of linseed. University of Wales. (Unpublished Ph. D. Thesis).
- Lemay, A., Dodin, S., Kadri, N., 2002. Flaxseed dietary supplement versus hormone replacement therapy in hypercholesterolemic menopausal women. *Obstet. Gynecol.* 100: 495-504.
- Mazza, G., 1998. Flaxseed products for disease prevention. In: *Functional foods, biochemical and processing aspects*. Lancaster, Pennsylvania: Technomic Publishing Company, 91-127.
- McDonald, B.E., 1994. Canola oil nutritional properties. Canola Council of Canada, Winnipeg, MB.
- Milder, I.E.J., Arts, I.C.W., Putte, B., Venema, D.P., Hollman, P.C.H., 2005. Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. *British Journal of Nutrition* 93: 393-402.
- Oomah, B.D., Mazza, G., 1993. Flaxseed proteins. A review. *Food Chem.* 48: 109-114.
- Oomah, B.D., Kenaschuk, E.O., Mazza, G., 1995. Phenolic acids in flaxseed. *J. Agric. Food Chem.* 43: 2016-2019
- Prasad, K., Mantha, S.V., Muir, A.D., Westcott, N.D., 2000. Protective effect of secoisolariciresinol diglucoside against streptozotocin-induced diabetes and its mechanism. *Mol. Cell. Biochem.* 206: 141-150.
- Simon, J.A., Fong, J., Bernert, J.T., Browner, W.S., 1995. Serum fatty acids and the risk of stroke. *Stroke* 26: 778-782.
- Singer, P., Berger, I., Wirth, M., 1986. Slow desaturation and elongation of linoleic and alfa linolenic acids as a rationale of eicosapentaenoic acid-rich diet to lower blood pressure and serum lipids in normal, hypertensive and hyperlipemic subjects. *Prostaglandins Leuko. Med.* 24: 173-193.
- Thompson, L.U., Rickard, S.E., Orcheson, L.J., Seidl, M.M., 1996. Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. *Carcinogenesis* 17: 1373-1376.
- Yarım, B., Kurt, O., 2001. Keten bitkisinde yapılan araştırmaların projelenmesi. OMU. Ziraat Fak. T.B.B., Yayınlanmamış Bitirme Tezi.

ANTIOXIDANT ACTIVITY AND PHENOLIC COMPOUNDS OF ETHANOL EXTRACT OF GRAPE SEED, ROSEMARY, GREEN TEA AND OLIVE LEAF

Burcu AKTAŞ¹, Hatice BASMACIOĞLU-MALAYOĞLU¹

¹Ege University, Faculty of Agriculture, Department of Animal Science, 35100, Bornova, Izmir/Turkey

Corresponding author: hatice.basmacioglu@ege.edu.tr

Abstract

Total antioxidant activities and phenolic compounds of rosemary (*Rosmarinus officinalis* L.), grape (*Vitis vinifera* L.) seed, green tea (*Camellia sinensis* L.) and olive leaf (*Olea europea* L.) extracts were investigated in this study. The total antioxidant activities of plant (herbal) extracts were analyzed by two different methods, including 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging activity and trolox equivalent antioxidant capacity (TEAC). Carnosic acid, rosmarinic acid in rosemary extract (RE), total catechins in green tea extract (GTE) and oleuropein in olive leaf extract (OLE) were identified by high performance liquid chromatography (HPLC) method. Condensed tannins of grape seed extract (GSE) was analyzed by Butanol-HCl method. The total phenol contents determined by the Folin-Ciocalteu method varied between 141.7 to 315.56 mg of gallic acid equivalents (GAE)/g. The high total phenol content of plant extracts can be due to the high content of phenolic compounds present in the structures. According to results obtained, RE exhibited the highest radical scavenging activity (94 %), and GTE exhibited highest TEAC activity (285.4 μ M trolox/100g dry sample). Additionally, obtained data, revealed low linear correlation ($r=0.47$) between total phenol content and DPPH and high linear correlation ($r=0.82$) between total phenol content and TEAC. In conclusion, plant extracts can be used as natural antioxidant agents due to their high antioxidant activities in pharmaceutical, food industries and animal nutrition.

Keywords: Plant extracts, antioxidant activity, DPPH, TEAC

INTRODUCTION

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Kähkönen et al., 1999). Crude extracts of herbs and spices, and other plants materials from natural sources due to their antimicrobial and antioxidant properties are assessed as a natural antioxidant due to contents of polyphenolic compounds (=phytochemicals). The utilization of plant extracts as phytochemicals in food, cosmetic, medicine industries and animal nutrition is gaining more attention due to increasing consumer demands both safer and more nutritional food products (Basmacıoğlu-Malayoğlu et al., 2011).

Grape seeds are considered rich sources of polyphenolic compounds, mainly monomeric catechin and epicatechin, gallic acid, and polymeric and oligomeric procyanidins (Monagas et al., 2003). Rosemary extract from leaves of rosemary is belonging to the *Lamiaceae* family. The principal components responsible for the antioxidant activity of rosemary are reported to relate to the presence of three phenolic compounds, carnosic acid, carnosol, and rosmarinic acid (Frankel et al., 1996). Green tea extract is a derivative of cultivated evergreen tea plant is belonging to the *Theaceae* family. Green tea extract contains six primary catechins namely (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG) (Yang et al., 2018). The active compound of olive leaf is oleuropein, a secoiridoid, which has reported to be a potent antioxidant endowed with anti-inflammatory properties and pharmacologic and health promoting properties including hypoglycemic, antimicrobial, antiviral, anti-tumor and angiogenic activities (Benavente-Garcia et al., 2000).

The aim of this study was to determine the radical scavenging capacity and antioxidant activities of, rosemary, grape seed, green tea and olive leaf extracts by two common antioxidant activity methods, and relationship between antioxidant activity methods and total phenol content.

MATERIAL AND METHODS

Grape seed was obtained from local juice-processing industry (Dimes A.Ş, Kemalpaşa-İzmir). Rosemary was collected from Mersin. Green tea was obtained from local tea industry (Çay-kur A.Ş, Rize). Olive leaves were collected from Edremit-Balıkesir. The plant extracts were obtained from commercial firm (Edremit/Balıkesir, Turkey).

Condensed tannins of GSE analyzed by Butanol-HCl method. Carnosic acid, rosmarinic acid in RE, total catechins in GTE and oleuropein in OLE were identified by HPLC method. The total phenols in the plant extracts were determined by Folin-Ciocalteu method described by Dorman et al. (2003) with some modifications. Determination of total phenols was carried out three times, the results are mean values and given as gallic acid equivalent (GAE) per gram of extract.

DPPH method was carried out as described by Amarowicz et al. (2004) with minor modifications. The radical scavenging activity (RSA) was carried out three times and calculated using the equation below;

$$\%RSA = 100 \times (1 - A_E/A_D)$$

A_E is the absorbance of the solution containing antioxidant extract whereas A_D is the absorbance of the DPPH• solution.

TEAC method was carried out as described by Re et al. (1999) with some modifications. The results were expressed as μ M Trolox per 100 g of plant sample.

Statistical analyses of the data were determined by Student's t-test. A probability value of $P < 0.01$ was considered to denote a statistically significant difference. Data were given as the mean $\pm$ standard deviation (S.D.).

RESULTS AND DISCUSSION

Four different plant extracts used in this study contained; GSE: 41.07 g/100 g condensed tannin, RE: 14.90 g/100 g carnosic acid and 35.59 g/100 g rosmarinic acid, GTE: 85.31 g/100g total catechins, OLE: 15.49 g/100g oleuropein.

Table 2. Major phenolic compound or compounds of plant extracts

Grape seed	Condensed tannin (g/100g)	
	41.07	
Rosemary	Carnosic acid (g/100g)	Rosmarinic acid (g/100g)
	14.90	35.59
Green tea	Total catechins (g/100g)	
	85.31	
Olive leaf	Oleuropein (g/100g)	
	15.49	

The high level of polyphenolics in green tea has been reported by several researchers (Cabrera et al., 2006; Tsai et al., 2008). Also, grape seed (Rababah et al., 2004), rosemary (Yesil-Celiktas et al., 2007) and olive leaves (Benavente-Garcia et al., 2000) exhibited antioxidant properties due to their phenolic compounds.

The total phenol content and antioxidant activities of the plant extracts for each method are shown in Table 3. The total phenols determined by the Folin-Ciocalteu method varied from 141.7 to 315.6 mg GAE/g extract. The level of total phenolics in green tea extract was $\approx$ 1.27, 2.00 and 2.24 fold higher than in rosemary extract, grape seed extract and olive leaf extract, respectively.

Table 3. Total phenol content and antioxidant activities of the plant extracts.

Plant extracts	Total phenol content (mg GAE/ g extract)	DPPH Radical scavenging activity (%)	TEAC μM trolox/100g extract
Grape seed	157.2 ± 1.00	61.7 ± 1.99	69.5 ± 1.77
Rosemary	248.5 ± 2.36	94.0 ± 1.10	58.8 ± 1.91
Green tea	315.6 ± 0.66	74.4 ± 0.83	285.4 ± 0.46
Olive leaf	141.7 ± 2.16	72.1 ± 1.06	43.7 ± 0.90

Data of total phenols are expressed as milligrams of gallic acid (GAE) equivalents per gram extract. Data of DPPH Radical scavenging activity are expressed as %, TEAC are expressed as micromoles of Trolox equivalents per gram plant extract.

GTE was found to have the highest total phenolics among 4 herbs. Similar to our results, Tsai et al. (2008) found the level of total phenolics in green tea higher than among the 12 herbs. The high total phenol value of GTE can be due to the high content of total catechin. RE also, exhibited high levels of total phenolics (248.5 mg GAE/g extract). Researchers (Perumalla and Hettiarachch, 2011) suggested that antioxidant activity of grape seed extract is twenty and fifty fold greater than those vitamins E and C respectively.

The antioxidant activities of herbal extracts ranked differently in TEAC and DPPH methods, although both of them were based on the measurement of electron transfer propensity of antioxidant (Table 3). Similar results were obtained by Tsai et al. (2008). They also suggested that the different observations using TEAC and DPPH methods due to the TEAC values were not strongly related to chemical structures or numbers of electrons that an antioxidant give away (Huang et al., 2005). On the other hand, significant differences between the results were likely due to genotypic and environmental differences within species, choice of parts tested, time of taking samples and determination methods (Shan et al., 2005; Can et al., 2018).

The results obtained in our study show low linear correlation ($r=0.47$) between total phenol content and DPPH and high linear correlation ($r=0.82$) between total phenol content and TEAC.

Some researchers (Katalinic et al., 2006) have demonstrated a high linear correlation between the content of phenolic compounds and their antioxidant activities while others (Wong et al., 2006) show low linear correlation. These results showed that antioxidant activity of plant extracts is not limited to phenolics. Therefore, qualitative and quantitative analysis of major individual phenolics in the plants could be helpful for explaining the relationships between the total phenol contents and total antioxidant activity in the species. It is also clear that the number of phenolic –OH groups present in the structure of an antioxidant molecule is not always the only factor determining its antioxidant activity. Position of phenolic –OH groups, presence of other functional groups in the whole molecule and ketonic groups also play important roles in antioxidant activities (Rice-Evans et al., 1996; Xu et al., 2016). Some researchers (Liu et al., 2007) suggested the solvent used in the extraction may also be important in the antioxidant activity of the extract, depending on the phenolic content and, the polarity and hydrophobicity of antioxidants were found to play important roles in their activity, especially in biomembrane systems (Wu et al., 2007).

CONCLUSION

The results confirm that plant extracts were rich in phenolic compounds and demonstrated good antioxidant activity measured by different methods. A positive correlation existed between antioxidant activity and total phenolics, measured by TEAC analysis in plant extracts, revealing that phenolic compounds were the dominant antioxidant component. These plants, rich in phenolic compounds could be utilized as natural antioxidants in food industry and animal nutrition.

ACKNOWLEDGEMENTS

This article is a part of the first author's doctoral thesis. The data presented in this article is obtained from the project financially supported by Ege University Scientific Research Projects Coordination Unit (Project number 2008-ZRF-032).

REFERENCES

- Amarowicz, R., Pegg, R.B., Moghaddam, P.R., Barl, B., Weil, J.A., 2004. Free-radical scavenging capacity and antioxidant activity of selected plant species from Canadian Prairies. *Food Chemistry*, 84: 551-562.
- Basmacıoğlu-Malayoğlu, H., Aktaş, B., Yeşil-Çeliktas, Ö., 2011. Total phenolic contents and antioxidant activities of the essential oils from some plant species. *The Journal of Agricultural Faculty of Ege University*, 48(3): 211-215.
- Benavente-García, O., Lorente, J., Lorente, J., Ortuño, A., Del Rio, J. A., 2000. Antioxidant activity of phenolics extracted from *Olea europaea* L. Leaves. *Food Chemistry*, 68: 457-462.
- Cabrera, C., Artacho, R., Gimenez, R., 2006. Beneficial effects of green tea – A review. *Journal of the American College of Nutrition*, 25(2): 79-99.
- Can A., Ayvaz H., Uysal-Pala, Ç., Condelli, N., Galgano, F., Tolve, R., 2018. The total of near and mid-infrared spectroscopy for rapid quantification of oleuropein, total phenolics, total flavonoids and antioxidant activity in olive tree (*Olea europaea*) leaves. *Journal of Food Measurement and Characterization*, 1-11.
- Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J., 2003. Characterisation of the antioxidant properties of deodorised aqueous extracts from selected *Lamiaceae* herbs. *Food Chemistry*, 83: 255-262.
- Frankel, E. N., Huang, S. W., Aeschbach, R., Prior, E., 1996. Antioxidant activity of a rosemary extract and its constituents, carnosic acid, carnosol, and rosmarinic acid, in bulk oil and oil-in-water emulsion. *Journal of Agricultural and Food Chemistry*, 44: 131-135.
- Huang, D., Ou, B., Prior, R. L., 2005. The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53: 1841-1856.
- Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, 47: 3954-3962.
- Katalinic, V., Milos, M., Jukic, M., 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*, 94: 550-557.
- Liu, X., Dong, M., Chen, X., Jiang, M., Lv, X., Yan, G., 2007. Antioxidant activity and phenolics of an endophytic *Xylaria* sp. from *Ginkgo biloba*. *Food Chemistry*, 105: 548-554.
- Monagas, M., Gómez-Cordovés, C., Bartolomé, B., Laureano, O., Ricardo da Silva, J. M., 2003. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *Agricultural Food Chemistry*, 51: 6475-6481.
- Perumalla, A.V.S. and Hettiarachchy, N. S., 2011. Green tea and grape seed extracts – Potential applications in food safety and quality. *Food Research International*, 44: 827-839.
- Rababah, T.M., Hettiarachchy, N.S., Horax, R., 2004. Total phenolics and antioxidant activities of fenugreek, green tea, black tea, grape seed, ginger, rosemary, gotu kola, and ginkgo extracts, vitamin E, and tert-butylhydroquinone. *Journal of Agricultural and Food Chemistry*, 52: 5183-5186.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved radical cation decolorization assay. *Free Radical Biology and Medicine*, 26: 1231-1237.
- Rice-Evans, C. A., Miller, N. J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, 20: 933-956.
- Shan, B., Cai, Y.Z., Sun, M., Corke, M., 2005. Antioxidant Capacity of 26 Spice Extracts and characterization of their phenolic constituents. *Journal of Agricultural and Food Chemistry*, 53: 7749-7759.
- Tsai, T. H., Tsai, T. H., Chien, Y. C., Lee, C. W., Tsai, P.J., 2008. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: A comparative study of green tea versus different herbs. *Food Chemistry*, 110: 859-864.
- Wong, C.-C., Li, H.-B., Cheng, K.-W., Chen, F., 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. *Food Chemistry*, 97: 705-711.
- Wu, C.-R., Huang, M.-Y., Lin, Y.-T., Ju, H.-Y., Ching, H., 2007. Antioxidant properties of *Cortex fraxini* and its simple coumarins. *Food Chemistry*, 104: 1464-1471.
- Xu, Y., Burton, S., Kim, C., Sismour, E., 2016. Phenolic compound, antioxidant, and antibacterial properties of pomace extracts from four Virginia-grown grape varieties. *Food Science & Nutrition*, 4(1):125-133.
- Yang, H., Xue, X., Li, H., Apandi, S. N., Tay-Chan, S.C., Ong, S.P., Tian, E. F. 2018. The relative antioxidant activity and steric structure of green tea catechins- A kinetic approach. *Food Chemistry*, 257: 399-405.
- Yesil-Celiktas, O., Bedir, E., Vardar-Sukan, F., 2007. In vitro antioxidant activities of *Rosmarinus officinalis* extracts treated with supercritical carbon dioxide. *Food Chemistry*, 101: 1457-1464.

DETERMINATION OF THRESHING AND SEPARATING PARAMETERS OF THE POPPY PLANT

Deniz YILMAZ¹, Mukadder KARAMAN¹

¹Department of Agricultural Machinery and Technologies Engineering, Faculty of Agricultural Sciences and Technologies, University of Applied Sciences, Isparta, Turkey

Corresponding author email: denizyilmaz@sdu.edu.tr

Abstract

Poppy (Papaver somniferum L.) plant grown as a traditional product in our country is an annual herbaceous plant cultivated in summer or winter. Seed and oil of poppy plant are used in the food industry. The alkaloids contained in the poppy capsules are used for medical purposes and the poppy pulps are used as animal feed. In addition, the poppy plants are also used as ornamental plants. Therefore, poppy plant is an industrial plant used in many ways and it is an economic value in terms of foreign trade. The harvest and post-harvest processes of the poppy plant is carried out by conventional methods or semi-mechanized systems in Turkey. This case leads to damage in the product, loss of labor and yield. In recent years, studies about harvesting, threshing, separating and cleaning mechanisms have been carried out in various forms for poppy plants. However, these disadvantages have not been fully overcome. In order to minimize these problems and increase the quality and yield, it is necessary to know the threshing and separating parameters of the poppy plant. In this study, the threshing and separation parameters of the poppy plant, an important product for our country, have been determined in an effort to improve agricultural mechanization possibilities. In order to determine the threshing parameters of the poppy plant, experiments have been carried out in 3 different feeding rates, 3 different drum-concave spans and 3 different drum speeds with a prototype designed for aromatic plants. The separating parameters have been determined in 3 different feeding rate, 3 different sieve type and 3 different sieve velocity. Each experiment has been conducted in 3 replicates. As a result of the study, the threshing efficiency, work efficiency, power requirement and specific energy consumption values of the prototype for poppy plants. For the separating parameters of the prototype, separating efficiency, work efficiency and specific power consumption values are determined.

Keywords: poppy, threshing, separating, agricultural mechanization.

INTRODUCTION

Poppy is the only perennial culture plant and is belong to the family of papaveraceae. The homeland of Poppy is Anatolia (Koç 2002), but it has been cultivated for centuries in many parts of the world. The species cultivated in Turkey is papaver somniferum L. (Davis et al., 1988; Anonymous, 2017). In the field of agricultural production, poppies have a different place and importance compared to many other plants. The main reason for this is that it gives two different main products with commercial value. One of them is poppy capsule which is the main raw material source of the pharmaceutical industry and the other one is poppy seed used in the food industry. Seeds and oils of poppy plants has been used as food, its residue used as animal feed and its alkaloids used for medicinal purposes. Seeds and oils of poppy plants have been used as food, poppy residue have been used as animal feed and alkaloids have been used as medicinal purposes. While Turkey has a share of 48% in terms of poppy cultivation area, has share of 18% in terms of morphine production.

Many medicinal and aromatic plants are collected as wild from nature and processed. In our country, mainly the subspecies variations of white and purple floral of P. somniferum ssp. anatolicum are used for agricultural purposes (Tanker, 2003). In recent years higher efficiency and quality production have been achieved by studies and the support of the government.

Harvesting and threshing mechanization one of the most important stage in the agricultural production. In recent years, the use of different harvesting and threshing systems for medicinal aromatic plants have been increased. However, disadvantages are encountered in the harvesting, threshing and cleaning of medicinal aromatic plants. This case leads to damage in the product, loss of labor and yield. Therefore, it is necessary to known physico mechanical properties, threshing and

separating parameters of medicinal and aromatic plants for overcome the disadvantages (Gökdoğan, 2015). Karaca (1996) was conducted studies on specific energy consumption, work capacity and cleaning efficiency for poppy capsules.

The aim of this study is to determine the threshing and separation parameters of poppy plants for the purpose of improvement of agricultural mechanization possibilities of poppy plants. According to the results threshing efficiency, work efficiency, power requirement and specific energy consumption of prototype have been determined for the threshing unit, while separating efficiency, work efficiency and specific power consumption have been determined for separating unit.

MATERIAL AND METHODS

This study was conducted in Harvesting Laboratory in University of Isparta Applied Science, Faculty of Agricultural Sciences and Technologies, Isparta-Turkey (37°50'01", 30°32'15"). The white and purple flowers-poppy plants (*papaver somniferum*, ssp, *anatolicum*) used in the experiments was supplied from the producers in the Balkan Plain in the Denizli province.

The poppy plants in different stalk height is the biggest problem in harvesting mechanization of poppy plants. In the traditional harvesting method, the capsules are picked by hand from the knuckle one by one (Fig. 1). Poppies used in the experiments were cut by hand from the field at 70 cm stalk height.

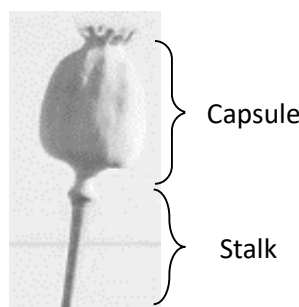


Fig. 1. The poppy plants

Poppy plants, threshing-separating prototype machine, torque meters, calipers and precision scales were used in the experiments. The threshing and separating machine consist of three basic units as threshing unit, separating unit and control unit (Fig. 2).

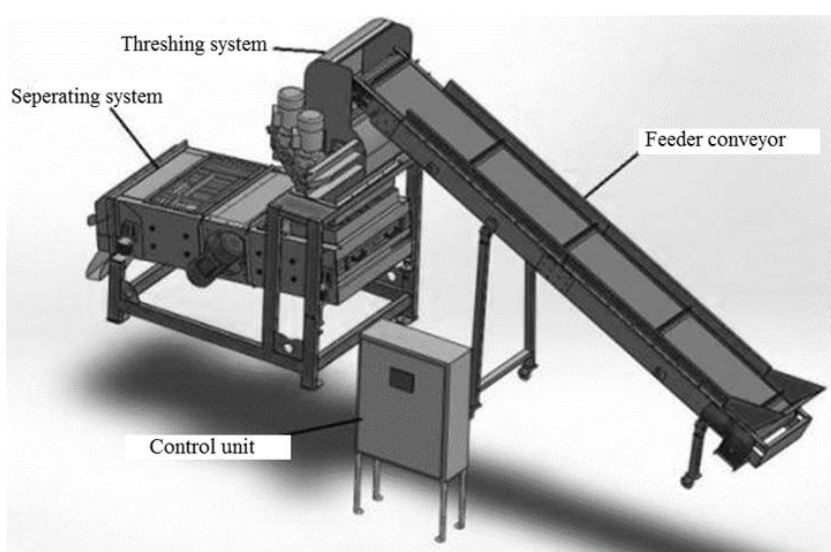


Fig 2. The prototype threshing and separating machine

In order to calculate the torque and the power consumed by the threshing unit a torque meter has been connected between the reducer electric motor and the drum shaft in the threshing system. The energy consumed by the machine (kW) were measured by the electrometer connected to the

power supply output and in order to calculate the power consumption, specific energy consumption, work efficiency and operating parameters of the machine. Preliminary tests were conducted to determine the threshing and separating parameters of poppy plants. Experiments were carried out after necessary preparations and adjustments on the prototype system with parameters obtained from the preliminary tests.

Preliminary tests

As a result of the preliminary experiments, it was determined that the cylinders should be operated at different directions and different speed. Hence one of the cylinders was operated at 35 min⁻¹, while the other one was operated at higher speeds. The distances of cylinders were chosen between 10 and 75 mm.

The sieve velocity were chosen as 35, 40 and 45 Hz. The inclination of the sieves used in separating unit was determined as 12%. The sieves used in experiments are 30 mm round hole, 35 mm round hole and 8-20 mm oblong (Fig. 3).

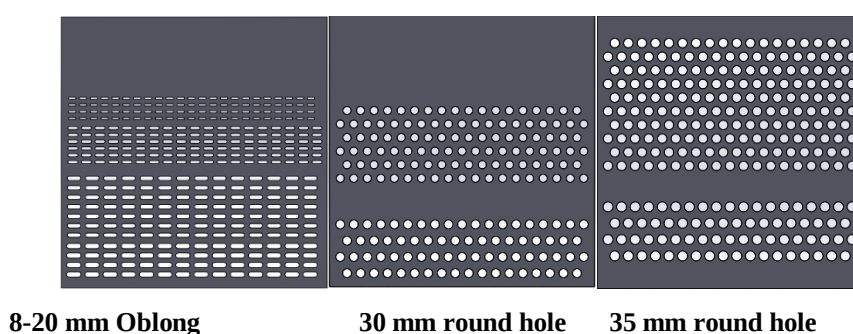


Fig 3. The sieves used in the experiment

Determination of threshing parameters

The threshing parameters of the prototype machine for the poppy plant have been determined. The threshing efficiency, work efficiency, the power requirement and specific energy consumption values of the machine have been determined based on working parameters obtained with preliminary tests. The working parameters of the machine determined with preliminary tests is three different feeding rate as 360, 540 and 720 kg/h; three different cylinder speed as 100, 200 and 300 min⁻¹; and three different cylinder distance as 14, 18 and 22. Each experiment was performed in 3 replicates.

Determination of separating parameters

In the separating unit, capsule fractures and poppy seeds falling into the sieves are separated. Seeds and capsules fell into the lower sieve are separated again and seeds are removed from capsules at the end of the sieve (Hacıyusufoğlu, 2013).

The separating efficiency, work efficiency and specific power consumption of the machine for poppy plant have been determined. In order to determine the separating parameters of the machine, the experiments were performed with 3 different sieve type as 30 mm round hole, 35 mm round hole and 8-20 mm oblong, 3 different sieve velocity as 15, 30, 45 Hz and 3 different feedings rates as 360, 540 and 720 kg/h. The experiments was carry out at %12 sieve inclination. Each experiment was performed in 3 replicates. The products obtained from the outlet of the machine are poppy seeds, fragmented capsules and poppy stalks.

RESULTS AND DISCUSSION

The operating parameters of the prototype for poppy plant have been determined by preliminary experiments and given in the Table 1.

Table 1. The operating parameters of the prototype for poppy plant

Operating parameters for threshing experiments			
Cylinder distance (mm)	Cylinder speed (min ⁻¹)	Feeding rate (kg/h)	Offset Power (kW)
14	100	360	0,5
18	200	540	0,8
22	300	720	1,0
Operating parameters for separating experiments			
Sieve type	Sieve velocity (Hz)	Feeding rate (kg/h)	
8-20 mm Oblong	15	360	
30 mm round hole	30	540	
35 mm round hole	45	720	

The Threshing Parameters of Machine for Poppy Plant

The threshing efficiency

The threshing efficiency was decreased with the increasing of cylinders distance, while threshing efficiency was increased with increasing of feeding rate. The threshing efficiency was increased with the changing of the cylinder speed 100 to 200 and 300 min⁻¹. The effect of cylinders distance × feeding rate × cylinder speed on the threshing efficiency was given in Fig. 4.

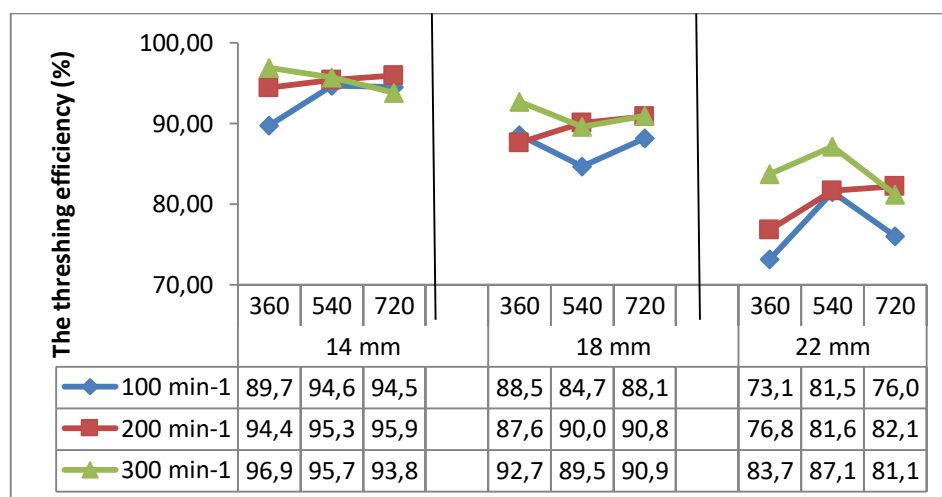


Fig 4. The effect of cylinders distance × feeding rate × cylinder speed on the threshing efficiency.

The highest threshing efficiency of prototype for poppy plant was found as %96,92 at 360 kg/h feeding rate, 300 min⁻¹ cylinder speed and 14 mm cylinders distance.

The work efficiency

According to the results of the threshing experiments of the machine the highest work efficiency was observed in 720 kg/h feeding rate, while the lowest work efficiency was found as 360 kg/h feeding rate. The increasing of the feeding rate, the work efficiency was increased, too. However, the work efficiency was decreased with the increase of the cylinder distances. In addition, it was seen that the values of the work efficiency were very close with each other with the changing of the cylinder speed. The effect of threshing parameters on the work efficiency was given in Fig. 5.

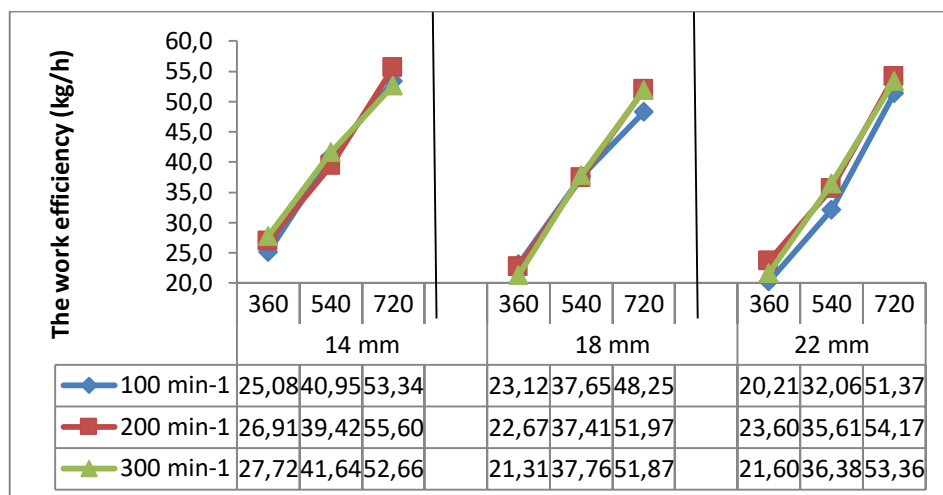


Fig 5. The effect of threshing parameters on the work efficiency.

The highest work efficiency of prototype for poppy plant was seen as %55,60 kg/h at 720 kg/h feeding rate, 200 min⁻¹ cylinder speed and 14 mm cylinders distance.

The power requirement

According the results the lowest and highest power requirement values of the threshing unit have been calculated as 0.020 kW and 1.081 kW, respectively. The power requirement value was decreased with the increasing of cylinders distance. On the other hand with the increasing of feeding rate and cylinder speed the power requirement was increased, too. The effect of cylinders distance × feeding rate × cylinder speed on the power requirement was given in Fig. 6.

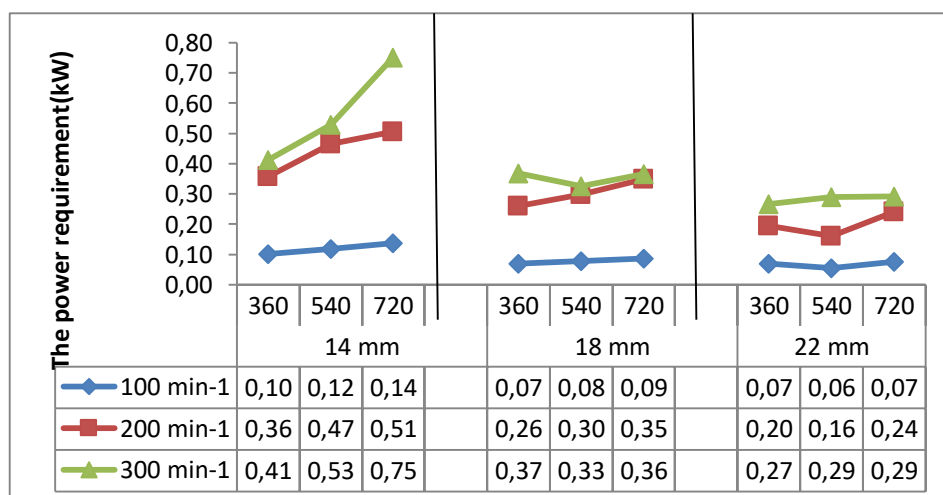


Fig 6. The effect of cylinders distance × feeding rate × cylinder speed on the power requirements

The highest power requirements value was found as 0.75 kW at the 14 mm cylinders distance, 720 kg/h feeding rate and 300 min⁻¹ cylinder speed.

The specific energy consumption

As a result of the study with the increasing of the cylinder distance and feeding rate The specific energy consumption value was decreased. The increasing of the cylinder speed was increased the specific energy consumption. The effect of cylinders distance × feeding rate × cylinder speed on the specific energy consumption was given in Fig. 7.

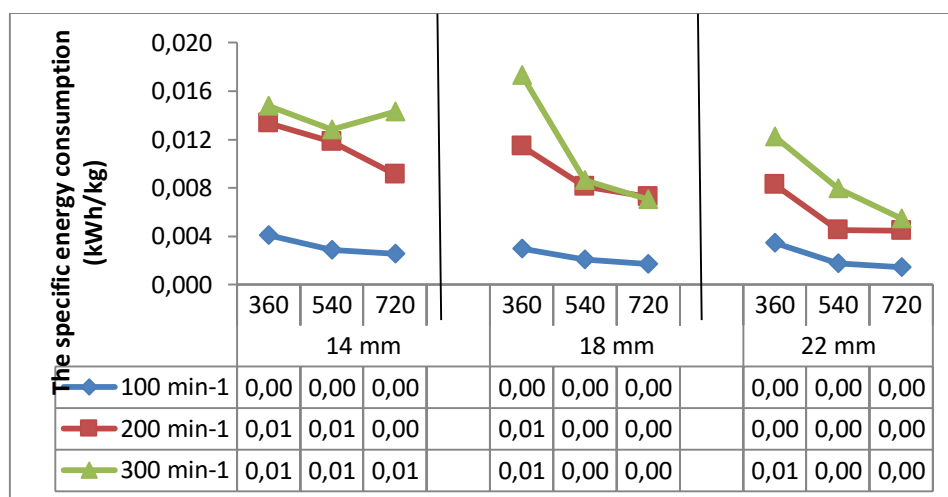


Fig 7. The effect of cylinders distance × feeding rate × cylinder speed on the specific energy consumption.

The highest specific energy consumption value was calculated as 0.014 kW at the 14 mm cylinders distance at 14 mm, 720 kg/h feeding rate and 300 min⁻¹ cylinder speed.

The Separating Parameters of Machine for Poppy Plant

The separating efficiency

According to the study results obtained from the separating experiments the efficiency of the separating unit was increased with the increasing of feeding rate. However, when the feed rate reached 720 kg/h, the separating efficiency was increased.

Depending on the sieve velocity, with increasing of the velocity after the limit of the 30 Hz, the separating efficiency of the machine for the poppy plant was decreased. In addition, according to the results separating processes with the 8-20 oblong sieve was not efficient in comparison to the other sieves. The separating efficiency of machine depending on the separating parameters was given in Fig. 8.

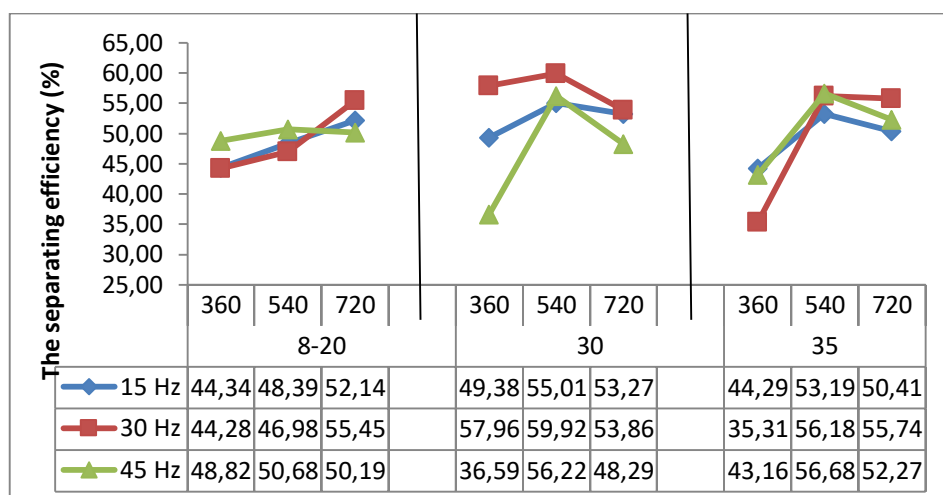


Fig 8. The effect of sieve velocity, feeding rate and sieve type on the separating efficiency for poppy plant.

The highest efficiency of separating unit for poppy plant was found as %59,92 at 540 kg/h feeding rate, 30 Hz sieve velocity and 30 mm round hole sieve type.

Work efficiency

The work efficiency values was found with round hole sieves highest than the oblong sieve type. The highest work efficiency values was calculated with the 35 mm round hole sieve. With the increasing of the feeding rate and the sieve velocity work efficiency values were increased, too. The effect of sieve velocity, feeding rate and sieve type on the work efficiency was given in Fig. 9.

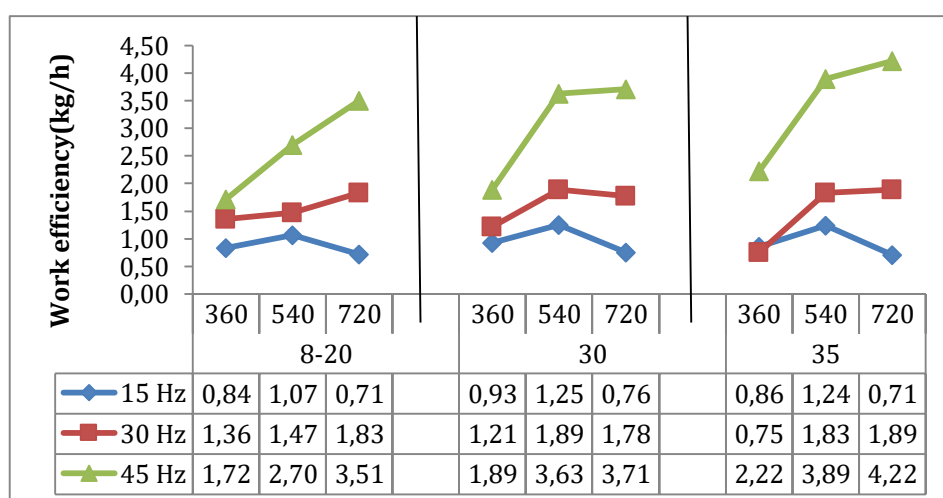


Fig 9. effect of sieve velocity, feeding rate and sieve type on the work efficiency.

The highest work efficiency values was determined as 4,22 kg/h with 35 mm round hole sieve at the 45 Hz sieve velocity and 720 kg/h feeding rate.

The specific power consumption

According to the study results the lowest specific power consumption value was observed with 35 mm round hole sieve type. With the increasing of the sieve velocity value the specific power consumption was decreased. The effect of sieve velocity, feeding rate and sieve type on the specific power consumption was given in Fig. 10.

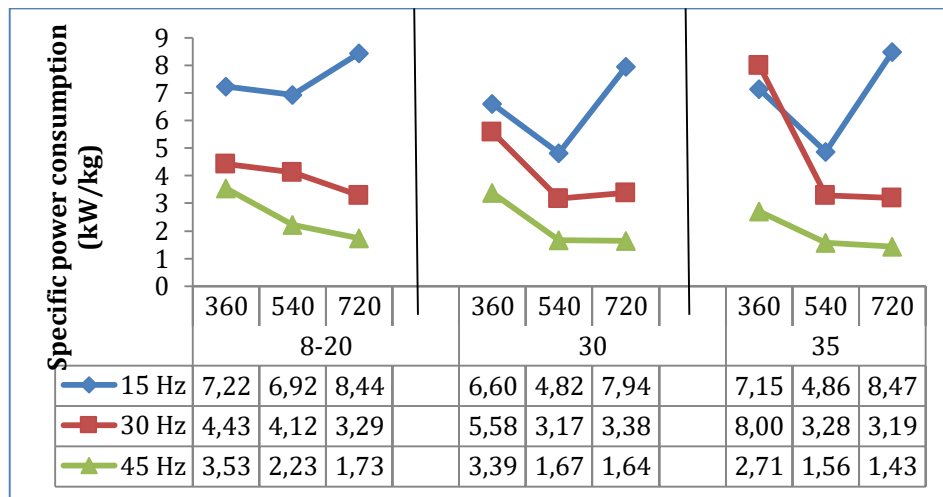


Fig 10. The effect of sieve velocity, feeding rate and sieve type on the specific power consumption.

The highest specific power consumption values was determined as 8.47 kW/kg with 35 mm round hole sieve at the 15 Hz sieve velocity and 720 kg/h feeding rate.

CONCLUSION

In this study, the threshing and separating parameters of a prototype machine for the poppy plant which has important cultivation areas and economic value and used seed and capsule were determined. The average threshing efficiency for poppy plants was varied between 80.37% and 94.58%. When the prototype examined in terms of threshing efficiency, it is recommended to operate the machine at 14 mm cylinder distance, 540 kg/h feeding rate and 200 min⁻¹ cylinder speed. On the other hand, it is suggested that for the high work efficiency, the prototype can be performed 14 mm cylinder distance, 720 kg/h feeding rate and 300 min⁻¹ cylinder speed for the poppy plants. For the lowest power requirements, the parameters should be selected as 22 mm cylinder distance and 100 min⁻¹ cylinder speed. The working parameters of the prototype for the minimum specific energy consumption should be selected as 22 mm cylinder distance 720 kg/h feeding rate and 300 min⁻¹ cylinder speed.

When the prototype examined in terms of separating efficiency, it is recommended to operate the machine at 12% sieve inclination, with 30 mm round hole sieve, 540 kg/h feeding rate and 30 Hz sieve velocity. According to the study results, the optimum sieve type for poppy plants can be said to be 30mm round hole sieve. Sieve types can be changed in order to increase the efficiency of separation unit. It is recommended that a vacuum or ventilation system can be used on separating unit for the stalk and capsule pieces and also to remove the dust accumulated in the seed.

REFERENCES

- Anonimous, 2017. 2016 Yılı Haşhaş Sektör Raporu. Toprak Mahsulleri Ofisi Genel Müdürlüğü. http://www.tmo.gov.tr/Upload/Document/hashassekt_raporu.pdf, (Erişim Tarihi: 15.08.2017).
- Davis, P.H., Mill, R.R. and Tan, K. 1988. Papaver L. Flora of Turkey and East Aegeani.
- Gökdoğan, M.E., 2015. Bazı Tıbbi ve Aromatik Bitkilerin Harmanlama ve Ayırma Düzeni İçin FizikoMekanik Özelliklerinin Belirlenmesi. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Tarım Makineleri ve Teknolojileri A.B.D. (Yayınlanmamış Yüksek Lisans Tezi) Isparta.
- Hacıyusufoğlu, A.F., 2013. Laboratuvar koşullarında haşhaş kapsül toplama sisteminin geliştirilmesi.
- Karaca, H., Konak, M. 1997. "Afyon Yöresinde Kullanılan Haşhaş Kapsülü Kırma-Ayırma Makinasının Bazı Yapısal ve İşletme Özelliklerinin Belirlenmesi ve Geliştirilmesi ", Tarımsal Mekanizasyon 17. Ulusal Kongresi 17-19 Eylül, 17, 644-652, (Konferans)
- Koç, H., 2002. Türkiye’de haşhaş ziraatının gelişimi. Tarım ve Köyişleri Bakanlığı Kocatepe Tarımsal Araştırma Enstitüsü Müdürlüğü, Afyon.
- Tanker, M., Tanker, N., 2003. Farmakognozi Cilt.1. Ankara Üniv., Eczacılık Fak., Yayınları No: 66. Ankara.

IN VITRO MICROPROPAGATION of MEDICINAL PLANTS via BIOREACTORS

Meltem BAYRAKTAR¹, Begüm GÜLER², Merve SEZEN², Aynur GÜREL³

¹Department of Genetic and Bioengineering Department, Faculty of Engineering and Architecture, Ahi Evran University, Kirsehir, Turkey

²Bioengineering Department, Graduate School of Nature and Applied Science, Ege University, İzmir, Turkey

³Bioengineering Department, Engineering Faculty, Ege University, Izmir/Turkey

Corresponding author email: mervesezen04@gmail.com

Abstract

Plants have been used for therapeutic purposes by human beings since ancient times. In vitro cultivation of plant explants for the production of pharmaceutically valuable compounds of commercial interest has gained popularity over the last few years because of the increasing global demand. In vitro micropropagation can be established under sterile conditions from explants such as plant shoot tip, node, meristem etc. In vitro micropropagation via semi-solid medium is expensive, labor-intensive and makes automation difficult. Although liquid medium has more advantages compared to semi-solid medium, some disadvantages like asphyxia, hyperhydricity and shear forces had to be eliminated by developing bioreactors. Thus, bioreactors have become the key step for in vitro commercial production of plants. Bioreactors are sterile vessels with liquid/gas inlet and outlet systems, while offering maximum possibilities to control environment conditions. Temporary and continuous immersion systems such as air-lift, bubble column, balloon type bubble and SETISTM bioreactors are used for in vitro micropropagation of medicinal plants such as *Echinacea purpurea*, *Hypericum perforatum*, *Lessertia frutescens*, *Catharanthus roseus*, *Panax ginseng*, *Eurycoma longifolia*.

Keywords: Medicinal plant, bioreactor, In vitro, micropropagation

INTRODUCTION

Medicinal and aromatic plants have been evaluated as colorants, agrochemicals, flavors, cosmetics, fragrances, biopesticides, food and food additives etc (Hussain et al., 2012). More than 50.000 of plant species all around the world are used for medicinal purposes with 5000–25.000 different metabolites and one-third of the flora on the earth have therapeutic properties (Ebrahimi and Mokhtari, 2017). Plant cell and tissue culture introduced as new sources of plant originated chemicals. Large-scale plant production through tissue and embryo cultures using bioreactors is promising for industrial plant propagation. Temporary and continuous immersion systems such as balloon type air-lift, rotary drum, column type bubble, balloon type bubble, SETISTM, RITA[®] and GrowtekTM bioreactors have been used for in vitro micropropagation of medicinal plants such as *Cymbopogon citratus*, *Hypericum perforatum*, *Securinega suffruticosa*, *Catharanthus roseus*, *Panax ginseng*, *Leucosium aestivum* (Hvoslef-Eide and Preil, 2005; Mujib et al., 2014).

Demand for Medicinal and Aromatic Plants

Medicinal and aromatic plants have a great role in supply of food supplements for personal care of humans alongside the therapeutically active substances. Therefore, their sector becomes a promising market and has a promising growth potential. According to BCC Reports; the overall global market for botanical and plant-derived drugs is expected to reach \$35.4 billion in 2020 with a compound annual growth rate of 6.6% from 2015 to 2020 (Roosta et al., 2017) and to PRNews Report; it will grow from \$29.4 billion in 2017 to around \$39.6 billion by 2022 with a compound annual growth rate of 6.1% ('Anonymous', 2017). This data shows the importance of large scale production of medicinal and aromatic plants through bioreactors.

Most of the demand for these plants is supplied by China and India from Asia; Egypt and Morocco from Africa; Poland, Bulgaria and Albania from Europe; Chile and Peru from South America. The U.S., Japan and Europe are the major consumers of these plants. Liquorice roots, Ginseng roots and Tragacanth are among the top MAPs (medicinal and aromatic plants) traded items. UN Comtrade database has recorded trade data for approximately 180 countries since 1962. According to data for 2015, total global MAPs' import and export value was \$3.02 and \$3.18 billion, respectively (Roosta et al., 2017).

Micropropagation via Bioreactors

Conventional micropropagation techniques have some disadvantages as intensive labor requirement at producing high amounts of plantlets and difficulty in controlling the chemical and/or physical properties in culture vessel. To overcome these problems, developing scale-up systems and automation of unit operations such as controlling of pH, oxygen, foaming, temperature and inoculation became necessary. Also, bioreactors reduce the risk of hyperhydricity phenomena by allowing to control the environment within the culture vessel. Bioreactors can be designed as requested by changing inner parts and operation parameters for every plant species and explant types for maximum growth and proliferation (Paek et al., 2001). The use of bioreactors in plant micropropagation is more promising method compared to conventional tissue culture techniques such as solid or semi-solid medium because of their advantages below:

- High amount of production can be produced.
- Scaling up is possible.
- Labor cost can be saved because handling the cultures is easy.
- The plantlets are in contact with medium and oxygen, which increases the uptake of nutrients and growth rate.
- The moving behaviour of explants results in reduced apical dominance and development of explants into plantlets.

Despite of these advantages, there are some disadvantages such as hyperhydricity, plantlet size variation caused by different growth stages and microbial contamination. The hyperhydricity phenomenon can be partly overcome by using modified medium with lowered concentrations of salts and sugar or increasing the light. Different types of plant explants have been successfully propagated in bioreactors (Table 1) (Hvoslef-Eide and Preil, 2005).

Table 1. Some of the medicinal and aromatic plants propagated in bioreactors.

Plant	Explant Type	Bioreactor	Reference
<i>Crescentia cujete</i>	Node	RITA®	Murch et. al., 2004
<i>Lilium</i> sp.	Bulblet	BTBB	Lian et. al., 2003
<i>Siberian Ginseng</i>	Somatic embryo	BTBB; air-lift	Hvoslef-Eide and Preil, 2005
<i>Phalaenopsis</i> sp.	PLB	Air-lift	Hvoslef-Eide and Preil, 2005
<i>Anoectochilus formosanus</i>	Nodal; shoot tip	BTBB	Hvoslef-Eide and Preil, 2005
<i>Chrysanthemum</i>	Node	BTBB; bubble column	Sivakumar et. al., 2005; Hvoslef-Eide and Preil, 2005
<i>Allium</i> sp.	Shoot	BTBB	Hvoslef-Eide and Preil, 2005
<i>Vitis</i> sp.	Node	BTBB	Hvoslef-Eide and Preil, 2005
<i>Saccharum</i> spp.	Shoot; embryo	Twin Flask; RITA®	Lorenzo et. al., 1998; Snyman et al., 2011

<i>Coffea arabica</i>	Shoot; embryo	RITA®	Roosta et. al., 2017; Alabarrán et al., 2005
<i>Lessertia frutescens</i> L.	Node	BTBB	Shaik et al., 2010
<i>Eleutherococcus sessiliflorus</i>	Somatic embryo	BTBB	Shohael et. al., 2005
<i>Anoectochilus formosanus</i>	Shoot	Flood and ebb	Wu et. al., 2007
<i>Camptotheca acuminata</i>	Embryo	RITA®	Sankar-Thomas et. al., 2008
<i>Phoenix dactylifera</i>	Shoot	RITA®	Fki et al., 2011
<i>Eucalyptus globulus</i>	Shoot	Twin Flask	González et al., 2011
<i>Dendrobium candidum</i>	Protocorm	BTBB	Cui et. al., 2014
<i>Cymbidium sinense</i>	Rhizome	BTBB	Gao et. al., 2014
<i>Citrus deliciosa</i>	Somatic embryo	RITA®	Cabasson et. al., 1997
<i>Schisandra lignans</i>	Shoot	BTBB; bubble column; RITA®	Szopa et. al., 2017

Bioreactor Types Used for Micropropagation of Medicinal and Aromatic Plants

The bioreactors can be divided into two systems: temporary and continuous immersion. In the continuous immersion, the explants are always in contact with the culture medium, while in the temporary immersion the bioreactor consists of two glasses or plastic vessels, one contains the plant material (plant culture vessel) and the other stores the culture medium (Mendonça et al., 2016) (Fig 1).

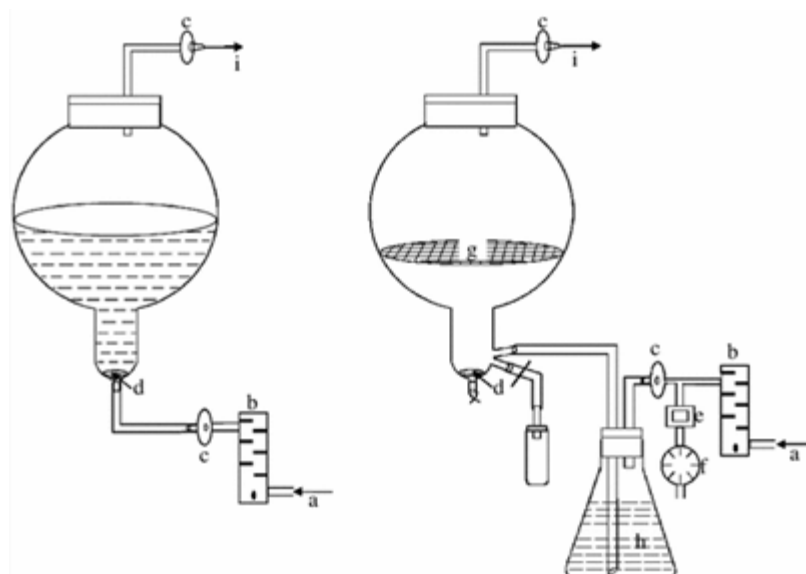


Fig 1. The schematic diagram of continuous (left) and temporary immersion (right) systems. a Air inlet, b air flow meter, c membrane filter, d sparger, e selenoid valve, f timer, g supporter (net), h medium reservoir, i air outlet (Gao et al., 2014)

1. Continuous Immersion Systems

Airlift Bioreactors: Airlift bioreactors consist a sparger that the air fed into the reactor with, a draft tube where the air uprise through the reactor, a riser region where the air goes upward inside the draft tube, a downcomer region where the degassed air downwards to the bottom of the vessel (Fig 2). These bioreactors have some advantages as; 1) preventing the bubbles from gathering around in one point inside the media, 2) increasing the interfacial area of plant explants and bubbles, 3) lowering the shear stress, 4) the movement of bubbles causes good mixing for the media, 5) its design and construction is simple (Huang et al., 2012; Paek et al., 2001).

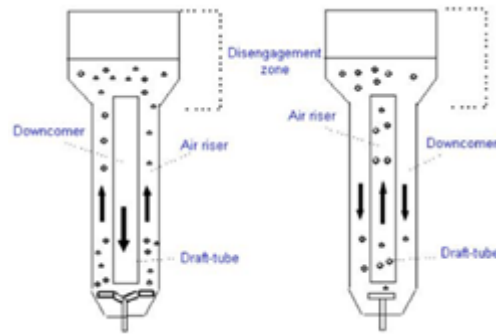


Fig 2. The schematic diagram of airlift bioreactors ('Anonymous', 2009).

Bubble Column Bioreactors: Bubble column bioreactors have similar properties with airlift bioreactors only they are lack of a draft tube within the vessel. The air is fed into the reactor directly from a sparger (Fig 3). The bubbles make the aeration and mixing. The advantages of these bioreactors are; 1) having good heat and mass transfer characteristics, 2) low operation costs due to lack of moving parts, 3) lower risk of contamination, 4) lower amount of energy consumption (Kantarıcı et al., 2015; Huang et al., 2012; Paek et al., 2001).

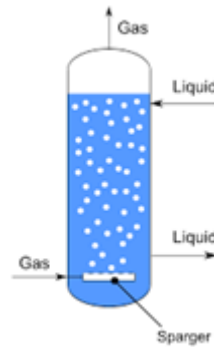


Fig 3. The schematic diagram of a bubble column bioreactor ('Anonymous', 2018).

Stirred Tank Bioreactors: Stirred tank bioreactors equipped with impellers provide high aeration rate and good mixing of the culture. When the air is given through the sparger, the impellers allow the transportation of gas molecules to the inner sides of the reactor (Fig 4). Despite the higher mixing rates, these bioreactors have higher energy consumption and shear stress due to the impellers, than air driven bioreactors such as airlift and bubble column. Along with these handicaps; complicated configuration, high risk of contamination and difficulty in optimizing variable conditions are other disadvantages of stirred tank bioreactors (Huang et al., 2012; Paek et al., 2001).

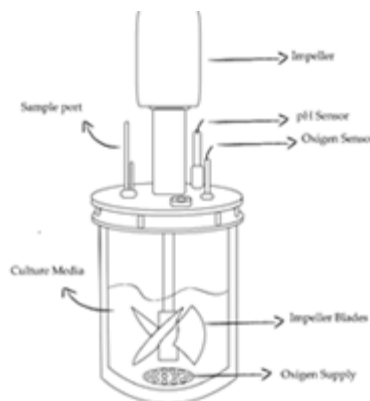


Fig 4. The schematic diagram of a stirred tank bioreactor (Calvalho et al., 2017).

Balloon Type Bubble Bioreactors: To overcome the foaming problem in airlift and bubble column bioreactors, balloon type bubble bioreactors are designed as; a 'Y'- or a 'T'-shaped tube at the bottom of the reactor for inoculum and harvesting (Fig 5), a sparger the air is fed into the reactor with and a concentric tube that reduces foaming by lifting the explants (Paek et al., 2001).

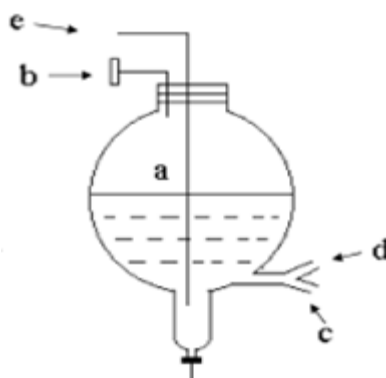


Fig 5. The schematic diagram of a balloon type bubble bioreactor. a body of the reactor, b air vent, c inoculum port, d sampling port, e medium exchange port (Thanh et al., 2005).

2. Temporary Immersion Systems

Temporary immersion bioreactors contains two vessels for holding the plant explants and the liquid medium. The transportation of the medium occurs as a pump pushes the medium into the vessel explants in and withdraw using the gravity. That movement of the liquid medium takes place in adjustable periods of time, provides intermittently expose of the medium rather than continuously. TIBs, specially designed for plant tissue culture, are consist advantages of both solid and liquid cultures (Huang et al., 2012; Zhang et al., 2012).

SETIS™ Bioreactors: SETIS™ bioreactors consist a culture vessel for explants (6 L), a media vessel (4 L), screw caps, air filters, silicone tubes and air pumps. Advantages are; 1) fully autoclavable, 2) maximum use of shelf space, 3) maximum light usage, 4) excellent drainage by gravity force (Fig 6) ('Anonymous', 2018).



Fig 6. A SETIS bioreactor ('Anonymous', 2018).

RITA® Bioreactors: In these bioreactors, the vessel contains plant explants is inside the vessel contains liquid medium. Besides of two vessels, there are parts as air vents, filters, central tube, disc tray and air pump (Fig 7). The system used generally for culture of somatic embryos, has some disadvantages as; being too small for micropropagation of large plants, the disc tray maintains high humidity that causes the vitrification of plants remain at the bottom and the roots of plants tangled with the disc tray and that situation makes the separation of plants from the system is difficult without harming the roots (Robert et al., 2006).

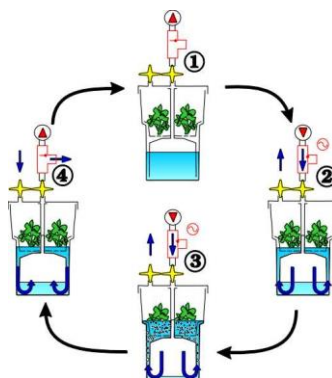


Fig 7. The phases of medium uptake in a RITA® bioreactor. 1) emersed stage, 2) beginning of the immersed stage, 3) immersed stage, 4) end of the immersed stage (Martre et al., 2001).

Twin-Flask System: The system contains two wide-mouth flasks, bottles or jars where one container is used for culture chamber and the other one for medium storage tank (Fig 8). Pressured air is applied through a silicone tube that connects two vessels, to the medium container so that explants exposed to the medium and withdraw with the absence of air (Watt, 2012). This type of bioreactors are easy to scale up, low-cost, easy to operate and construct and accessible. The handicups are unavailability for changing the medium, high risk of vitrification and being not suitable for forced ventilation and CO₂ enrichment (Georgiev et al., 2014; Ducos et al., 2009).

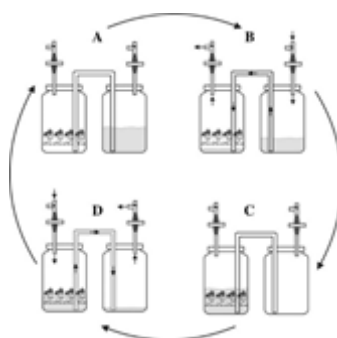


Fig 8. Design and operation steps of Twin-Flask Bioreactor (Georgiev et al., 2014).

CONCLUSION

Medicinal and aromatic plants are widely used for traditional purposes by folks and have high amount of market share in industry. The high demand was supplied from wild life for a long time but this situation harms the natural sources. At this point, plant tissue culture techniques step up and bring a new hope for the production of needed plants. Alongside with these techniques, usage of bioreactors provides the necessary large amounts of plants for commercial productions. The changeability of designing bioreactors makes them suitable for every kind of explant structure. For these reasons, producers should show more interest in bioreactors and grow their own plants with high yield in smaller vessels.

REFERENCES

- Anonymous, 2017. <https://markets.businessinsider.com/news/stocks/global-botanical-and-plant-derived-drugs-markets-2017-2022-market-will-grow-from-29-4-billion-in-2017-to-approx-39-6-billion-by-2022-with-a-cagr-of-6-1-1009779208>
- Anonymous, 2009. <https://www.slideserve.com/perrin/what-is-a-fermenter>
- Anonymous, 2018. <http://chanab.com/MassTransfer/>
- Anonymous, 2018. <http://www.setis-systems.be/about/about-setis>
- Alabarrán J., Bertrand B., Lartaud M., Etienne H., 2005. Cycle characteristics in a temporary immersion bioreactor affect regeneration, morphology, water and mineral status of coffee (*Coffea arabica*) somatic embryos. Plant. Cell.Tiss.Organ.Cult., vol. 81, p. 27-36
- Carvalho L. S., da Silva O. B., de Almeida G. C., de Oliveira J. D., Parachin N. S., Carmo T. S., 2017. Production Processes for Monoclonal Antibodies, Fermentation Processes, p. 180-197
- Ducos J. P., Terrier B., Courtois D., 2009. Disposable Bioreactors for Plant Micropropagation and Mass Plant Cell Culture, Adv Biochem Engin/Biotechnol, vol. 115, p. 89-115
- Ebrahimi M., Mokhtari, A., 2017. Engineering of Secondary Metabolites in Tissue and Cell Culture of Medicinal Plants: An Alternative to Produce Beneficial Compounds Using Bioreactor Technologies, Springer International Publishing
- Gao R., Wu S. Q., Piao X. C., Park S. Y., Lian M. L., 2014. Micropropagation of *Cymbidium sinense* using continuous and temporary airlift bioreactor systems, Acta Physiol Plant, vol. 36, p.117-124
- Georgiev V., Schumann, A., Pavlov A., Bley T., 2014. Temporary immersion systems in plant biotechnology, Eng. Life Sci., vol. 14, p. 607-621
- Huang T. K. and McDonald K. A., 2012. Bioreactor systems for in vitro production of foreign proteins using plant cell cultures, Biotechnology Advances, vol. 30, p.398-409
- Hussain , S., Fareed, S., Ansari, S., Rahman, A., Ahmad, I., Z., Saeed, M., 2012. Current Approaches Toward Production of Secondary Plant Metabolites, Journal of Pharmacy and Bioallied Sciences, vol. 4
- Hvoslef-Eide, A., K., Preil, W., 2005. Liquid Culture Systems For In Vitro Plant Propagation, Springer International Publishing
- Kantarci N., Borak F., Ulgen K. O., 2005. Bubble column reactors, Process Biochemistry, vol. 40, p.2263-2283
- Lorenzo, J. C., Gonz'alez, B. L., Escalona, M., Teisson, C., Espinosa , P., Borroto, C.,1998. Sugarcane shoot formation in an improved temporary immersion system, Plant Cell, Tissue and Organ Culture, vol. 54, p.197-200
- Martre P., Lacan D., Just D., Teisson C., 2001. Physiological effects of temporary immersion on *Hevea brasiliensis* callus, Plant Cell, Tissue and Organ Culture, vol. 67, p.25-35
- Mujib, A., Ali, M., Isah, T., Dipti, 2014. Somatic Embryo Mediated Mass Production of *Catharanthus roseus* in Culture Vessel (bioreactor) – A comparative study, Saudi Journal of Biological Sciences, vol. 21, p:442-449
- Murch, S. J., Liu, C., Romero, R. M., Saxena, P. K., 2004. In vitro culture and temporary immersion bioreactor production of *Crescentia cujete*, Plant Cell, Tissue and Organ Culture, vol. 78, p. 63-68
- Paek K. Y., Hahn E. J., Son S. H., 2001. Application Of Bioreactors For Large-Scale Micropropagation Systems Of Plants, In Vitro Cell. Dev. Biol.Plant, vol. 37, p.149-157
- Robert M. L., Herrera-Herrera J. L., Herrera-Herrera G., Herrera-Alamillo M. A., Fuentes-Carrillo P., 2006. A New Temporary Immersion Bioreactor System for Micropropagation, Methods in Molecular Biology, vol. 318, p. 121-129
- Roosta, R., A., Moghaddasi, R., Hosseini, S., S., 2017. Export Target Markets of Medicinal and Aromatic Plants, Journal of Applied Research on Medicinal and Aromatic Plants , vol. 7, p:84-88
- Shohael, A. M., Chakrabarty, D., Yu, K. W., Hahn, E. J., Paek, K. Y., 2005. Application of bioreactor system for large-scale production of *Eleutherococcus sessiliflorus* somatic embryos in an air-lift bioreactor and production of eleutherosides, Journal of Biotechnology, vol. 120, p. 228-236
- Sivakumar G., Kim S. J., Hahn E. J., Paek K. Y., 2005. Optimizing Environmental Factors For Large-Scale Multiplication Of Chrysanthemum (*Chrysanthemum Grandiflorum*) In Balloon-Type Bioreactor Culture
- Snyman S. J., Nkwanyana P. D., Watt M. P., 2011. Alleviation of hyperhydricity of sugarcane plantlets produced in a RITA® and characterisation of acclimated plants. South Afr. J. Bot, vol. .77, p. 685-692
- Thanh N. T., Murthy H. N., Yu K. W., Hahn E. J., Paek K. Y., 2005. Methyl jasmonate elicitation enhanced synthesis of ginsenoside by cell suspension cultures of *Panax ginseng* in 5-l balloon type bubble bioreactors, Appl Microbiol Biotechnol, vol. 67, p.197-201
- Watt M. P., 2012. The status of temporary immersion system (TIS) technology for plant micropropagation, African Journal of Biotechnology Vol. 11(76), pp. 14025-14035
- Zhang B., Song L., Bekele L. D., Shi J., Jia Q., Zhang B., Jin L., Duns G. J., Chen J., 2018. Optimizing factors affecting development and propagation of *Bletilla striata* in a temporary immersion bioreactor system, Scientia Horticulturae, vol. 232, p.121-126

SOME PROPERTIES OF SOILS IN WHERE DIFFERENT AUTUMN-FLOWERING COLCHICUM (*Colchicum* spp.) SPECIES NATURAL GROWN IN TURKEY

Erdinç UYSAL¹, Erdal KAYA¹

¹Atatürk Central Horticultural Research Institute, Yalova, Turkey

Corresponding author email: erdincuysal@hotmail.com

Abstract

This study is conducted in order to determine some properties of the soils on which 34 species of the autumn-flowering Colchicum genus that are natural grown in flora of Turkey. The study material comprises 250 samples of soil collected from 56 provinces in 9 years, from 2006 to 2014. The soils were analysed in terms of calcareous content, pH, salinity, organic matter, available phosphorus and potassium and the results were evaluated. According to the results of the study; no high levels of salt were found. The soil reaction varied from 4.66 to 8.72 and was neutral or slightly alkaline. Even though a large amount of the samples had medium or low levels of calcareous content, there was also soil that contained high or very high calcareous content. Soil samples were mostly highly organic matter. The available phosphorus values of the soils were found on average 18 mg kg⁻¹ and the potassium 306 mg kg⁻¹.

Keywords; *Colchicum, autumn-flowering, flora of Turkey, soil*

INTRODUCTION

Turkey is one of the richest gene centres in the world in terms of biodiversity with its geographical situation. There are 9582 flowery plants and fern species classified in 1225 genus from 163 families. 3155 species among these plants (34.3 %) have been classified as endemic ones (Ozhatay, 2011).

Colchicum species are classified in the group of geophytes. Geophyte is the name of those fern plants having a special underground storage organ such as bulbs, corms or rhizomes (Anonymous, 1996). The majority of the biological richness in Anatolia is composed of plants belonging to the botanical group of geophytes species. Geophytes species, which have widespread habitats all over the world except Australia, are seen especially in the Balkans, Anatolia and Caucasus. The soil on the Anatolian peninsula, with its location where three gene generations fall upon each other, offers habitat for various groups of geophytes plants. There are approximately 40 genus and 700 species of such plants in Turkey (Arslan et al., 1996; Güner et al., 2002).

The genus Colchicum belongs to the family Colchicaceae (Toplan et al., 2016). While the species number is constantly changing approximately 100 species of the genus Colchicum is distributed unequally around the world (Düşen and Sümbül, 2007). Turkey is a major centre of diversity and speciation with the high endemism rate. In Turkey, there are approximately 50 species of Colchicum 22 of which endemic (Güner et al., 2002).

Colchicum species are divided into two groups according to flowering time. Autumn-flowering species usually have bigger corm and seed than spring flowering ones (Sütlüpmar, 1983).

Colchicum species, which are widely used as medication for many years. Some of them are cultivated to be used for pharmaceutical industry. Tropolone alkaloids content of the species help in the treatment of gout, amyloidosis, cirrhosis, Behcet's disease, psoriasis, Hodgkin lymphoma, myeloid leukemia and skin cancers (Toplan et al., 2016).

It is important to cultivate in the cultural environment the plants that are rare in the nature but are needed by the people. It is very important for the cultivation to know the climate and soil demands of the cultivated plants. Within this study, soil samples from different provinces offering habitat to autumn-flowering Colchicum genus were collected from places where 34 species grew in 250 populations and they were analysed to get information about the soil characteristics.

MATERIAL AND METHODS

250 soil samples collected from the habitats of autumn-flowering *Colchicum* species in Turkey constitute the material of this study. Information about the *Colchicum* species and the locations from where the soil samples were taken in 56 provinces are shown on Table 1 and Fig 1.

The soil samples were collected between 2006 and 2014 in the habitats of these species in compliance with general rules in this respect with stainless spades from a depth of 0-20 cm (Jackson 1962) and labelled in polyurethane sacks.

Table 1. Information about the plants species names that the soil samples were taken.

No	Species name
1	<i>C. balansae</i> Planchon
2	<i>C. baytopiorum</i> C. D. Brickell
3	<i>C. bivonae</i> Guss.
4	<i>C. boissieri</i> Orph.
5	<i>C. chalcedonicum</i> Azn. ssp. <i>chalcedonicum</i>
6	<i>C. chlorobasis</i> K.Perss.
7	<i>C. cilicicum</i> (Boiss.) Dammer
8	<i>C. davisii</i> C.D.Brickell
9	<i>C. decaisnei</i> Boiss.
10	<i>C. dolichantherum</i> K.Perss.
11	<i>C. heldreichii</i> K.Perss.
12	<i>C. hirsutum</i> K.Perss.
13	<i>C. ignescens</i> K.Perss.
14	<i>C. imperatoris-friderici</i> Siehe ex K.Perss.
15	<i>C. inundatum</i> K.Perss.
16	<i>C. kotschy</i> Bornm.
17	<i>C. lingulatum</i> Boi.&Spr.ssp. <i>rigescens</i> K.Pr
18	<i>C. macrophyllum</i> B. L. Burt
19	<i>C. marasicum</i> nova
20	<i>C. micaceum</i> K.Perss.
21	<i>C. micranthum</i> Boiss.
22	<i>C. paschei</i> K.Perss.
23	<i>C. persicum</i> Baker
24	<i>C. polyphyllum</i> Boiss. & Heldr.
25	<i>C. sanguicolle</i> K.Perss.
26	<i>C. sieheanum</i> Hausskn. ex Stef.
27	<i>C. soboliferum</i> C.A.Meyer apud Fisch.&Mey.
28	<i>C. speciosum</i> Steven
29	<i>C. stevenii</i> Kunth
30	<i>C. sz.</i> ssp. <i>branchyphyllum</i> K.Pers.
31	<i>C. szovitsii</i> Fisch. Et Mey.subsp. <i>szovitsii</i>
32	<i>C. turcicum</i> Janka
33	<i>C. umbrosum</i> Steven
34	<i>C. variegatum</i> Linnaeus

As Kacar (1994) put it, the samples were prepared for analysis in the laboratory; pH degree was quantified by means of pH-metre with glass electrode in soil-water mixture in ratio 1:2,5 (Anonymous, 1981) and electrical conductivity was measured with EC-metre in the same mixture. Hızalan and Ünal (1966), put it that the lime ratio of the samples was identified by means of Scheibler calcimetre; content ratios in terms of organic substances, on the other hand, was identified using the modified method of Walkley-Black, so Jackson (1960). While available phosphorus was identified with the method reported by Olsen et al. (1954), 1 N ammonium acetate (pH 7) extraction was used for the quantification of the available potassium ratio (Anonymous 1980).



Fig 1. Information about the locations from where the soil samples were taken.

RESULTS AND DISCUSSION

The highest, lowest and average values concerning the analysis results are shown on Table 2, classifications about physical and chemical characteristics of the soil samples are indicated on Table 3.

Table 2. The highest, lowest and average values concerning the results of analysis

Soil Properties	Minimum	Maximum	Average
pH	4.66	8.72	7.21
EC ₂₅ (µmhos cm ⁻¹)	49	1200	203
CaCO ₃ (%)	0.00	54.76	5.11
Organic matter (%)	0.69	32.52	8.32
Available P (mg kg ⁻¹)	3	145	18
Available K (mg kg ⁻¹)	38	1400	306

pH value of the soils range from 4.66 to 8.72. According to the classification (Eyüpoğu, 1999) it was found that 5.2 % of the soil samples had medium, 14.0 % light acidity, 36.0 % neutral, and 44.0 % of the samples had light and 0.8 % strong alkaline. The results showed that the majority of the soils had neutral and light alkaline characteristics. Kandemir (1997) carried out pH analysis on different soil samples collected in different locations within the scope of a study on some endemic iris species and it appeared thereby as a result that the soils in question had pH values varying between 5,85 – 7,60 and neutral and light alkaline characteristics in general. Topçuoğlu et al. (1996) reported that they found the pH of the soil studied between 7.20-7.98 and that they were light alkaline in a study conducted by *Orchis mascula* species. It's obvious that there is a similarity between the results of these studies.

Table 3: Classifications about physical and chemical characteristics of the soil samples.

Soil Properties	Limiting value	Interpretation	%
pH (Eyüpoğlu 1999)	4,5-5,5	Moderately acid	5,2
	5,6-6,5	Slightly acid	14,0
	6,6-7,5	Neutral	36,0
	7,6-8,5	Slightly alkaline	44,0
	>8,5	Strongly alkaline	0,8
EC ₂₅ (µmhos cm ⁻¹ , Dellavalle 1992)	<400	Non saline	98,4
	400-800	Slightly saline	0,8
	801-1200	Moderately saline	0,8
	1201-1600	Saline	0,0
	1601-3200	Strongly saline	0,0
CaCO ₃ (%, Hızalan and Ünal 1966)	<1,0	Very low	55,6
	1,0-5,0	Low	19,2
	5,1-15,0	Medium	14,0
	15,1-25,0	High	5,2
	>25,0	Very high	6,0
Organic matter (%, Anonymous 1985)	<1,0	Very low	0,8
	1,0-2,0	Low	2,4
	2,1-3,0	Medium	7,6
	3,1-4,0	High	8,0
	>4,0	Very high	81,2
Available P (mg kg ⁻¹ , Olsen et al. 1954)	<3,0	Very low	0,4
	3,0-7,0	Low	12,0
	7,1-20,0	Medium	58,0
	>20,0	High	29,6
Available K (mg kg ⁻¹ , Pizer 1967)	<100	Very low	3,6
	100-150	Low	13,2
	151-200	Medium	14,4
	201-250	Good	13,6
	251-320	High	18,8
	>320	Very high	36,4

When analysed in terms of electrical conductivity (Dellavalle 1992), it was observed that 98.4 % of the soils collected were non-saline soils, on the other hand only 1.6 % had slightly and medium saline values. In similar studies with different geophyte genuses, it is seen that the areas investigated are composed of non saline soils. A study was conducted in order to determine some properties of the soils on which different species of *Anemone* genus that are natural grown in flora of Turkey (Uysal ve Kaya, 2017). For this purpose 71 soil samples were taken from different locations. According to the results of the study; all of the taken soil samples were considered within non saline or slightly saline soil class. In another study in peony, researchers reported that peony growing areas had low salinity content (Uysal ve Kaya, 2010).

The soils were also analysed in terms of their lime content and according to the classification made (Hızalan ve Ünal, 1966), it was observed that 74.8 % of them contained low or very low lime. On the other hand, 14 % of the soils contained lime in medium degrees and the others had high or very high lime content. Uysal et al. (2013) and Kandemir (1997) carried out similar studies for iris respectively and they obtained similar results as in our study indicating that the soils in question

possessed lime varying from very little to very high degrees. Although lime contents of the soils analysed vary, the fact that some soils contain about 55 % lime makes us think that the plant is resistant to high degrees of lime in the soil.

It was also demonstrated (Anonym 1985) that the soils contained little, medium, high organic matter with percentage degrees of 3.2, 7.6 and 89.2 respectively. Uysal and Kaya (2010) showed in a study they executed for peony that almost all the soils collected had high degrees of organic substances. The results of a similar study carried out by Uysal et al. (2013) for bulbous irises certified that 61 % of the soils analysed had high organic substance content. The results indicate that colchicums prefer soils with high organic matter content.

According to the classification of soil available phosphorus content (Olsen et al., 1954), it was determined that the soil had 12.4 % low or very low, 58 % moderate, 29.6 % high phosphorus.

The soils surveyed for available K are classified according to Pizer (1967). According to this, it is determined that 16.8% of the soils is low or very low, 28% is medium or good, and 55.2% has high and very high potassium content.

The phosphorus and potassium contents of soils are found to be different from those of soils, while the phosphorus contents are found in the soil in the middle levels and the potassium content is high in the large area.

Phosphorus values ranged from 6.65 to 18.02 mg kg⁻¹ and potassium values ranged from 360 to 955 mg kg⁻¹ in a study conducted to determine some physical and chemical properties of the different orchid species grown naturally in the Van region (Çığ ve Yılmaz, 2015). Researchers have shown that phosphorus varies between moderate to high levels in the soil, while potassium is present at high levels.

Uysal et al. (2013) identified available phosphorous and potassium values within the scope of a study they carried out to specify the soil characteristics of locations where bulbous irises have habitats in Turkey. The results they obtained are similar to those values we got in our study concerning colchicums. Accordingly 58 % of the soils had medium and 18 % high phosphorous content. While 26 % of the soils contained potassium in medium degree, 54 % of them had high and very high potassium in their content.

CONCLUSION

Soil samples were collected from the natural habitats of autumn-flowering *Colchicum* genus existing in the flora in Turkey within the scope of this study so as to get information about the characteristics of the soils in their natural environment.

As a result of the study; it was determined that nearly all of the soil samples contained low levels of salt and high organic matter content.

The results of the study also showed that the soils had neutral and light alkaline characteristics

Although lime generally found soil samples containing low levels, it was also found that the plant could grow in soils with very high levels of lime.

Available phosphorous and potassium contents of the analysed soils were specified as 3-145 mg kg⁻¹ and 38-1400 mg kg⁻¹ respectively.

ACKNOWLEDGEMENTS

This research has been supported by TUBITAK (The Scientific and Technological Research Council of Turkey). Project Number: 105G068 and 110G007

REFERENCES

- Anonim, 1980. Soil and Plant Testing and Analysis as a Basis of Fertilizer Recommendations. F.A.O. Soils Bulletin 38/2, p.95.
- Anonim, 1981. The Analysis of Agricultural Materials. Second Edition Ministry of Agri. Fisheries and Food RB 427, Replaces Technical Bulletin 27, p. 226.
- Anonim, 1985. Agricultural Analysis Handbook. Hach Com. 22546-08, p.2/65, 2/69.
- Anonim, 1996. Türkiye'nin Ekonomik Değeri Olan Geofitlerin Üretimi ve Doğal Populasyonları Hakkında Rapor. Doğal Çiçek Soğancıları Derneği, İstanbul.
- Arslan N., Ekim T., Koyuncu M., 1996. Development on Conservation and Propagation of Natural Howerbulbs in Turkey. 7th International Symposium on Flowerbulbs. March 10-16, Herzliya, İsrail.
- Çığ A., Yılmaz H., 2015. Van yöresinde doğal olarak yetişen farklı orkide türlerine ait toprakların bazı fiziksel ve kimyasal özellikleri. Toprak Bilimi ve Bitki Besleme Dergisi 3 (1): 1 - 8
- Dellavalle N.B., 1992. Determination of Specific Conductance in Supertanat 1:2 Soil:Water Solution. In Handbook on Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc. Athens,GA.
- Düşen O.D., Sumbül H., 2007. A Morphological Investigation of Colchicum L.(Liliaceae) Species in the Mediterranean Region in Turkey. Turk J Bot 31: 373-419.
- Eyüpoğlu F., 1999. Türkiye Topraklarının Verimlilik Durumu. Toprak ve Gübre Araştırma Enstitüsü Yayınları, Genel yayın No: 220, Teknik Yayınlar No: T.67, Ankara
- Güner A., Özhatay N., Ekim T., Başer K., 2002. Flora of Turkey and East Aegean Islands. XI volumes, ISBN: 1-55297-673-4. USA. 224.
- Hızalan E., Ünal H., 1966. Topraklarda Önemli Kimyasal Analizler. A.Ü. Ziraat Fakültesi Yayınları: 278.
- Jackson M.L., 1960. Soil chemical analysis, Prentice- Hall, Inc. Englewood,Cliffs-NJ.
- Jackson M.L., 1962. Soil chemical analysis. Prentice-Hall of India Private Limited, New Delhi.
- Kacar B., 1994. Bitki ve Toprağın Kimyasal Analizleri:III. Toprak Analizleri. Ankara Üniversitesi Ziraat Fakültesi Eğitim,Araştırma ve Geliştirme Vakfı Yayınları No:3 İsbn:975-7717-04-5. Ankara.
- Kandemir N., 1997. Bazı Endemik İris (Iridaceae) Türleri Üzerinde Morfolojik, Anatomik ve Ekolojik Bir Araştırma. Yayınlanmamış Doktora Tezi, Ondokuzmayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.
- Olsen S.R., Cole V., Watanable F.S., Dean L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate. U.S.D.A. Circular no. 939. Washington D.C.
- Ozhatay N., 2011. Grower's Handbook, İstanbul 80 p
- Pizer N.H., 1967. Some Advisory Aspect: Soil Potassium and Magnesium. Teck.Bull. No:14:184
- Sütlüpnar N., 1983. Türkiye'nin Sonbaharda Çiçek Açan Colchicum Türleri Üzerinde Araştırmalar Doğa, Bilim Derg. Temel Bilim, 7: 355-59.
- Topçuoğlu B., Kasap Y., Alpaslan M., Yalçın R., 1996. Kahramanmaraş yöresinde doğal florada yetişen salep bitkisinin bazı bitki besin maddesi içerikleri ile salep bitkisinin yetiştiği toprakların bazı fiziksel ve kimyasal özellikleri. Ankara Üniversitesi Tarım Bilimleri Dergisi 2(3):7-10.
- Toplan G.G., Güreç Ç., Mat A., 2016. Importance of Colchicum species in modern therapy and its significance in Turkey. J. Fac. Pharm. İstanbul, 46 (2):129-144
- Uysal E., Kaya E., 2010. Türkiye Florasında Mevcut Şakayık (Paeonia spp.) Türlerinde Toprakların Verimlilik Durumlarının Belirlenmesi. I. Ulusal Toprak ve Su Kaynakları Kongresi 1-4 Haziran 2010 Eskişehir, s. 835-842.
- Uysal E., Erken K., Kaya E., Erken S., Gülbağ F., 2013. Türkiye Florasında Mevcut Soğanlı İris (Iris Spp.) Türlerinde Toprakların Verimlilik Durumlarının Belirlenmesi, 5. Süs Bitkileri Kongresi, 6-9 Mayıs 2013, s. 723-728 Yalova
- Uysal E., Kaya E., 2017. Türkiye Florasında Doğal Olarak Yetişen Farklı Dağ Lalesi (Anemone spp) Türlerine Ait Toprakların Bazı Fiziksel ve Kimyasal Özellikleri. 5. Uluslararası Katılımlı Toprak ve Su Kaynakları Kongresi, 12-15 Eylül 2017, Bildiriler Kitabı (1): 176-184, Kırklareli.

SOME SOIL PROPERTIES OF DIFFERENT ASPHODELINE (*Asphodeline* spp.) SPECIES NATURAL GROWN IN FLORA OF TURKEY

Erdoğan UYSAL¹, Erdal KAYA¹

¹Atatürk Central Horticultural Research Institute, Yalova/Turkey

Corresponding author email: erdincuyasal@hotmail.com

Abstract

In this study, it is aimed to determine some properties of the soils on which 14 species of the *Asphodeline* genus that are natural grown in flora of Turkey. For this purpose, during the 4 years (2011-2014), 70 soil samples taken from 25 different provinces were used as materials. The soils were analysed in terms of calcareous content, pH, salinity, organic matter, available phosphorus and potassium. According to the results of the study, the soil reaction was found to be neutral or slightly alkaline. In terms of salinity, although a small number of high salinity soils were identified, it was generally found that the samples were non saline or slightly saline. Even though a large amount of the samples had medium or low levels of calcareous content, there was also soil that contained high or very high calcareous content. Soil samples were mostly highly organic matter. The available phosphorus values of the soils were found on average 13 mg kg⁻¹ and showed a wide range of variation. It has been observed that the soils analyzed for potassium contents have medium and high values.

Keywords; *Asphodeline*, flora of Turkey, soil properties

INTRODUCTION

Turkey is one of the richest gene centres in the world in terms of biodiversity with its geographical situation. Geophytes are an important part of this richness. Geophyte is the name given to herbs which stay dormant underground for the greater part of the year and have specialised food-storing underground stems such as bulbs, corms, rhizomes and tubers (Giray, 2001).

The genus *Asphodeline* (Liliaceae) is known as "Ciriş otu, Kiriş otu, Deli Kiriş" like the genus *Asphodelus* and *Eremurus* in Turkey (Tuzlacı, 1985).

The genus *Asphodeline* Rchb. (Liliaceae) is represented by 14 species distributed in the Mediterranean area (Ulubelen and Tuzlacı, 1985). The genus *Asphodeline* is presented in Turkey by 20 taxa of which 11 are endemic. Because of the high endemism ratio, Turkey is the main gene center for *Asphodeline* (Tuzlacı, 1987).

The edible use of its roots, shoots, flowers, and leaves has been known for a long time (Todorova-Nikolova et al., 2010). There are some literature about *Asphodeline*'s medicinal properties;

In a study *Asphodeline lutea* Reichb. (Liliaceae) flowers collected from İzmir were found to contain sugars, tannins, steroidal saponozites, anthracene derivatives and flavonoids. Luteolin-7-glucoside, luteolin, apigenin-7-ramno glucoside, and apigenin were isolated from flowers extract. The flavonoid content was 4 % (Atasü, 1980).

In another study four *Asphodeline* species with yellow flowers growing in Turkey had chemically been investigated (Koçoğlu and Sütülpınar, 1998). As a result the existence of two flavonoids (apigenin and luteolin), six anthracene derivatives (aloe-emodin, asphodelin, chrysophanol, isochrysophanol, physcion and rhein) and a phenolic acid (chlorogenic acid) had been shown in the roots of the species.

It is very important for the cultivation to know the climate and soil demands of the cultivated plants. Within this study, soil samples from different provinces offering habitat to *Asphodeline* genus were collected from different places and they were analysed to get information about the soil characteristics.

MATERIAL AND METHODS

70 soil samples collected from the habitats of *Asphodeline* species in Turkey constitute the material of this study. The soil samples were collected between 2011 and 2014 in the habitats of these species in compliance with general rules in this respect with stainless spades from a depth of 0-20 cm (Jackson 1962) and labelled in polyurethane sacks.

As Kacar (1994) put it, the samples were prepared for analysis in the laboratory; pH degree was quantified by means of pH-metre with glass electrode in soil-water mixture in ratio 1:2,5 (Anonymous, 1981) and electrical conductivity was measured with EC-metre in the same mixture. Hızalan and Ünal (1966), put it that the lime ratio of the samples was identified by means of Scheibler calcimetre; content ratios in terms of organic substances, on the other hand, was identified using the modified method of Walkley-Black, so Jackson (1960). While available phosphorus was identified with the method reported by Olsen et al. (1954), 1 N ammonium acetate (pH 7) extraction was used for the quantification of the available potassium ratio (Anonymous 1980).

RESULTS AND DISCUSSION

Information about the plants and the locations from where the soil samples were taken in 25 provinces and the results of the analysis about the physical and chemical properties of soil samples are shown on Table 1. Classifications about physical and chemical characteristics of the soil samples are indicated on Table 2.

When analysed in terms of electrical conductivity (Dellavalle 1992), it was observed that 87.1 % of the soils collected were non-saline soils. The percentage of the soils classified as slight saline was 10; on the other hand 1.4 % had saline and 1.4 % high saline values. In a similar study of the *polygatum* plant from the Liliaceae family, soil samples taken from different regions of Turkey were examined (Uysal and Kaya, 2013). According to the results 93% of the soils surveyed are found non-saline. The rest was classified as light, medium and high saline soils.

The average pH value of the soil samples was found to be 7.89. In reference to Eyüpoğlu (1999) it was found that 14.3 % neutral, and 82.9 % of the samples had light, and 2.9 % strong alkaline. The results showed that the majority of the soils had light alkaline characteristics. Kandemir (1997) carried out pH analysis on different soil samples collected in different locations within the scope of a study on some endemic iris species and it appeared thereby as a result that the soils in question had pH values varying between 5,85 – 7,60 and neutral and light alkaline characteristics in general. Topçuoğlu et al. (1996) reported that they found the pH of the soil studied between 7.20-7.98 and that they were light alkaline in a study conducted by *Orchis mascula* species. It's obvious that there is a similarity between the results of these studies.

Table 1. Information about the plants and the locations from where the soil samples were taken and some physical and chemical properties of soil samples

No	Location	Species name	EC µmhos cm ⁻¹	pH	Lime (CaCO ₃) %	Organic matter %	Availab le P mg kg ⁻¹	Availab le K mg kg ⁻¹
1	İzmir	<i>Asphodeline lutea</i> (L.)	150	7,87	4,47	5,11	8,07	170
2	İçel	<i>Asphodeline lutea</i> (L.)	147	8,07	10,44	21,12	44,91	453
3	Aydın	<i>Asphodeline lutea</i> (L.) Reichb	548	7,58	16,25	14,36	118,65	495
4	Çanakkale	<i>Asphodeline lutea</i> (L.) Reichb	268	7,29	1,19	7,30	5,84	125
5	Antalya	<i>Asphodeline lutea</i> (L.) Reichb	212	7,85	3,03	10,56	28,26	373
6	Antalya	<i>Asphodeline lutea</i> (L.) Reichb	233	7,93	12,11	12,70	22,78	500
7	Aydın	<i>Asphodeline lutea</i> (L.) Reichb	186	7,64	0,00	13,06	7,27	203
8	Bursa	<i>Asphodeline lutea</i> (L.) Reichb	440	8,00	41,30	7,66	4,99	153
9	Antalya	<i>Asphodeline lutea</i> (L.) Reichb	196	8,06	13,93	6,60	15,74	278
10	Muğla	<i>Asphodeline anatolica</i> E. Tuzlaci	191	7,95	24,41	13,06	8,39	243
11	Antalya	<i>Asphodeline anatolica</i> E. Tuzlaci	180	7,92	13,83	9,68	7,54	285
12	Antalya	<i>Asphodeline anatolica</i> E. Tuzlaci	155	8,29	9,91	6,60	3,95	580
13	Muğla	<i>Asphodeline anatolica</i> E. Tuzlaci	151	7,50	0,00	11,28	2,58	223
14	Muğla	<i>Asphodeline anatolica</i> E. Tuzlaci	112	7,46	0,00	6,60	2,15	118

15	Muğla	<i>Asphodeline anatolica</i> E. Tuzlaci	193	7,90	29,83	9,68	4,08	280
16	İçel	<i>Asphodeline baytopae</i> E. Tuzlaci	156	8,08	5,22	12,00	34,06	423
17	İçel	<i>Asphodeline baytopae</i> E. Tuzlaci	188	7,93	11,25	17,88	40,97	520
18	Hatay	<i>Asphodeline baytopae</i> E. Tuzlaci	177	7,69	14,45	16,52	12,16	213
19	Hatay	<i>Asphodeline baytopae</i> E. Tuzlaci	337	7,66	1,40	12,00	5,14	380
20	Antalya	<i>Asphodeline brevicaulis</i> (Bertol.) J. Gay ex Baker	237	8,30	7,01	23,28	5,44	213
21	Muğla	<i>Asphodeline brevicaulis</i> (Bertol.) J. Gay subsp. <i>brevicaulis</i>	138	8,04	0,00	3,30	5,44	150
22	Muğla	<i>Asphodeline brevicaulis</i> (Bertol.) J. Gay subsp. <i>brevicaulis</i>	209	7,73	0,41	4,24	6,32	168
23	İçel	<i>Asphodeline cilicica</i> E. Tuzlaci	203	8,10	11,85	10,56	12,25	358
24	Adana	<i>Asphodeline cilicica</i> E. Tuzlaci	251	7,78	14,39	2,52	3,52	148
25	İçel	<i>Asphodeline cilicica</i> E. Tuzlaci	680	7,75	43,18	11,10	6,65	230
26	K. maraş	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>ovoidea</i>	303	7,46	11,67	10,22	11,72	415
27	Konya	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>damascena</i>	142	8,26	29,62	2,52	5,20	138
28	Sivas	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>damascena</i>	182	8,34	19,63	7,18	7,14	394
29	Kayseri	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>damascena</i>	147	7,65	0,58	3,46	5,49	241
30	Adana	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>rugosa</i>	154	7,93	18,31	22,20	6,90	308
31	Adana	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>rugosa</i>	160	8,13	3,22	4,68	4,20	320
32	Sivas	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>damascena</i>	263	8,00	9,87	1,02	2,35	239
33	Sivas	<i>Asphodeline damascena</i> (Boiss.) Baker subsp. <i>damascena</i>	2720	7,72	20,90	2,42	2,86	125
34	Erzurum	<i>Asphodeline dendroides</i> (Hoffm.) Woronow	1208	7,83	27,46	2,32	5,89	385
35	Kayseri	<i>Asphodeline globifera</i> J. Gay ex Baker	201	8,38	40,16	3,70	7,91	290
36	Adana	<i>Asphodeline globifera</i> J. Gay ex Baker	195	7,33	0,39	4,56	1,67	108
37	Osmaniye	<i>Asphodeline globifera</i> J. Gay ex Baker	189	6,85	0,58	8,08	2,34	195
38	K. maraş	<i>Asphodeline globifera</i> J. Gay ex Baker	420	7,17	16,33	26,12	23,44	440
39	Adana	<i>Asphodeline globifera</i> J. Gay ex Baker	202	7,89	4,54	6,60	5,44	213
40	Yozgat	<i>Asphodeline globifera</i> J. Gay ex Baker	683	7,65	19,36	6,12	4,82	235
41	Adana	<i>Asphodeline globifera</i> J. Gay ex Baker	210	7,83	46,58	5,66	7,62	170
42	Nevşehir	<i>Asphodeline globifera</i> J. Gay ex Baker	286	8,21	29,89	1,96	5,14	700
43	Kayseri	<i>Asphodeline globifera</i> J. Gay ex Baker	196	8,03	18,91	9,40	5,66	340
44	K. maraş	<i>Asphodeline peshmeniana</i> E. Tuzlaci	250	8,20	3,68	4,82	12,96	321
45	Niğde	<i>Asphodeline prismatocarpa</i> J. Gay	196	7,90	13,43	13,42	54,53	509
46	Karaman	<i>Asphodeline rigidifolia</i> (Boiss.) Baker	204	8,10	19,74	11,28	24,13	554
47	İçel	<i>Asphodeline rigidifolia</i> (Boiss.) Baker	167	8,15	17,77	7,66	15,04	552
48	İçel	<i>Asphodeline rigidifolia</i> (Boiss.) Baker	197	8,15	16,39	11,64	62,93	938
49	İçel	<i>Asphodeline rigidifolia</i> (Boiss.) Baker	148	7,85	12,19	4,24	19,87	400
50	Karaman	<i>Asphodeline rigidifolia</i> (Boiss.) Baker	280	8,10	51,82	4,42	5,61	450
51	Karaman	<i>Asphodeline sertachae</i> Tuzlaci	164	8,35	11,23	5,52	8,45	200
52	Karaman	<i>Asphodeline sertachae</i> Tuzlaci	382	7,75	11,19	21,12	35,09	438
53	Karaman	<i>Asphodeline sertachae</i> Tuzlaci	326	7,90	10,99	5,52	7,96	225
54	Antalya	<i>Asphodeline sertachae</i> Tuzlaci	147	8,09	4,78	5,66	4,60	218
55	Erzurum	<i>Asphodeline</i> sp.	465	8,41	3,55	1,02	2,18	313
56	Antalya	<i>Asphodeline</i> sp.	287	7,68	2,74	11,64	30,67	733
57	Erzincan	<i>Asphodeline</i> sp.	164	8,38	1,58	2,62	1,67	235
58	Adana	<i>Asphodeline</i> sp.	227	7,88	1,94	4,06	8,33	153
59	Eskişehir	<i>Asphodeline</i> sp.	219	7,90	10,53	8,08	5,17	160
60	Amasya	<i>Asphodeline</i> sp.	158	6,64	0,00	10,22	10,79	370
61	Kastamonu	<i>Asphodeline</i> sp.	304	7,45	1,94	17,40	10,48	168
62	İçel	<i>Asphodeline taurica</i> (Pallas) Kunth	214	7,82	23,71	9,14	12,02	371
63	İçel	<i>Asphodeline taurica</i> (Pallas) Kunth	212	7,85	26,35	15,80	27,68	366
64	Ankara	<i>Asphodeline taurica</i> (Pallas) Kunth	204	8,53	4,50	2,64	2,82	393
65	Çankırı	<i>Asphodeline taurica</i> (Pallas) Kunth,	168	8,24	4,29	3,92	2,99	144
66	Erzincan	<i>Asphodeline tenuior</i> (Fischer) Ledeb var. <i>puberulenta</i> E. Tuzlaci	172	7,54	1,57	4,94	4,27	423
67	Erzincan	<i>Asphodeline tenuior</i> (Fischer) Ledeb var. <i>puberulenta</i> E. Tuzlaci	199	7,66	0,48	2,82	1,84	155
68	Erzurum	<i>Asphodeline tenuior</i> (Fischer) Ledeb var. <i>puberulenta</i> E. Tuzlaci	551	8,67	9,42	0,92	1,35	213

69	Antalya	<i>Asphodeline turcica</i> Tuzlacı	180	8,20	14,04	15,80	4,29	320
70	Antalya	<i>Asphodeline turcica</i> Tuzlacı	204	7,94	13,68	7,30	7,00	140
Minimum			112	6.64	0.00	0.92	1.35	108
Average			288	7.89	12.88	8.72	13.11	314
Maximum			2720	8.67	51.82	26.12	119	938

Table 2: Classifications about physical and chemical characteristics of the soil samples.

Soil Properties	Limiting value	Interpretation	%
pH (Eyüpoğlu 1999)	4,5-5,5	Moderately acid	0,0
	5,6-6,5	Slightly acid	0,0
	6,6-7,5	Neutral	14,3
	7,6-8,5	Slightly alkaline	82,9
	>8,5	Strongly alkaline	2,9
EC ₂₅ (µmhos cm ⁻¹ , Dellavalle 1992)	<400	Non saline	87,1
	400-800	Slightly saline	10,0
	801-1200	Moderately saline	0,0
	1201-1600	Saline	1,4
	1601-3200	Strongly saline	1,4
CaCO ₃ (%, Hızalan and Ünal 1966)	<1,0	Very low	14,29
	1,0-5,0	Low	22,86
	5,1-15,0	Medium	31,43
	15,1-25,0	High	17,14
	>25,0	Very high	14,29
Organic matter (%, Anonymous 1985)	<1,0	Very low	1,4
	1,0-2,0	Low	4,3
	2,1-3,0	Medium	10,0
	3,1-4,0	High	5,7
	>4,0	Very high	78,6
Available P (mg kg ⁻¹ , Olsen et al. 1954)	<3,0	Very low	17,1
	3,0-7,0	Low	35,7
	7,1-20,0	Medium	28,6
	>20,0	High	18,6
Available K (mg kg ⁻¹ , Pizer 1967)	<100	Very low	0,0
	100-150	Low	11,4
	151-200	Medium	15,7
	201-250	Good	20,0
	251-320	High	8,6
	>320	Very high	44,3

The lime contents of the studied soils varied between 0-52%. According to the classification made (Hızalan ve Ünal, 1966), it was observed that 37% of them contained low or very low lime. On the other hand, 49 % of the soils contained lime in medium degrees and the others had high or very high lime content. Uysal and Kaya (2017) and Kandemir (1997) carried out similar studies for anemone and iris respectively and they obtained similar results as in our study indicating that the soils in question possessed lime varying from very little to very high degrees. Although the soil samples

have different lime contents, soils with high lime content were found in important order. This result gives rise to thought that *Asphodeline* could be resistant to soils with high lime content.

If the study results are examined, the contents of organic matter, which are on average 8.72%, are considerably high. In a study with different species of *Anemone* genus that are natural grown in flora of Turkey, it was determined that all of the soil samples studied contained high organic matter (Uysal and Kaya, 2017). The results of a similar study carried out by Uysal and Kaya (2013) for *Polygonatum*. According to the results, all of the soil samples analysed had high organic matter content. The results indicate that asphodelines prefer soils with high organic matter content.

According to the classification of soil available phosphorus content (Olsen et al., 1954), it was determined that the soil had 52.8 % low, 28.6 % moderate, 18.6 % high phosphorus.

The soils surveyed for available K are classified in reference to Pizer (1967). According to this, it is determined that 11.4% of the soils is low or very low, 35.7% is medium or good, and 52.9% has high and very high potassium content.

The phosphorus and potassium contents of soils are found to be different from those of soils, while the phosphorus contents are mostly found in the soil in low levels and the potassium content is high in the large area.

Phosphorus values ranged from 6.65 to 18.02 mg kg⁻¹ and potassium values ranged from 360 to 955 mg kg⁻¹ in a study conducted to determine some physical and chemical properties of the different orchid species grown naturally in the Van region (Çiğ ve Yılmaz, 2015). Researchers have shown that phosphorus varies between moderate to high levels in the soil, while potassium is present at high levels.

Uysal and Kaya (2017) identified available phosphorous and potassium values within the scope of a study they carried out to specify the soil characteristics of locations where anemone species have habitats in Turkey. The results they obtained are similar to those values we got in our study concerning asphodelines. Accordingly 79 % of the soils had medium and low phosphorous content. Otherwise 66 % of the soils contained potassium in high degree.

CONCLUSION

The main aim of this study is to learn information about the characteristics of the soils in asphodeline plant's natural environment.

As a result of the study; it was determined that most of the soil samples contained low levels of salt and high organic matter content.

The results of the study also showed that the soils had slightly alkaline characteristics.

Although lime found soil samples containing different levels, it was also found that the plant could grow in soils with very high levels of lime.

Available phosphorous and potassium contents of the analysed soils were specified as 1-119 mg kg⁻¹ and 108-938 mg kg⁻¹ respectively.

ACKNOWLEDGEMENTS

This research has been supported by TUBITAK (The Scientific and Technological Research Council of Turkey). Project Number: 110G007

REFERENCES

- Anonim, 1980. Soil and Plant Testing and Analysis as a Basis of Fertilizer Recommendations. F.A.O. Soils Bulletin 38/2, p.95.
- Anonim, 1981. The Analysis of Agricultural Materials. Second Edition Ministry of Agri. Fisheries and Food RB 427, Replaces Technical Bulletin 27, p. 226.
- Anonim, 1985. Agricultural Analysis Handbook. Hach Com. 22546-08, p.2/65, 2/69.

- Atasü, 1980. Flavonoids of *Asphodeline lutea* Reichb. Flowers. Ankara Ecz. Fak. Mec., 10: 102-111.
- Çığ A., Yılmaz H., 2015. Van yöresinde doğal olarak yetişen farklı orkide türlerine ait toprakların bazı fiziksel ve kimyasal özellikleri. Toprak Bilimi ve Bitki Besleme Dergisi 3 (1): 1 - 8
- Dellavalle N.B., 1992. Determination of Specific Conductance in Supertanat 1:2 Soil:Water Solution. In Handbook on Reference Methods for Soil Analysis. Soil and Plant Analysis Council, Inc. Athens,GA.
- Eyüpoğlu F., 1999. Türkiye Topraklarının Verimlilik Durumu. Toprak ve Gübre Araştırma Enstitüsü Yayınları, Genel yayın No: 220, Teknik Yayınlar No: T.67, Ankara
- Hızalan E., Ünal H., 1966. Topraklarda Önemli Kimyasal Analizler. A.Ü. Ziraat Fakültesi Yayınları: 278.
- Jackson M.L., 1960. Soil chemical analysis, Prentice- Hall, Inc. Englewood,Cliffs-NJ.
- Jackson M.L., 1962. Soil chemical analysis. Prentice-Hall of India Private Limited, New Delhi.
- Kacar B., 1994. Bitki ve Toprağın Kimyasal Analizleri:III. Toprak Analizleri. Ankara Üniversitesi Ziraat Fakültesi Eğitim,Araştırma ve Geliştirme Vakfı Yayınları No:3 Isbn:975-7717-04-5. Ankara.
- Kandemir N., 1997. Bazı Endemik İris (Iridaceae) Türleri Üzerinde Morfolojik, Anatomik ve Ekolojik Bir Araştırma. Yayınlanmamış Doktora Tezi, Ondokuzmayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.
- Koçoğlu T.K., Sütülpınar N., 1998. Chemical Investigations on the Roots of Yellow Flowering *Asphodeline* Species in Turkey. İstanbul Ecz. Fak. Mec., 32: 37-42.
- Olsen S.R., Cole V., Watanable F.S., Dean L.A., 1954. Estimation of Available Phosphorus in Soils by Extraction With Sodium Bicarbonate. U.S.D.A. Circular no. 939. Washington D.C.
- Pizer N.H., 1967. Some Advisory Aspect: Soil Potassium and Magnesium. Teck.Bull. No:14:184
- Todorova-Nikolova G., Lazarova I., Mikhova B., Kostova I., 2010. Anthraquinone, naphthalene and naphthaquinone components of *Asphodeline lutea*. Chem. Nat. Comp. 46, 322–323.
- Topçuoğlu B., Kasap Y., Alpaslan M., Yalçın R., 1996. Kahramanmaraş yöresinde doğal florada yetişen salep bitkisinin bazı bitki besin maddesi içerikleri ile salep bitkisinin yetiştiği toprakların bazı fiziksel ve kimyasal özellikleri. Ankara Üniversitesi Tarım Bilimleri Dergisi 2(3):7-10.
- Tuzlacı E., 1985. Marmara Üniversitesi Eczacılık Dergisi, 1: 69.
- Tuzlacı E., 1987. Revision of the genus *Asphodeline* (Liliaceae). A new infragenericclassification. Candollea 42: 559-576.
- Ulubelen A., Tuzlacı E., 1985. Sesquiterpene lactones, flavonoids andanthraquinones from *Asphodeline globifera* and *Asphodeline damascena*. Phytochemistry 24: 2923-2924.
- Uysal E., ve E. Kaya, 2013. Türkiye Florasında Mevcut Mührüsüleyman (*Polygonatum spp.*) Türlerinin Yetiştği Toprakların Bazı Verimlilik Durumlarının Belirlenmesi, III. Ulusal Toprak ve Su Kaynakları Kongresi, 22-24 Ekim 2013, s. 724-728, Tokat.
- Uysal E., Kaya E., 2017. Türkiye Florasında Doğal Olarak Yetişen Farklı Dağ Lalesi (*Anemone spp*) Türlerine Ait Toprakların Bazı Fiziksel ve Kimyasal Özellikleri. 5. Uluslararası Katılımlı Toprak ve Su Kaynakları Kongresi, 12-15 Eylül 2017, Bildiriler Kitabı (1): 176-184, Kırklareli.

PROPAGATION OF MASTIC TREE: FROM SEED TO TISSUE CULTURE

Murat İSFENDİYAROĞLU

Ege University Faculty of Agriculture, Department of Horticulture, 35100, İzmir, Turkey

Corresponding author email: murat.isfendiyaroglu@ege.edu.tr

Abstract

Mastic tree (*Pistacia lentiscus* L.) is a natural element of Mediterranean maquis vegetation. Although, *Pistacia lentiscus* var. *chia* is the unique variety from which the gum mastic is economically obtained, only grown in Chios island of Greece. It's a known fact that the mastic tree was also grown on a limited scale in Çeşme Peninsula in the past. Gum mastic is being used in food, cosmetic and [pharmaceutical industries](#) in currency. In recent years, it was attempted to restart the growing in Çeşme Peninsula. Mastic tree is a dioecious plant and only male plants are used in mastic production. So the mastic tree doesn't propagated by seed. Mastic tree has been propagated by wood cuttings. Cuttings have been directly sticking into the soil where the plantation be established. Leafy semi-hardwood cuttings have also rooted at high percentages up to 76%. Success in propagation by grafting/budding is time dependent and limited. Air layering is also used in nursery tree production. Micropropagation by in vitro proliferated shoots from mature mother plants was unsuccessful. Although, success rates are quite high in regeneration of explants obtained from in vitro axenic embryos besides in vitro micrografting. Testing the genetic stability and gender in new plantlets obtained are obligatory. As for the micrografting, heterogeneity of seedling rootstocks and possible scion/stock interactions have to be considered. In this paper, different propagation methods of mastic tree were exposed and discussed.

Keywords: Mastic tree, propagation, cutting, layering, micropropagation

INTRODUCTION

Mastic tree (*Pistacia lentiscus* L.) is an evergreen shrub or small tree, belonging to Sumac family (*Anacardiaceae*). It is a leading component of Mediterranean maquis vegetation (Zohary, 1995). It is the unique species evaluated for gum mastic. Mastic is a resin obtained from *P. lentiscus* var. *chia* Duham. which is differed from the wild species with some morphological features (Browicz, 1987; Zakynthinos and Rouskas, 1998) The oldest information on mastic goes back to Herodotus (5th century B.C.). In traditional Greek medicine, mastic had been used particularly for gastrointestinal disorders. Ancient Greek and Roman physicians mentioned about the therapeutic properties of mastic and recommended its use (Browicz, 1987; Belles, 2008; Paraschos et al., 2012). Today, mastic resin and mastic oil are the products with high added value and used in many industrial applications (Chadzopulu et al., 2011; Freedman, 2011; Paraschos et al., 2012). In Chios island, about 140t/year mastic is being produced from nearly 2 million trees in currency. Figures of mastic production and the number trees did not change for a long time (Belles, 2008). Mastic tree is a dioecious tree and only male trees have been used through the centuries because of their high quality resin. So, mastic tree is propagated clonally by the hardwood cuttings (Baytop, 1968; Browicz, 1987; Belles 2008, Zografou et al., 2010). Despite the mastic tree and mastic gum have been associated with Chios through the history, mastic tree cultivation had been also conducted in the adjacent Western Anatolia in the past (Baytop, 1968; İsfendiyaroğlu, 2003). Remnants of old plantations on Çeşme Peninsula pointed out the former growing activities on a limited scale, but gradually lost its importance in time. In recent years, some attempts were started aiming on the protection and rehabilitation of the current genetic material, by some governmental institutions and NGOs. Moreover, establishing the new plantations and restart the mastic production in alternative lands are encouraged. Recent planting activities in Turkey seemed to be increased the demand to nursery tree production in the coming years. So, prediction of the most proper propagation method is of great importance. In this review, current and potential propagation methods of mastic tree were presented and discussed.

Propagation of Mastic Tree

Seed Propagation: Mastic tree is a dioecious plant and pollinated by wind (Zohary, 1952). In *Pistacia* species, viable pollens drift as much as half a mile or sometimes more, cause seedling diversity (Joley and Opitz, 1971). Gum mastic is being obtained only from male trees (Browicz, 1987; Belles, 2008). There are several cultivated male clones differing in quality and quantity of mastic resin that show genetic diversity (Browicz, 1987; Belles, 2008; Zografou et al., 2010). So, the seeds should not be used to propagate the mastic tree, because of genetic variability. Seed propagation can be used in reforestation and environmental rehabilitation programmes (Piotto and Di Noi, 2003). And it is also considered in breeding works and evaluating as ornamental nursery tree. Marked differences were pointed out among the *P. lentiscus* genotypes, related with the extend of fruiting, percentage of aborted ovaries, viability of seeds, percentage and speed of germination (Grundwag, 1975; Mulas et al., 1998; Piotto et al., 2003; Fascella et al., 2004). The low seed setting in *Pistacia* species is attributed mainly to degeneration of female reproductive structures (Grundwag, 1975; 1976). In mastic tree, fruit colour is strongly associated with the seed viability and black fruits usually contain viable seeds, whereas the red ones contain non-viable seeds in relation with parthenocarpy or ovary abortion (Garcia-Fayos and Verdu, 1998). Seeds of *P. lentiscus* do not show dormancy. But the seeds are rich in oily substances, therefore the seed viability could be lost in a comparatively short course of time (Garcia-Fayos and Verdu, 1998; Piotto et al., 2003). In fact, seeds showed a significant and strong decrease in germinability over time (6 months) under standard storage conditions (21°C and 6% moisture content) (Garcia-Fayos and Verdu, 1998). Pulp removal is a necessary treatment for germination success (Piotto, 1995; Garcia-Fayos and Verdu, 1998; Piotto et al., 2003). Mastic tree seeds are quite small (1000 seeds = 10-25g), so it is impossible to scarify them by hand. Short durations of cold stratification (3-4 weeks at +3-4°C) seemed to be sufficient for completing the dormancy removal in seeds (Piotto, 1995; Piotto et al., 2003; Fascella et al., 2004). Increases in time of cold stratification generally resulted with the decreases in percent germination of seeds (Garcia-Fayos and Verdu, 1998; Karakır and İsfendiyaroğlu, 1999; Fascella et al., 2004). Combined effects of scarification (50 sec) with cold stratification (7 and 21 days) did not significantly affect the percent germination of *P. lentiscus* seeds, and germination rates ranged between 75 and 81% (Piotto, 1995). The effects of acid (1N HCl for 20 min) scarification or soaking with GA₃ (1000 ppm for 20 min) after cold stratification seemed to be partially genotype dependent, but generally increased the germination of seeds compared to untreated ones (Fascella et al., 2004). GA₃ applications (24 h) ranged between 0 and 200 ppm did not significantly change the germination of mastic tree seeds that scarified with H₂SO₄ (10 min) and stratified at +2°C, throughout 60 days (Karakır and İsfendiyaroğlu, 1999). In case of seed propagation, mature black fruits have to be collected in October-November and subsequently subjected to mesocarp removal. Scarification is useful to speed up germination and homogenize the seedling emergence. Alternatively, cold stratification may be applied. Sowing in autumn, without any pretreatment or spring sowing of either scarified or cold stratified (2-3 weeks) seeds were recommended (Piotto et al., 2003; Prada and Arizpe, 2008). Mean height and stem diameter were measured as 12.5 cm and 2 mm respectively in 11 month old seedlings were grown under controlled conditions (Karakır and İsfendiyaroğlu, 1999).

Cutting Propagation: Mastic tree has been propagated clonally by the hardwood cuttings through the history. Thick branches are collected from the mature trees and planted at their final places where the tree is grown. The planting holes must be dug one year earlier, at a depth of 70-80 cm and let to aerated. Cuttings are planted in a depth of 40-50 cm and a little sideling. The planting time is December so that the highest use of precipitation. During the first summer after planting, cuttings are regularly watered and fertilized (Browicz, 1987; Belles, 2008). Wood cuttings are also rooted in containers filled with good aerated soil based mixtures in lath house or in simple greenhouses. Leafy cuttings from mature trees of *Pistacia* species are considered very difficult-to-root (Joley and Opitz, 1971). Cutting collection time is crucial for rooting success and cuttings could be rooted in a very short period of the year. In this period, the effect of applied auxins is more pronounced (Avanzato and Damiano, 1990; Dunn et al., 1996b). Rooting of *Pistacia* cuttings from

mature stock plants is limited even with the high auxin concentrations and auxin combinations (Al Barazi and Schwabe, 1982; Dunn et al., 1996a). Ontogenetic juvenility in *Pistacia* species seems to be lost after a few years generally, making the rooting of cuttings more difficult (Lee et al., 1976; Al Barazi and Schwabe, 1982; Pair and Khatamian, 1983). Semi-hardwood cuttings of mastic tree showed a short period of rootability (İsfendiyaroğlu, 2000; Viola et al., 2004; Pignatti and Crobeddu, 2005). Even the effects of exogenously applied auxins and their combinations are more pronounced in proper time of collection (İsfendiyaroğlu, 2000). In leafy semi-hardwood cuttings from mature *P. lentiscus* var. *chia* trees, the highest rooting (76.6%) was obtained when they were collected in mid-February and treated with 20 g.l⁻¹ IBA as quick-dip, in pure perlite (coarse), with bottom-heating (25°C), under mist. In the second year, mid-February cuttings also gave the highest percent rooting 43.3% with IBA-NAA mix at 10+2.5 g.l⁻¹. Lower rooting rates were obtained with IBA treatments up to 40 g.l⁻¹ (İsfendiyaroğlu, 2000; 2003). Micro-anatomical observations showed that, mastic tree cuttings showed indirect rhizogenesis and root primordia originated from basal callus tissue. The emergence of adventitious roots was observed after 45th day or onward (Karakır and İsfendiyaroğlu, 1999). In leafy semi-hardwood cuttings of selected mastic tree clones, February seems to be the most proper collection period. IBA and its mixtures with low amounts of NAA as quick-dip is necessary to obtain the highest number of rooted cuttings. The rooted cuttings showed a rapid shoot growth after transplanting to the soil based growing medium and reached up to 50 cm high at the end of the active growing season (Karakır and İsfendiyaroğlu, 1999).

Propagation by Layering: Air layering seems to be an alternative method to propagate mastic tree when the limited quantity of nursery tree is needed. It was basically an ancient method used to propagate a number of tropical and subtropical trees and shrubs. Today, this method is useful for producing a few plants of relatively large size for special purposes (Nelson, 1987; Hartmann et al., 2002). In mastic tree, branches that more than 1.5 cm in diameter found to be most suitable for layering. Girdling the stem by removing a strip of bark 0.5-1 cm wide completely around the stem is necessary. A 15x25 cm black polybag passes from the distal end of the branch up to girdled area, after evenly cutting its closed bottom edge. The lower end of the bag should be twisted and filled with about 1 l of moist peat. After then, both ends of polybag are wrapped with electrical tape. Making layering in mid-summer and removal of rooted branches from the mother plant at the beginning of spring are recommended. Rooted layers are transplanted in 4 l containers after a slight pruning on the top. In this method, success is ranged between 85-90%, but the number of layers is closely related with the canopy size of the mother plant used. Excessive application of layers could be detrimental for mother plants was suggested (Tutar et al., 2016b).

Propagation by Budding/Grafting: In the history of mastic culture, clonal propagation by budding/grafting did not take place in practice. However, it is possible to meet a few examples of grafted trees scattered around the Çeşme peninsula (Western Turkey). In recent years, some experimental works conducted on prediction of proper method of budding/grafting onto other *Pistacia* rootstocks (Tutar et al., 2016a; Parlak, 2018). Among the five different *Pistacia* seedling rootstocks assessed, the highest bud-take (45.3%) was obtained from *P. atlantica* with “T” budding was done at the end of summer (Tutar et al., 2016a). From mid-February to mid-March was the best period for chip budding and cleft grafting onto *P. atlantica* and *P. lentiscus* seedlings, under controlled conditions was reported. And the highest graft-take (47.8%) was observed in cleft grafts of mastic tree/*P. atlantica* combination carried out in 15 February (Parlak, 2018). *P. atlantica* seedlings seem to be the most proper rootstock for the clonal propagation of mastic tree, because of its high vigor. However, mastic resin and essential oil yields did not measured in the mentioned rootstock studies. Moreover, chemical composition of essential oils from budded/grafted trees should be clarified, because of the probable scion/stock interactions (Hartmann et al., 2002; Mudge et al., 2009). In fact, inconsistent figures were obtained in relation with the essential oil content of mastic resins, when the self- rooted trees compared with the trees that were budded onto different rootstocks (Tutar et al., 2016a).

Micropropagation: Early works showed that, browning of plant explants caused by phenolic exudation was the major constrain in micropropagation of *P. lentiscus* (Fascella et al., 2004; Ruffoni

et al., 2004; Mascarello et al., 2007). Propagation from axenic seeds of female mastic trees was proposed as a novel protocol to obtain nursery trees in large quantities (Yıldırım, 2012; Kılınç et al., 2015; Koç et al., 2014). Although the genetic stability of regenerated plants were studied in terms of polymorphism (Kılınç et al., 2015), gender of the new plantlets remain obscure. Root regeneration of *in vitro* proliferated shoots from mature mother plants of mastic tree was unsuccessful. So, micrografting of mastic tree onto seedling rootstocks of different *Pistacia* species was developed (Onay et al., 2016). In this method, shoots (one-year-old wood) from mature mastic trees were collected in mid-May and forced to sprout axillary buds in solid (peat, perlite) and liquid media *in vivo*. And shoot tips were proliferated by subcultures for using as a source of scion. The highest grafting success (100%) was recorded from *in vitro* regenerates from both male and female genotypes. Despite the high frequency of regeneration was achieved by this protocol, low frequency of genotypic variation in regenerated plants has to be considered. Moreover, all the *Pistacia* species used as seedling rootstock in this study were deciduous by contrast with mastic tree, and possible disorders could be derived from scion/stock interactions (Simons, 1987; Garner, 2007; Hartmann et al., 2002; Mudge et al., 2009) were ignored in this protocol.

In conclusion, among the different methods assessed, propagation by leafy semi-hardwood cuttings seems to be the most proper method in mass production of mastic tree clones. Despite the success of rooting depends on a limited cutting collection time, rooting rate of cuttings were at the commercially acceptable levels, even the mother plants were in mature phase. Rejuvenation of mother plants seems to be crucial for the high root regeneration in cuttings. Determination of auxin sensitive phase of rooting in cuttings should be determined for high efficiency of applied auxins and their combinations. Further nutrition programs can be developed for rapid growth of rooted propagules.

REFERENCES

- Al Barazi, Z., Schwabe, W.W., 1982. Rooting softwood cuttings of adult *Pistacia vera*. J. Hort. Sci., 57 (2): 247-252.
- Avanzato, D., Damiano, C., 1990. Study of rooting of *Pistacia atlantica*. Desf. Abstracts of Contributed papers 2. Posters. In: XXIII. International Hort. Congr., Firenze, Italy, August 27-September 1, No. 4032.
- Baytop, T., 1968. Türkiye’de sakız (mastix) elde etme imkanları. İstanbul Üniv. Eczacılık Fakültesi Mecmuası, 4 (1): 31-35 (In Turkish).
- Belles, C., 2008. Mastiha Island, 2nd ed. (Chios, Greece: G.N.Merousis Publishers), p. 333.
- Browicz, K., 1987. *Pistacia lentiscus* cv. Chia (*Anacardiaceae*) on Chios island. Plant Syst. Evol., 155 (1-4):189-195.
- Chadzopulu, A., Koukoulia, A., Theodosopoulou, E., Adraniotis, J., 2011. Unique mastic resin from Chios. Prog. Health Sci., 1 (1):131-135.
- Dunn, E.D., Cole, J.C., Smith, M.W., 1996a. Position of cut, bud retention and auxins influence rooting of *Pistacia chinensis*. Sci. Hortic., 67: 105-110.
- Dunn, D.E., Cole, J.C., Smith, M.W., 1996b. Timing of *Pistacia chinensis* Bunge. rooting using morphological markers associated with calendar date and degree-days. J. Am. Soc. Hortic. Sci., 12: 269-273.
- Fascella G., Airò M., Zizzo G.V., Ruffoni B., 2004. Prime osservazioni sulla coltivazione in vitro di Lentisco (*Pistacia lentiscus* L.). Italus Hortus 11: 141-143 (in Italian).
- Freedman, P., 2011. Mastic: a Mediterranean luxury product. Med. Hist. Rev., 26 (1): 99-113.
- Garcia-Fayos, P., Verdu M., 1998. Soil seed bank, factors controlling germination and establishment of a Mediterranean shrub: *Pistacia lentiscus* L. Acta Oecologica, 19: 357-366.
- Garner, R.J., 2007. The Grafters Handbook. Cassell Illustrated, London, U.K., p. 323.
- Grundwag, M., 1975. Seed set in some *Pistacia* L. (*Anacardiaceae*) species after inter-and intraspecific pollination. Isr. Jour. Bot., 24: 205-211.
- Grundwag, M., 1976. Embryology and fruit development in four species of *Pistacia* (*Anacardiaceae*). Bot. J. Linn Soc., 73: 355- 370.
- Hartmann, H.T., Kester, D.H., Davies, F.T. Jr., 2002. Plant Propagation, Principles and Practices, 7th ed., Prentice Hall Inc. Englewood Cliffs, New Jersey, p. 647.
- İsfendiyaroğlu, M., 2000. Cutting Propagation of Mastic Tree (*Pistacia lentiscus* var. Chia Duham.), NUCIS Newsletter, 9: 42-44.
- İsfendiyaroğlu, M., 2003. Effects of some physical and biochemical factors on the rooting of mastic tree (*Pistacia lentiscus* var. Chia Duham.) Cuttings. Ege Üniv. Ziraat Fak. Derg., 40 (1): 25-32.
- Karakır, M.N., İsfendiyaroğlu, M., 1999. Sakız Ağacı (*Pistacia lentiscus* L.)’nın Vegetatif Yöntemlerle Çoğaltılması ve Kök Oluşumunun Anatomik-Fizyolojik İncelenmesi Üzerine Araştırmalar. İzmir (Doktora Projesi) TÜBİTAK, TOGTAG-1511 Nolu proje, s: 97 (in Turkish).

- Kılınç, F.M., Süzerer, V., Çiftçi, Y.Ö., Onay, A., Yıldırım, H., Uncuoğlu, A.A., Koc, I., Metin, Ö.K., 2015. Clonal micropropagation of *Pistacia lentiscus* L. and assessment of genetic stability using IRAP markers. *Plant Growth Regul.*, 75 (1): 75–88.
- Koç, İ., Onay, A., Özden Çiftçi, Y., 2014. In vitro regeneration and conservation of the lentisk (*Pistacia lentiscus* L.). *Turk. J. Biol.*, 38: 653-663.
- Joley, L.E., Opitz, K.W., 1971. Further experience with propagation of *Pistacia*. Combined Proceedings of the International Plant Propagators' Society, 21: 67–76.
- Mascarello, C., Fascella, G., Zizzo, G.V., Mantovani, E., Ruffoni, B., 2007. In vivo and in vitro propagation of *Pistacia lentiscus* L. *Acta Hort.*, 764: 299-306.
- Mudge, K., Janick, J., Scofield, S., Goldschmidt, E.E., 2009. A history of grafting. *Horticultural Reviews*, 35: 437–49.
- Mulas, M., Brigaglia N., Cani M.R., 1998. Evaluation of *Pistacia lentiscus* L. genetic resources to select ecotypes having high efficiency in the colonisation of marginal lands. *Acta Hort.*, 457: 279-286.
- Nelson, W.L., 1987. Innovations in air layering. *Comb. Proc. Intl. Plant Prop. Soc.*, 37: 88-89.
- Lee, C.I., Paul, J.L., Hackett, W.P., 1976. Effect of Ca saturation and air filled porosity of sphagnum peat on root regeneration of *Pistacia chinensis* Bunge and *Liquidambar styraciflua* L., *J. Amer. Soc. Hort. Sci.*, 101 (3): 233-236.
- Onay, A., Tilkat, E., Süzerer, V., Karakaş Metin, Ö., Özden Çiftçi Ö., Kılınç, F.M., Koç, I., Şakiroğlu, M., Yıldırım, H., Altinkut Uncuoğlu, A., Çalar, N., Akdemir, Ö.F., 2016. Rejuvenation of mature lentisk by micrografting and evaluation of genetic stability. *Turkish J. Biol.*, 40: 781–796
- Pair, J.C., Khatamian, H., 1983. Propagation and growing of the Chinese pistache. *Proc. Int. Plant Prop. Soc.*, 32: 497-503.
- Paraschos, S., Mitakou, L., Skaltsounis, A., 2012. Chios gum mastic: a review of its biological activities. *Curr. Med. Chem.*, 19 (14): 2292-2302.
- Parlak, S., 2018. Clonal propagation of mastic tree (*Pistacia lentiscus* var. *chia* Duham.) in outdoor beds using different rootstock and grafting techniques. *J. For. Res.*, 29(4): 1061–1067.
- Pignatti, G., Crobeddu, S., 2005. Effects of rejuvenation on cutting propagation of Mediterranean shrub species. *Italian Society of Silviculture and Forest Ecology*, pp. 290-295
- Piotto, B., 1995. Influence of scarification and prechilling on the germination of seeds of *Pistacia lentiscus*. *Seed Science and Technology*, 23: 659-663
- Piotto, B., Di Noi, A., 2003. Why Propagate Trees and Shrubs of the Mediterranean Flora from Seed. In: B. Piotto and A. Di Noi, editors, *Seed Propagation of Mediterranean Trees and Shrubs*. Agency for the Protection of the Environment and for Technical Services (APAT), Rome. p.7.
- Piotto, B., et al., 2003. Fact Sheets on the Propagation of Mediterranean Trees and Shrubs from Seed. In: B. Piotto and A. Di Noi, editors, *Seed Propagation of Mediterranean Trees and Shrubs*. Agency for the Protection of the Environment and for Technical Services (APAT), Rome. pp.11-78.
- Prada, M. A., Arizpe, D., 2008. *Pistacia lentiscus* L. In: *Riparian tree and shrub propagation handbook*. pp. 90-93.
- Ruffoni, B., Mascarello, C., Fascella, G., Airo, M., Amoretti, M., 2004. La micropropagazione del Lentisco (*Pistacia lentiscus* L.). In: *Atti II Convegno nazionale "Piante mediterranee: Valorizzazione delle risorse e sviluppo sostenibile"*, Agrigento, pp.277-280 (in Italian).
- Simons, R.K., 1987. Compatibility and Stock-Scion Interactions as related to Dwarfing. In: *Rootstocks for Fruit Crops*. Eds. Rom, R.C. and Carlson, R.F., John Wiley & Sons, Inc. U.S.A., pp.79-106
- Tutar, M., Şafak, C., Aksoy, D., Çiçek, F., 2016a. Damla sakızına (*Pistacia lentiscus* L. var. *chia* Duham.) anaç Olarak kullanılabilecek *Pistacia* Türleri. *Bahçe*, 45: 230-235.
- Tutar, M., Aksoy, D., Şafak, C., Çiçek, F., 2016b. Damla sakızının (*Pistacia lentiscus* L. var. *chia* Duham.) havai daldırma yöntemiyle üretilmesi. *Bahçe*, 45: 867-866.
- Viola, F., Forio, L.R., Cocozza, M.A., 2004. Propagazione agamica di alcune specie della macchia mediterranea. *Italus Hortus* 11: 186-1.
- Yıldırım, H., 2012. Micropropagation of *Pistacia lentiscus* L from axenic seedling-derived explants. *Sci Hortic*. 137: 29-35.
- Zakynthinos, G., Rouskas, D., 1998. Wild and cultivated *Pistacia* species in Greece. Towards a comprehensive documentation and use of *Pistacia* genetic diversity in Central and West Asia, North Africa and Europe, S. Padulosi, and A. Hadj-Hassan, eds. (14-17 December 1998, Irbid, Jordan), Report of the IPGRI Workshop, pp.88-92.
- Zografou, P., Linos, A., Hagidimitriou, M., 2010. Genetic diversity among different genotypes of *Pistacia lentiscus* var. *chia* (mastic tree). Paper presented at: XIV GREMPA Meeting on Pistachios and Almonds. (Zaragoza, Spain: CIHEAM).
- Zohary, M., 1952. A monographical study of the genus *Pistacia*. *Palestine J. Bot.*, Jerusalem Series, 5: 187-228.
- Zohary, D., 1995. The genus *Pistacia* L. In: Padulosi S, Caruso T, Barone E ed(s). *Taxonomy, distribution, conservation and uses of Pistacia genetic resources: report of a workshop*, 29-30 June 1995, Palermo, Italy. Rome: IPGRI pp.1-11.

A STUDY ON VOLATILES, IN VITRO ANTICHOLINESTERASE INHIBITOR AND ANTIMICROBIAL ACTIVITY OF ENDEMIC *Allium tuncelianum*

Rezzan ALIYAZICIOGLU¹, Ufuk OZGEN², Nuriye KORKMAZ¹, Seyda AKKAYA¹, Sila Ozlem SENER², Merve BADEM¹, Nevin ULAS COLAK³, Sengul ALPAY KARAOGLU⁴

¹Department of Biochemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

²Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

³Department of Analytical Chemistry, Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

⁴Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53100, Rize, Turkey

Corresponding author email: rezzan@ktu.edu.tr

Abstract

The genus *Allium* has been belongs to the Alliaceae family and consist of 700 species of bulbous perennials and biennials. 164 of which grows in Turkey and 65 of them are endemic. *Allium tuncelianum* has being grown in Tunceli province. In this study, analysis the volatiles by using GC-MS, anticholinesterase inhibitory and antimicrobial activity of the methanolic extracts from the aerial parts of the plant was carried out. The result of antimicrobial activity being studied by agar diffusion method was determined moderate antibacterial and anticandidal activity, *Yersinia pseudotuberculosis*, *Staphylococcus aureus*, *Bacillus cereus*, *Mycobacterium smegmatis*, *Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*. To analyse inhibitory activities of AChE and BuChE of the plant was used modified colorimetric Ellman's method. As a result of the study, the IC₅₀ value has been found as 338,844 and 337,824 µg/mL for inhibitory activity of AChE and BuChE, respectively. Volatiles of the plant were determined as β-trans-caryophyllene, sesquicineole, pelargonaldehyde, β-phellandrene and α-phellandrene by GC-MS. According to the results, the plant has been moderate anticholinesterase inhibitor, antibacterial and anticandidal activity and volatile ingredients.

Keywords: *Allium tuncelianum*, anticholinesterase inhibitor activity, antimicrobial activity, GC-MS

INTRODUCTION

Turkey, as in many plant species in terms of *Allium* species is a rich country. There are about 500 *Allium* species in the world, about 164 of them are seen in Turkey (Yazar, 2006). *Allium tuncelianum* is an endemic plant species that is found only in Tunceli province especially on the slopes of Munzur mountains (Yanmaz and Ermis, 2005). *A. tuncelianum* has 25-60 cm long flower stalk, light purple flowers, capsule-shaped fruit, 1-2 mm, diameter 3-cornered and wrinkled seeds (Yazar, 2006). The people of Tunceli know the plant as “rock garlic” (Yanmaz and Ermis, 2005). Today, plants are frequently used in the prevention and treatment of diseases.

Plant essential oils are important components of plant chemistry. Essential oils from plants or plant droplets can easily pass through the cell membrane. They can also be absorbed easily and profusely from the lungs and the skin. In addition, the pharmaceutical value of essential oils taken as medicines or food additives in the body is quite high. Plant essential oils have been used for many years as antimicrobial, antioxidant, anti-inflammatory, carminative, antispasmodic, and simulant. In recent years, mutagenic and antimutagenic activity of essential oils has been found to have potential use in the treatment and prevention of many diseases, including cancer (Erdoğan, 2012).

Cholinesterase inhibitors catalyze the hydrolysis of acetylcholine which is a neurotransmitter. There are two cholinesterase enzymes in the body as acetylcholinesterase and butyrylcholinesterase. Cholinesterase inhibitors enhance central and peripheral cholinergic function by inhibiting

acetylcholinesterase degradation. Cholinesterase inhibitors are clinically used in diseases such as Myasthenia Gravis, Glaucoma, and Alzheimer's Disease (Alasehirli, 2005).

In recent years, demand to the plants is increased due to the various side effects of synthetic drugs used in the treatment of diseases caused by pathogenic bacteria and resistance to drugs (Kara et al., 2016). It is known that various essential oils have antimicrobial properties. An essential feature of essential oils and their components is their hydrophobic structure. With these properties, the essential oils easily transfers through the cell membrane, causing the bacterial to break down. (Rowing and Calm, 2017).

Purpose of this study was carried out analysis the volatiles by using GC-MS, anticholinesterase inhibitory and antimicrobial activity of the methanolic extracts from the aerial parts of *Allium tuncelianum*.

MATERIAL AND METHODS

The plant was collected from the Erzincan region (2000 m) in August and a sample of herbarium was prepared (Herbarium no: 10960). The aerial part of the plant was dried in the shade and comminuted. At least 500 g of the extract was prepared with 1000 mL of methanol and the extracts were extracted for 8 hours and 3 times on a shaker at 50 °C and the extracts were combined. At the end of this period, the extract is filtered with filter paper. The filtrate was concentrated at 30 °C in an evaporator until a dense extract was obtained.

For gas chromatography analyses, a Shimadzu QP2010 ultra GC–MS, fitted with a PAL AOC-5000 plus auto sampler, and Shimadzu 2010 plus FID and Shimadzu Class-5000 Chromatography Workstation software was used. The gas chromatographic conditions were as followed: GC oven fitted with a RTX-5M 30 m capillary column (0.25 mm I.D., 0.25 mm film thickness, Restek, USA) with carrier gas helium at flow rate of 1.0 mL min⁻¹, operating under an initial temperature at 60°C (2min) to 240°C (5 min) at 5°C/min, with He as carrier gas (1 ml/min), the temperature of the injector and detector were 250°C and 230°C, respectively. The ionization voltage was 70 eV. Identification of compounds was based on a comparison of their mass spectra with Standards (Adams, 2004) Confirmation of compound identities was obtained using retention index (Adams, 2004.). The datum analysis was performed on a NIST library (Shimadzu, Kyoto, Japan) and Wiley libraries of mass spectra (National Institute of Standards and Technology, 2008). After analysis on a Restek RTX-5M column, quantification was performed as peak area using integration data.

For SPME sampling, *Allium tuncelianum* puree was prepared by blending 100 g plant for 3 min without water in a Waring blender. The plant peree (1 g) was placed into a 10-mL headspace vial, and the flask was closed with a septum. The SPME headspace volatiles were collected using a Supelco 57348 2 cm, 50/30 µm DVB/Carboxen/PDMS Stable-Flex fiber (precondition of the fiber at 250 °C in the injection port of a GC for 10 min) for 30 min (highest concentration of aroma compounds without qualitative change of the composition; tested by 10 min extraction steps up to 30 min). After sampling, the SPME device was placed into the injector of the GC and the GC-MS instruments through the whole GC analysis time of 62 min using RTX-5M column. The identified compounds are listed in Table 2 and Table 3.

Some modification with Ellman method was used for cholinesterase activity. The Ellman method is a colorimetric method in which, after AChE breaks down acetylcholine, the thiolcholine reacts with 5,5'-dithiobis- (2-nitrobenzoic acid) (DTNB) to give the yellow colored 5-thio-2-nitrobenzoate anion. 96 well microplates were used in this method. Acetylcholinesterase enzyme will be used as an enzyme for acetylcholinesterase inhibition activity, and acetylthiocholine iodide as a substrate. Butyrylcholine iodide is used as the butyrylcholinesterase enzyme substrate obtained from horse serum as an enzyme for butyrylcholinesterase inhibition activity (Ellman et al., 1961).

The agar well diffusion method was used to determine if the plant's antimicrobial activity of the methanol extract of the plant (Perez et al., 1990; Woods et al., 2003). The test Microorganisms used for antimicrobial activity were given in Table 1. Ampisilin and Flukonazol were used positive control.

Table 1. Test microorganisms for antimicrobial activity.

Test Microorganisms	
Gram negative bacteria	<i>Escherichia coli</i> (ATCC 25922), <i>Yersinia pseudotuberculosis</i> (ATCC 911), <i>Pseudomonas aeruginosa</i> (ATCC 27853)
Gram positive bacteria	<i>Staphylococcus aureus</i> (ATCC 25923), <i>Enterococcus faecalis</i> (ATCC 29212), <i>Listeria monocytogenes</i> (ATCC 43251), <i>Bacillus cereus</i> (702 Roma)
No gram bacteria	<i>Mycobacterium smegmatis</i> (ATCC607)
Fungals	<i>Candida albicans</i> (ATCC 60193), <i>Saccharomyces cerevisiae</i> (RSKK 2519)

Data were expressed means $\pm$ standard deviations of three parallel measurements. % inhibition values were calculated using linear regression analysis (Microsoft Excel programme for Windows, versus 2003). Standart Deviation (SD) was calculated with SPSS (Version 22.0, Chicago, IL, USA) software.

RESULTS AND DISCUSSION

A. tuncelianum, naturally grown in Tunceli province of Turkey, was investigated by gas-phase chromatography and GC-MS using Solid Phase Micro Extraction Method (SPME). As a result of solid phase micro-extraction method, fifteen essential oil component was determined. According to this evaluation, the main components of this specie was found to be p-Cymene and sesquicineole in the ratios of 20.24 % and 16.65 %, respectively. The list of identified volatile constituents as well as their grouping into three classes, namely monoterpenes , sesquiterpenes and others (aldehyde and hydrocarbones).

Table 2. Qualitative determination MS.

Retention Time	Area %	Height	Height %	A/H	Name	Retention İndeks	Class
8.350	3.33	20268	3.40	4.51	5-Hexenal (CAS)	806	Aldehit
10.705	3.83	19945	3.35	5.27	1-Hexanol	875	alkol
15.963	5.44	29226	4.91	5.11	Phellandrene <alpha>	1007	monoterpen
16.789	9.67	52182	8.77	5.09	Cymene <para>	1026	monoterpen
17.032	3.32	16155	2.71	5.64	beta.-Phellandrene	1032	monoterpen
19.171	3.62	17814	2.99	5.58	Diallyl disulphide	1081	alkan
20.055	6.48	34316	5.76	5.19	Pelargonaldehyde	1102	aldehit
27.943	3.21	15262	2.56	5.78	Benzene, 1-Methoxy-4-(2-Propenyl)- Estragole	1287	monoterpen
31.830	1.98	12414	2.09	4.37	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.) γ -Cadinene-	1383	monoterpen
32.209	2.07	11531	1.94	4.94	Tridecane	1393	alkan
33.505	5.63	35273	5.93	4.38	trans(.beta.)-caryophyllene	1426	seskiterpen
33.906	4.29	23885	4.01	4.93	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)- 2-Norpinene	1437	monoterpen
34.517	2.47	16313	2.74	4.16	Farnesene <(E)-, beta>	1452	seskiterpen
36.634	3.17	20150	3.38	4.32	.beta.-Bisabolene (CAS)	1508	seskiterpen
36.901	22.03	135680	22.79	4.45	sesquicineole	1516	seskiterpen

Table 3. Quantitative determination FID.

Ret.Time	Area	Height	Conc	% Area	Name
8.678	1096.2	202.4	6.19357	6.1936	5-Hexenal (CAS)
15.980	1554.4	311.9	8.78266	8.7827	Phellandrene <alpha->
16.803	3583.1	645.2	20.24488	20.2449	Cymene <para->
17.041	1677.6	211.6	9.47865	9.4787	beta.-Phellandrene
20.076	1050.7	183.9	5.93638	5.9364	Pelargonaldehyde
33.539	1430.3	271.4	8.08130	8.0813	trans(.beta.)-caryophyllene
36.945	2947.3	451.9	16.65257	16.6526	sesquicineole

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that causes a significant disruption of normal brain structure and function. At the cellular level, AD is characterized by a progressive loss of cortical neurons, especially pyramidal cells, that mediate higher cognitive functions. Acetylcholinesterase inhibitors help improve memory function and attention in AD patients by interfering with the breakdown of acetylcholine, thereby increasing the levels of the neurotransmitter at the synapse (Korolev, 2014). The % inhibition values of the methanolic plant extract were submitted in Table 4.

Table 4. Acetylcholinesterase, and butyrylcholinesterase inhibitor activities (% inhibition).

Samples	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
AChEI of the plant	19,412 ± 1,365	28,023 ± 3,021	37,083 ± 3,654	42,896 ± 2,982
BChEI of the plant	29,107 ± 1,025	41,588 ± 2,014	46,840 ± 2,015	55,174 ± 1,265
Galantamine for AChEI	68,663 ± 2,265	74,286 ± 3,245	79,371 ± 6,154	83,275 ± 4,302
Galantamine for BChEI	42,536 ± 1,023	57,662 ± 2,002	69,787 ± 2,351	79,463 ± 2,365

AChEI: Acetylcholinesterase Inhibitor Activity

BChEI: Butyrylcholinesterase Inhibitor Activity

The half-maximal inhibitory concentrations (IC₅₀) of the plant were found 346,74 µg/mL for AChEI and 123,03 µg/mL for BChEI. The results of AChEI and BChEI of the plant were found near to the result of galantamine. We haven't meet any cholinesterase enzyme study about the plant in literature studies. The results of this work will lead to further work.

Table 5. The half-maximal inhibitory concentrations (IC₅₀) of the plant.

Samples	IC ₅₀ (µg/mL)
AChEI of the plant	346,74
BChEI of the plant	123,03
Galantamine for AChEI	1,25
Galantamine for BChEI	35,48

The methanolic extract was screened for antimicrobial activity against three bacteria strains (*Yersinia pseudotuberculosis*, *Staphylococcus aureus* and *Mycobacterium smegmatis*) and three yeast strains (*Candida albicans*, *Candida tropicalis* and *Saccharomyces cerevisiae*) using the agar diffusion method. For positive control, ampicillin (Amp.) and fluconazole (Flu.) were used. Control drug activity results are presented as follows: Amp. against Ec: 10 mm, Yp: 10 mm, Kp: 10 mm, Pa: 18 mm, Sa: 35 mm, Ef: 10 mm, Bc: 15 mm; Flu. against Ca: 25 mm, Ct: 25 mm, Sc: 25 mm. The screening results were assessed in terms of diameter of the inhibition zone. Antimicrobial drugs are frequently used in the treatment of diseases. Due to overuse and misuse of these drugs, the bacteria create resistance to the synthetic drugs. Due to the formation of resistance, the trend towards natural medicines has increased (Ventola, 2015). Any antimicrobial work about *A. tuncelianum* hasn't found. Since the plant is effective against various microorganisms, it is a preliminary study for the development of antimicrobial medicines from the plant.

Table 6. Microorganisms and inhibition zone.

Sample	Microorganisms and Inhibition Zone (mm)										
	Gram- Negative				Gram-Positive			No Gram Ms	Yeast Fungi		
	Ec	Yp	Kp	Pa	Sa	Ef	Bc		Ca	Ct	Sc
A. tuncelianum	8	12	6	8	10	6	-	13	14	18	20
Amp.	10	10	10	18	35	10	15	35			
Flu.									25	25	25

Ec: *Escherichia coli* ATCC 25922, **Yp:** *Yersinia pseudotuberculosis* ATCC 911, **Kp:** *Klebsiella pneumonia* subsp. *pneumonia* ATCC18883, **Pa:** *Pseudomonas aeruginosa* ATCC 27853, **Sa:** *Staphylococcus aureus* ATCC 25923, **Ef:** *Enterococcus faecalis* ATCC 29212, **Bc:** *Bacillus cereus* 702 Roma, **Ms:** *Mycobacterium smegmatis* ATCC607, **Ca:** *Candida albicans* ATCC 60193, **Ct:** *Candida tropicalis* ATCC 13803, **Sc:** *Saccharomyces cerevisiae* RSKK 251, **Amp.:** Ampicillin, **Flu.:** Fluconazole, (—): no activity

CONCLUSION

This study is the first study on the plant in terms of volatile oil, cholinesterase inhibitor and antimicrobial activity studies. Because of the toxicity and side effects of synthetic drugs, researchers have turned to naturally occurring compounds and having started with the plant. Our study showed that the plant has a strong antimicrobial effect and moderate cholinesterase inhibitor effect. And also the plant has rich essential oil contents. Our study has preliminary study feature about with using the plant on the prevention and treatment of various diseases.

ACKNOWLEDGEMENTS

Sıla Ozlem Sener, Merve Badem and Nuriye Korkmaz would like to acknowledge the scholarship by the Turkish Scientific and Technical Research Council.

REFERENCES

- Adams R.P., 2004. Identification of essential oil components by gas chromatography-mass spectroscopy. Allured Carol Stream IL, USA.
- Alaşehirli B., 2005. The Inhibitors of Cholinesterase (Anticholinesterases). *Türkiye Klinikleri Journal of Internal Medical Sciences Pharmacology*, 1(18): 47.
- Ellman G.L., Courtney K.D., Andres V. Jr., Feather-Stone R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem Pharmacol.*, 7: 88-95.
- Erdoğan E.A., 2012. Mutagenic and Antimutagenic Effects of the Essential Oils-Bitki Uçucu Yağlarının Kullanım Alanları ve Muhtemel Genetik Etkileri. *Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi*, 2(2): 21-24.
- Kara A.A., Algur Ö.F., Köseoğlu M.Ş., 2016. Bazı Şifalı Bitkilerin *Helicobacter pylori* üzerindeki Antimikrobiyal Aktiviteleri. *Cumhuriyet Science Journal*, 37(2): 129-140.
- Korolev I. O., 2014. Alzheimer's disease: a clinical and basic science review. *Medical Student Research Journal*, 4: 24-33.
- Kürekci C., Sakin F., 2017. Uçucu Yağlar: Antimikrobiyal Açından Uçucu Yağlar: In-Vitro ve In-Vivo Çalışmalar. *Türkiye Klinikleri Journal of Animal Nutrition and Nutritional Diseases-Special Topics*, 3(1): 15-20.
- Perez C., Pauli M., Bazerque P., 1990. An antibiotic assay by the well agar method. *Acta Biologica et Medicine Experimentalis*, 15: 113-115.
- Ventola C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics*, 40(4): 277.
- Woods G.L., Brown-Elliott B.A., Desmond E.P., Hall G.S., Heifets L., Pfyffer G.E., Ridderhof J.C., Wallace R.J., Warren N.C., Witebsky F.G., 2003. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes. Approved Standard NCCLS document M24-A, 23: 18.
- Yanmaz R., Ermiş S., 2005. Tunceli Sarımsağı (*Allium tuncelianum* Kollman, Özhatay, Matthew, Şiraneci) Tohumlarındaki Çimlenme Probleminin Çözülmesi Üzerinde Araştırma, Ankara Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü, Ankara.
- Yazar E., 2006. Tunceli sarımsağında (*Allium tuncelianum* (Kollman, N.Özhatay, D. Matthew, Ş. Şiraneci) in vitro kök ve sürgün ucu kültürü yoluyla çoğaltma, Yüksek Lisans Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara.

POTANTIAL RISK OF ARSENIC ACCUMULATION BY MEDICINAL PLANTS

Esra ERSOY ÖMEROĞLU, Aslı BAYER

Ege University, Faculty of Science, Department of Biology, Basic and Industrial Microbiology
Section, Bornova-Izmir, Turkey

Corresponding author email: esraerso@gmail.com

Abstract

Medical plants and preparations of these plants are widely used by people all over the world. The reason for the popularity of medical plants is that they are easy to access, have a relatively low cost, are therapeutically effective, and are not considered to have toxic side effects. The widespread view is that consumption of natural products and herbal medicines is harmless and that there are no side effects and that the expected medicinal effect can not be achieved, even if they are harmless. Because of this, medicinal plants are used as the most important life-saving medication for the vast majority of the world population and for this reason it appears that commercialization of medical plants has increased in recent years. 80% of the world population uses aromatic medicinal plants to meet their health needs. However, human activities cause an increase in environmental pollution based on heavy metals in agricultural soils and as a result; in medical and aromatic plants and spices, accumulation of heavy metals such as Cu, Cd, Fe, Mn, Pb, Zn and As occurs. For this reason, the World Health Organization has established acceptable limits for the amount of toxic substances in medical plants. One of these heavy metals is arsenic and can be found widely in nature in various forms of oxidation. The most important way of exposure to As is to take food by mouth and accumulation in the human body may lead to the development of resistance to various antimicrobial agents as well as to the increase in various types of cancer. Therefore, it should be checked whether there are heavy metal contents such as As and plant formulation contents of products obtained from medical plants and necessary precautions should be taken.

Keywords: Medicinal plants, arsenic, accumulation.

INTRODUCTION

Heavy metals and environmental pollution are seen as an emerging and serious problem worldwide. Arsenic abundant in the earth crust (As), is a toxic element ubiquitous in the environment and the organisms (Rahimi et al., 2012). Concurrently arsenic being the most abundant 20th element in the earth's crust and in the biosphere is noteworthy (Bhattacharya, 2017). Arsenic can be found in nature in various oxidation forms, such as elemental As (0), arsenic As (-III), arsenate As (V) and arsenite As (III). Most common forms are As(V) and As(III) (Rahimi et al., 2012).

The transfer of arsenic to terrestrial and aquatic environments takes place with both natural and anthropogenic activities. The most important anthropogenic inputs are observed as, incineration of municipal solid wastes, fossil fuels in coal and fuel firing power plants, release of metal pollutants and the direct use of arsenic-containing herbicides in industrial and agricultural areas (Rahimi et al., 2012).

Heavy metals released into the air, soil and environment absorbed by the roots and leaves of plants, cause dysfunction in plant metabolism and then constitute serious risks about human health (Lajayer et al., 2017). Chronic exposure to inorganic arsenic results in negative health effects including cardiovascular and neurological effects, as well as causing cancer in the skin and internal organs (Rahimi et al., 2012).

Phytotoxic effects of arsenic in plants are; root plasmolysis, discoloration and subsequent necrosis of the leaf tip and edges and a decrease in water motility consequently. Other effects that can be observed as reduction in growth, purple coloration, leaf fading, and the arrest of seed germination (Rahimi et al., 2012). Besides having an important place in their use as nutrients, plants and plant materials also have some important chemical compounds that can be used in the medical field (Pyne et al., 2017).

Plants can be easily exposed to heavy metals during or after sowing (Stanojkovic-Sebic et al., 2014). The vast majority of the world's population uses medicinal plants as the primary source of health care. The World Health Organization (WHO) reported that approximately 80% of the population use only medicinal plants to treat many diseases (Stanojkovic-Sebic et al., 2014). Aside from being considered as an important source of natural products, medicinal plants have an important place in the socio-cultural, spiritual and health areas of India for rural and tribal life (Pyne et al., 2017).

The low cost of herbal medicines and general opinion on the lack of side effects cause an increase in the use of these products (Rahimi et al., 2012). However, recent studies have reported lead, arsenic, mercury and cadmium poisoning from traditional Indian herbal products and Asian herbal medicines. Furthermore other noteworthy products containing toxic heavy metals to form significant health concerns are some of the medicinal plants in Brazil, nutritional supplements in Poland, Argentina and Mexico (Arpadjan et al., 2008).

Many countries have established quality and safety standards for herbal remedies clearly indicating the maximum permissible value for heavy metals in herbal medicine. However, these standards are generally based on quality standards for foods and are not based on research on herbal medicines (Rahimi et al., 2012). This has led to the need to improve the quality standards for herbal remedies by reviewing and revising the maximum permissible values by conducting research based on medicinal plants (Rahimi et al., 2012). A study in the Gondar region of Ethiopia, which is thought to serve these purposes; Examples of 10 medicinal plants commonly used for medical purposes were selected (Atinafu et al., 2015). Examples are shown in table 1 (Table 1).

As, Pb and Cd levels were analyzed in the plant samples and the results are shown in the table. It was determined that 10 samples examined had detectable levels of all metals examined and values are summarized in table 2 (Table 2) (Atinafu et al., 2015).

Of the medicinal plants analyzed, 80% contain arsenic at a level far below the maximum permissible limit (1 mg/kg) set by the WHO. However, DIG and AGA samples contain arsenic above the permissible limit, which may cause health hazards (Fig. 1) (Atinafu et al., 2015).

International Standards Related to Heavy Metals in Medical Plants and Plant Based Products

In recent years, due to having reasonable prices, natural and safe properties, use of aromatherapy has been increasing significantly in developing and developed countries. However, because of low quality of raw materials facilities, the negative effects of herbal medicines have increased. As a result of these effects, health, safety and quality assurance of medicinal plants and herbal products have become the main concerns of the WHO. Recently, several pharmacopoeias worldwide have been established for medicinal plants and herbal products such as in the United States, Europe, England and Italy. WHO (2005) regulates the maximum limit for toxic metals in medicinal plants as 0.3, 1 and 10 mg / kg for Cd, As and Pb, respectively (Lajayer et al., 2017).

Table1. List of medicinal plants analyzed for their heavy metal content and traditional use (Atinafu et al., 2015).

Plant Species	Code Given	Local Name (in Amharic)	Parts used	Application & route of administration
<i>Acalypha acrogyna</i>	GUL	Gullo	Leaf	All types of cancer, orally
<i>Adansonia digitata</i>	BAM	Bamba	Bark	Cholera, bloating of stomach, orally
<i>Brucea antidyseptica</i>	ABA	Abalo	Leaf	Leprosy, scabies and other skin diseases, topically
<i>Calpurnia aurea</i>	DIG	Digta	Leaf	Ascariasis, gastric ulcer, oral
<i>Carissa spinarum L</i>	AGA	Agam	Leaf	Snake bite/external

<i>Croton macrostachyus</i>	BIS	Bsana	Bark	Gonorrhea, colic, orally	Abdominal
<i>Dodonaea angustifolia</i>	KIT	Kitkita	Leaf	Jaundice, Taeniasis, orally	Malaria and
<i>Maytenus ovatus</i>	ATA	Atat	Leaf	All types of cancer, oral	
<i>Maytenus undata</i>	CHE	Checho	Leaf	Eye infection, externally	
<i>Piliostigma thonningii</i>	WAN	Wanza	Bark	Wound heal after male, female circumcision, externally	

Table 2. Heavy metal content of medicinal plants in mg per kg of sample in dry weight (Atinafu vd., 2015).

Sample code	Heavy metal content (mg/kg $\pm$ SD)		
	As	Cd	Pb
BIS	0.037 $\pm$ 0.002	1.55 $\pm$ 0.01	0.37 $\pm$ 0.002
AGA	1.89 $\pm$ 0.006	0.01 $\pm$ 0.002	15.66 $\pm$ 0.013
ABA	0.04 $\pm$ 0.001	6.75 $\pm$ 0.03	35.97 $\pm$ 0.011
KIT	0.19 $\pm$ 0.004	0.01 $\pm$ 0.001	8.68 $\pm$ 0.014
GUL	0.10 $\pm$ 0.002	2.31 $\pm$ 0.007	6.53 $\pm$ 0.013
ATA	0.09 $\pm$ 0.001	2.25 $\pm$ 0.004	20.60 $\pm$ 0.018
WAN	0.03 $\pm$ 0.001	1.48 $\pm$ 0.009	3.43 $\pm$ 0.011
DIG	2.06 $\pm$ 0.008	1.37 $\pm$ 0.003	2.25 $\pm$ 0.012
BAM	0.06 $\pm$ 0.003	0.001 $\pm$ 0.0003	0.002 $\pm$ 0.0001
CHE	0.09 $\pm$ 0.002	1.72 $\pm$ 0.004	0.002 $\pm$ 0.0001
MPL	1	0.3	10

*MPL – Maximum permissible limit.

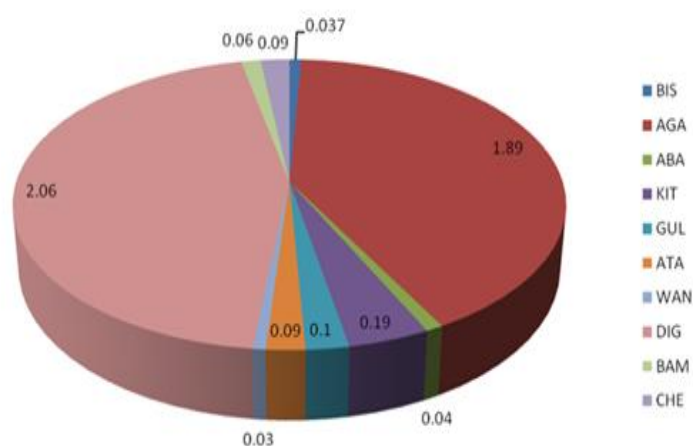


Fig. 1. Arsenic (As) content of medicinal plants in mg/ kg of samples (Atinafu vd., 2015).

CONCLUSION AND FUTURE APPROACHES

As a result, heavy metal pollution as an element of environmental stress has been significantly increased as the accumulation of secondary metabolites in medicinal plants. Therefore, there are notable changes in the quality and quantity of the relevant products. For this reason, suitable soil management and collection of medicinal plants from heavy metal-free sites are required to reduce the heavy metal intake of these plants (Lajayer et al., 2017).

Interest in the therapeutic use of medicinal and aromatic plants is increasing rapidly worldwide as well as in Turkey. In our country, the rate of use of medicinal plants in all age groups is very common. This results in significant increases in the incidence of various neurological diseases, cardiovascular diseases, renal and respiratory diseases, hematological diseases, and particularly skin, lung, liver, bladder and kidney cancers. However, in our country, lack of research on the heavy metal content of medicinal plants thus absence of any standards is a matter of concern. Therefore, multidisciplinary studies are of great importance.

In order to eliminate the deficiencies in the literature, it will be beneficial to determine the arsenic content of medicinal and aromatic plants especially grown in these regions by sampling from the areas contaminated with arsenic. The use of arsenic-resistant microorganisms can provide remediation of contaminated areas regarding arsenic, as another approach, would be reducing accumulation in medicinal plants which tend to accumulate arsenic. It is thought that the use of organisms with arsenic accumulating properties or arsenic toxicity decreasing enzyme systems in the bioremediation processes in order to be cost effective and widely applicable may be an approach that will reduce these concerns.

REFERENCES

- Arpadjan, S., Çelik, G., Taşkesen, S., Güçer, Ş., 2008. Arsenic, cadmium and lead in medicinal herbs and their fractionation. *Food and Chemical Toxicology*, 46: 2871-2875.
- Atinafu, T., Mekonnen, T., Somasundaram, J., 2015. Determination of some toxic heavy metal accumulation in medicinal plants commonly used in Gondar area district, Northwestern Ethiopia. *International Journal of Pharmacy and Analytical Research*, 4(4): 399-405.
- Bhattacharya, S., 2017. Medicinal plants and natural products in amelioration of arsenic toxicity: a short review. *Pharmaceutical Biology*, 55(1): 349-354.
- Lajayer, B.A., Ghorbanpour, M., Nikabadi, S., 2017. Heavy metals in contaminated environment: destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. *Ecotoxicology and Environmental Safety*, 145: 377-390.
- Pyne, S., Santra, S.C., 2017. Accumulation of arsenic, copper and iron in common medicinal plants of Murshidabad district, West Bengal, India. *International Journal of Experimental Research and Review*, 9: 54-62.
- Rahimi, M., Farhadi, R., Balashahri, M.S., Abedi, M.R., Nasab, K.A., 2012. Effect of arsenic on medicinal plant. *International journal of Agronomy and Plant Production*, 3: 755-758.
- Stanojkovic-Sebic, A., Pivic, R., Josic, D., Dinic, Z., Stanojkovic, A., 2014. Heavy metals content in selected medicinal plants commonly used as components for herbal formulations. *Journal of Agricultural Sciences*, 21: 317-325.

DETERMINATION of VOLATILES, ANTIMICROBIAL and ANTIOXIDANT ACTIVITY of EXTRACTS from FLOWERS of CALENDULA (*Calendula officinalis* L.)

Didem ŞAHİNGİL, Ali Adnan HAYALOĞLU

Department of Food Engineering, Engineering, Faculty Inonu University, 44280 Malatya, Turkey,

*Corresponding author email: didem.sahingil@inonu.edu.tr

Abstract

Calendula officinalis is a member of the richest family of flowering plants, Asteraceae. In this study, the volatile profile of *Calendula officinalis* flowers by SPME-GC/MS technique, antimicrobial activity (against Gram-positive and Gram-negative pathogen) and antioxidant properties of ethanol, methanol and aqueous extracts of *Calendula officinalis* (Aynısafa) *Calendula officinalis* flowers obtained from the Marmara region were used for this study. Antimicrobial effects of methanolic, ethanolic and aqueous extracts of *Calendula officinalis* flowers against Gram negative and Gram positive bacteria and yeast were determined by disc diffusion method (0.6 mm disc diameter). Six different concentrations (25-250 mg/mL) were studied for determination of minimum inhibitory concentration. Volatiles in *Calendula officinalis* flowers were determined by SPME-GC/MS method. Antimicrobial activity was tested against both Gram negative and Gram positive bacteria using methanolic and ethanolic extracts of *C. officinalis* flowers. At the end of the test, the antimicrobial effect was observed for only against Gram positive bacteria in aqueous extracts. As a result, it was determined that ethanol extracts of *Calendula officinalis* flower had higher antimicrobial activity than methanol and aqueous extracts. A total of 121 volatile components (29 ester, 27 terpene, 6 aldehyde, 13 ketone, 12 alcohol, 19 hydrocarbon and 15 acids and Miscellaneous compounds) were determined from the head space of *Calendula officinalis* flowers.

Keywords: *Calendula officinalis*, antimicrobial, antioxidant activity, GS-MS aroma profile

INTRODUCTION

The World Health Organization (WHO) has catalogued 20,000 plant species studied for medicinal purposes (Gulluce and Aslan, 2006). Natural products from plants include phenolic compounds that could delay or inhibit the inception of infectious diseases (Robards and Prenzlara, 1999). Literature surveys show that plant-based drugs play a promising role in the treatment of infectious diseases (Ahmad and Beg, 2001). Efficacy and safety of plant extracts against some several pathogens is also well-documented (Abudunia and Ansar, 2014). The use of plant derived natural compounds as part of herbal preparations and alternative sources of drugs continues to play major roles in the general wellness of people all over the world. The search for new antioxidant, antimicrobial and anticancer agents are becoming an important objective for scientists.

Calendula officinalis is a member of the richest family of flowering plants, Asteraceae. It is grown and cultivated in a wide range of the world including Central, South, Eastern Europe, West Asia, Germany and USA. In various countries of the world, pot marigold, calendula, ringerblume, souci des jardins are known by their names. In Turkey, it is also known by the names of ointment, gold, velvet, orange, thyme, dead flower, velvet flower, medical nergiz, okgeğözü.

The objectives of this study were:

- to quantify the GS-MS volatile profile of calendula flowers;
- to test its the antimicrobial capacities against some human pathogen strains and
- to determine the antioxidant activity

MATERIAL AND METHODS

The *Calendula officinalis* flower samples were provided by from the Marmara Region of Turkey (harvested 2017). For the aqueous extract (AE), CO flowers powder (200 g) was extracted

with boiling water (500 mL) for 30 min. The infusion was filtered and then freeze-dried (aqueous extract). For the organic extracts, ethanolic and methanolic extracts were obtained by soxhlet extraction of 200 g of CO flowers during 6 h in about 500 mL of solvents. Then, the extract was filtrated and then were evaporated using vacuum evaporator until dryness. This extracts were concentrated to dryness and the residue was kept at 4 °C.



Fig. 1. *Calendula officinalis* plant.

Determination of volatiles in *Calendula officinalis* flower

Volatile compounds identified in the samples obtained from *Calendula officinalis* flower were put immediately in glass bottles in a freezer at -18°C. A duplicate 3.0 g portion of the sample was then placed in a 15 mL vial (Supelco, Bellefonte, PA, USA), followed by 10 µL of internal standard containing 300 ppm of 2-methyl-3-heptanone and 300 ppm 2-methylpentanoic acid in methanol (Sigma-Aldrich Co., St. Louis, MO, USA), and 100 ppm Methyl heptanoate allowed to equilibrate at 40°C for 30 min. Extraction of volatiles was carried out using Head Space Solid Phase MicroExtraction(HS-SPME) technique.

Determination of Antimicrobial Activity (Against Gram Positive and Gram Negative Pathogens and Antioxidant Activity of Extracts of Methanolic, Ethanolic and Aqueous Extracts

Antimicrobial (Gram-positive and Gram-negative pathogen) and antioxidant properties of ethanol, methanol and aqueous extracts of *Calendula officinalis* (Aynısafa) *Calendula officinalis* flowers obtained from the Marmara region were used in the research.

Staphylococcus epidermidis RSKK 0802, *Staphylococcus aureus* 1021/06008, *Staphylococcus warneri* 95052, *Staphylococcus xylosus*, *Shigella flexneri* RSKK 184, *Bacillus cereus* RS 863, *Escherichia coli* (Microbiology laboratory of Inonu University Turgut Özal Medical Center), *Enterococcus faecalis*, *C. albicans* ATCC 04055, *Streptococcus mutans*, *Enterobacter*, *Salmonella*, *Pseudomonas putida* were investigated on antimicrobial effect on 13 different microorganisms. Antimicrobial effects of methanolic, ethanolic and aqueous extract of *Calendula officinalis* flowers on Gram negative and Gram positive bacteria and yeast were determined by disc diffusion method (0.6 mm disc diameter) and 6 different concentrations (mg / ml) were studied for determination of minimum inhibitory concentration. The antimicrobial effects of methanolic, ethanolic and aqueous extras of *Calendula officinalis* flowers were determined by measuring the inhibition zone diameter mm (IZ).

ABTS Radical Cation Scavenging Activity

For the ABTS assay, ABTS stock solution was prepared by reacting 7 mM ABTS with 2.45 mM potassium persulfate solution. The stock solution was left in the dark at room temperature for 16 h, and it was diluted with ethanol to reach an absorbance of 0.70 ($\pm$ 0.02) at 734 nm. A 100 µL of the sample extract was mixed with 2400 µL of ABTS•+ solution, and the absorbance was measured at 734 nm after 10 min, against ethanol. The results were expressed mg/g trolox equivalent of sample by means of a curve for Trolox (1-100 ppm) (Xu et al., 2010).

RESULTS AND DISCUSSION

Determination of Volatiles in *Calendula officinalis* Flower

Calendula officinalis flowers having 121 aroma components; 29 ester, 27 terpene, 19 aldehyde ketone, 12 alcohol, 19 hydrocarbon and 15 acid and Miscellaneous components. Aliphatic acids and aldehydes were the most abundant chemical classes in all the samples whereas aromatic alcohols distinguish the samples from floral. Linalool, one of the terpene alcohol found in many flowers and spice plants were the most abundant in all the samples. Among sulfur compounds, Butane,1-isothiocyanate was the major one, followed by benzyl-isothiocyanate.

Table 1. Aldehydes and Ketones in samples (µg/kg).

Acetone	0,85±0,10
Ethyl hexyl ketone	0,20±0,02
2-Methyl-1-butanol	0,39±0,09
3-Methyl-1-butanol	0,48±0,11
2-Pentanone	0,51±0,06
Hexanal	2,88±0,73
2-Heptanone	0,32±0,13
2-Hexenal, (E)-	0,35±0,15
Octanal	0,71±0,31
6-Methyl-5-hepten-2-one	3,19±1,25
n-Nonanal	1,60±0,86
Furfural	0,40±0,07
Isocaryophyllen	0,82±0,40
3,5-Octadien-2-one	0,51±0,30
Benzaldehyde	2,06±0,81
Propanal, 2-methyl-3-phenyl-	7,60±3,04
Perillaldehyde	0,56±0,28
Dihydropseudoionone	0,50±0,21
Methyl 2-pyrrolyl ketone	1,37±0,39

Table 2. Esters in samples(µg/kg).

Methyl acetate	7,30±0,74
Ethyl acetate	1,08±0,49
Methyl propanoate	0,95±0,02
Methyl butyrate	2,33±0,42
Methyl 2-methylbutyrate	2,40±0,85
Methyl n-pentanoate	3,65±0,65
Methyl 2-methylvalerate	1,86±0,55
2-Methylbutyl acetate	2,82±1,24
Methyl caproate	28,21±5,24
Isoamyl acetoacetate	0,97±0,43
Capronate <ethyl->	2,46±0,81
Isoamylbutyrate	1,54±0,46
Ethanoate <hexyl->	3,56±1,43
Heptanoic acid, methyl ester	17,74±3,72
• Tert-butyl hexanoate	0,81±0,22
Methoxyacetic acid, 2-pentadecyl ester	0,95±0,01
Anisate <para-methyl->	0,95±0,23
Octanoic acid, methyl ester	12,8±5,66
Butyl caproate	0,88±0,12
Butanoic acid, 2-methyl-, hexyl ester	1,09±0,28
Ethyl n-heptadecanoate	1,38±0,45
Methyl nonanoate	2,65±1,04
Linalyl acetate	5,46±2,13
Cyclohexanol, 5-methyl-2-(1-methylethyl)-, acetate	23,70±7,20
Menthol acetate	2,97±0,88
Methyl caprate	4,17±1,36
Methyl myristate	1,64±0,82
Methyl palmitate	1,12±0,37
Diethyl Phthalate	15,88±3,41

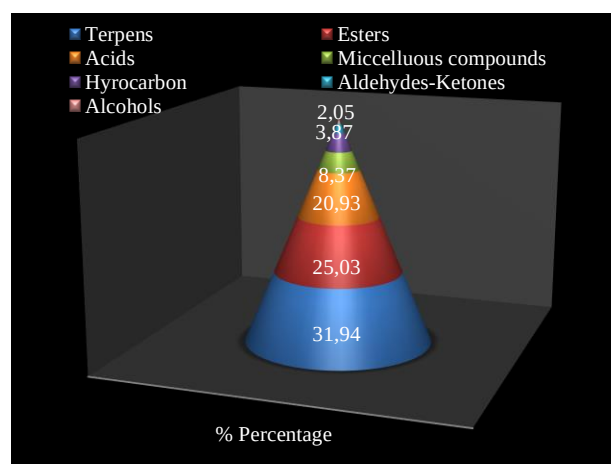


Fig 2. Percentage of Volatile compounds in *C. officinalis* flower

Table 3. Alcohols in samples (µg/kg).

Ethanol	2,69±0,67
2-Ethylnon-1-en-3-ol	0,43±0,12
Benzene, ethyl-	0,84±0,32
Tridecanol, 2-ethyl-2-methyl-	0,29±0,05
1-Hexanol	0,84±0,21
2-Butoxyethanol	0,63±0,19
1-Dodecanol	0,50±0,03
Sulcatol	0,92±0,37
1-Hexanol, 2-ethyl-	1,08±0,37
trans-p-Menthan-7-ol	2,26±0,67
2-Hexanol	0,41±0,07
Benzeneethanol	1,81±0,61

Table 4. Miscellaneous compounds in samples (µg/kg)

Hydroxylamine	4,70±0,96
neo-Menthol	3,02±1,46
3-p-Menthanol	0,76±0,08
Methyleugenol	1,27±0,62
Guaiacwood acetate	0,38±0,20
Cubenol	0,37±0,17
Veridiflorol	0,65±0,36
t-Muurolol	1,21±0,64
Carvacrol	3,21±1,63
t-Muurolol	1,51±0,70

Table 5. Terpenes in samples (µg/kg).

Trichloromethane	0,42±0,09
α-pinene,	3,44±1,32
α-Thujene	0,63±0,29
Toluene	57,41±4,85
Beta-Pinene	0,92±0,29
o-Xylene	0,41±0,06
delta-Carene	0,62±0,24
β-Myrcene	0,63±0,23
dl-Limonene	9,95±2,10
1,8-Cineole	3,78±1,36
gamma-Terpinene	3,68±0,52
Styrene	5,08±1,01
Cymene <para->	1,47±0,60
alpha-Cubebene	4,62±2,73
Isomenthone	2,68±1,40
alpha-Copaene	10,28±4,08
Linalool	8,30±3,48
alpha-Gurjunene	1,65±0,95
germacrene-d	2,35±1,40
Cedrene <beta->	2,49±1,29
Caryophyllene	9,30±3,25
β-Cyclocitral	0,48±0,30
α-Cubebene	1,14±0,66
Cadina-1(6),4-diene	1,66±0,98
<10betaH->	
Z-Citral	1,08±0,72
endo-Borneol	1,59±0,81

Table 6. Hydrocarbons in samples (µg/kg)

2,2-dimethoxybutane	0,35±0,01
Nonane, 5-(2-methylpropyl)	1,66±0,43
3-Ethyl-3-methylheptane	0,61±0,16
Dodecane	0,42±0,24
Benzene, propyl-	0,37±0,10
Eicosane	3,67±1,43
Dodecane, 4,6-dimethyl-	0,37±0,12
Dodecane, 4,6-dimethyl-	1,24±0,56
Heptadecane	0,30±0,15
Heptane, 3,3,5-trimethyl-	0,43±0,29
Nonane, 3-methyl-5-propyl-	0,93±0,51
Heneicosane	0,68±0,24
Hexadecane	0,37±0,16
Naphthalene,	7,93±3,39
(1.alpha.,4a.alpha.,8a.alpha.)-	
Naphthalene,	- 3,23±1,36
(1.alpha.,4a.beta.,8a.alpha.)-	
Benzene, 1-methoxy-4-(1-propenyl)-	3,59±0,73
Pentacosane	0,28±0,12
2-Pentadecanone, 6,10,14-trimethyl-	0,36±0,16
Phenol, 2-methoxy-4-(2-propenyl)- (CAS)	0,83±0,39
Eugenol	

Determination of Antimicrobial Activity (Gram positive and Gram negative pathogens) and Antioxidant Activity of Extracts of Methanolic, Ethanolic and Aqueous

To demonstrate the antimicrobial activity of the CO extract, *Candida* species, *E. coli* species and *Salmonella* and other pathogens were tested. Table 7 indicates that all extracts of CO flowers show inhibitory activity against *Salmonella enteritidis* and *E. coli* ATCC except for aqueous extracts. Moreover, all extract showed an inhibitory effects against *Staphylococcus* species. Antimicrobial activity was observed against both Gram negative and Gram positive bacteria obtained by methanolic and ethanolic extract of officinalis flowers, whereas antimicrobial effect was observed only on Gram positive bacteria in aqueous extracts. As a result, it was determined that *Calendula officinalis* flower's ethanol extracts had higher antimicrobial activity than methanol and aqueous extracts. However, none of inhibitory effect of CO extracts was observed for *C. albicans* ATCC 04055. Similarly, the aqueous-methanolic extracts from *C. officinalis* also showed antimicrobial activity against tested germs (Rigane et al., 2013).

The phenolic compounds and flavonoids found in CO could be responsible for its antimicrobial activity. Following these results, we could conclude that aqueous-methanolic-ethanolic flower extract from CO has very interesting antibacterial activities against the tested microorganisms. MIC values of methanolic and aqueous extracts were between 50-200 mg/mL and were bactericidal for all bacteria.

Table 7 Antibacterial activity of different *Calendula officinalis* extracts against clinical bacterial pathogens.

Antibacterial activity of <i>Calendula officinalis</i> extracts in term of inhibition zone (IZ) in mm				
Bacteria	Methanol extract	Ethanol extract	aqueous extracts	Oxytetracycline
<i>Staphylococcus epidermidis</i>	2.81	2.65	2.92	3.43
<i>Staphylococcus aureus</i>	2.80	2.90	2.70	3.40
<i>Staphylococcus warneri</i> 95052	1.50	2.50	2.20	2.80
<i>Staphylococcus xylosus</i>	1.60	2.00	1.30	3.20
<i>Shigella flexneri</i> RSKK 184	1.00	0.70	ND	0.70
<i>Bacillus cereus</i> RS 863	1.60	2.51	2.60	3.01
<i>Escherichia coli</i>	1.50	1.60	ND	3.30
<i>Enterococcus faecalis</i>	1.00	1.00	ND	1.60
<i>C. albicans</i> ATCC 04055	ND	ND	ND	ND
<i>Streptococcus mutans</i>	1.50	2.51	2.52	3.50
<i>Enterobacter</i>	0.80	0.80	ND	2.10
<i>Salmonella enteritidis</i>	1.00	0.60	ND	2.20
<i>Staphylococcus hominis</i>	0.60	ND	2.00	3.00

ND: not determined

Antioxidants Properties

In order to estimate the in vitro antioxidant scavenging activities of CO extracts, the ABTS scavenging assay was determined. Figure 3 shows the percentage of scavenging activity of CO methanolic extract, aqueous extract and ethanolic extract on scavenging activity of ABTS. The radical scavenging effects of different concentrations of methanol, water and hexane extracts of CO and Gallic acid (GA) are given here. The antioxidant activities of the extracts were compared with GA, which is a synthetic antioxidant. Both water and methanol extracts had radical scavenging effects.

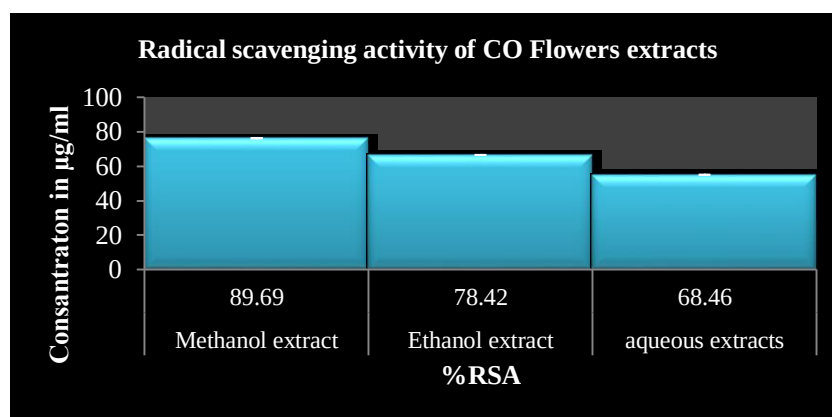


Fig. 3 Radical scavenging activity of CO Flowers extracts. Methanolic extract, Aqueous extract, Ethanolic extract.

However, this effect was not as great as the radical scavenging effect of GA. The radical scavenging effect of the methanol extract appeared to be closer to that of GA at 120 mg/mL. The magnitude of the radical scavenging effect at all concentrations was ranked as GA > ME > EE > AE. Radical scavenging activity has been demonstrated before in numerous studies (Ercetin et al., 2012; C'etkovic et al., 2004). EC₅₀ registered in our assay was lower and with potent antioxidant activities compared to previous studies by Ercetin et al. (2012).

Our study demonstrates that antioxidant and antimicrobial activity were observed for methanol, ethanol and aqueous CO flowers extracts, which might be correlated to its leading phenolic and flavonoid content. All extracts of CO flowers showed an inhibitory activity against the tested bacteria. In fact, we were able to demonstrate the antioxidant and antimicrobial activities of methanol, ethanol and aqueous extracts. Further work is underway to isolate bioactive components of CO flowers extract to determine potential pharmacological agents. Also, clinical studies are now required to examine the antimicrobial principles of *C. officinalis* in detail for various applications.

REFERENCES

- Ahmad I., Beg A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J Ethnopharmacol.* 74:113-123.
- Abudunia AM, Ansar M., 2014. Evaluation of antibacterial activity of extracts from *Calendula arvensis* flowers. *JCPR* ;6:156-161.
- C'etkovic' GS, Djilas SM, C'anadanovic'-Brunet JM, Tumba VT., 2004. Antioxidant properties of marigold extracts. *Food Res Int*, 37:643-650.
- Ercetin T., Senol F.S., Orhan I.E., Tokerb G., 2012. Comparative assess-ment of antioxidant and cholinesterase inhibitory properties of the marigold extracts from *Calendula arvensis* L. and *Calendula officinalis*. *Ind Crop Prod*, 203-208.
- Gulluce M., Aslan A., 2006. Screening the antioxidant and antimicrobial properties of the lichens *Pamelia saxatilis* *Platismatia glauca*. *Ramalina pollinaria*. *Ramalina polymorpha* and *Umbilicaria nylanderiana*. *Phytomedicine*.13:515-521.
- Robards K., Prenzlera PD., 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem.* 66:401-436.
- Rigane G., Ben Younes .S, Ghazghazi H., Ben Salem R., 2013. Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. *J Int Food Res*, 20:3001-3007.
- Xu, C., Zhang, Y., Cao, L., Lu J., 2010. Phenolic compounds and antioxidant properties of different grape cultivars grown in China, *Food Chemistry*, 119, 1557-1565.

USE OF MINT OIL FOR PROTECTING AND CONTROL AGAINST PHYTOPATHOGENIC FUNGI ON FRUITS AND VEGETABLES DURING POSTHARVEST STORAGE

Rezzan KASIM¹, M.Ufuk KASIM¹, Aysun ÇAVUŞOĞLU¹

¹Kocaeli University, Vocational School of Arslanbey, 41285, Kartepe-Kocaeli, Turkey

Corresponding author e-mail: rkasim@kocaeli.edu.tr

Abstract

Essential oils; obtained from leaves, fruits, barks or root parts of plants are volatile, strongly aromatic, usually colorless or light yellow, which are easily crystallized and are liquid at room temperature, and naturally produced compounds. Essential oils in the plant provide transfer the chemical substances to the cell, constitute the structure of the hormones in the plant cells and at the same time stimulate the defense mechanism of the plant through transfer of information between the cells and the plant defense mechanism, and thus increase the resistance against diseases and pests harmful effects. Mint Essential Oil is mainly composed menthol, menthone, mentholuras, limonene, b-pinene, methyl acetate, germacrene-d, trans-sabinene hydrate, isomenthone, 1,8-cineole, pulegone. It is found that mint oil had very strong antifungal activity against dermatophytes with Candida albicans, and Mentha arvensis L. and Mentha piperita L. showed strong antifungal activity against mycotoxin-producing Aspergillus flavus and A. parasiticus. In this review study, the mechanism of action of mint oil, one of the plant essential oils, against phytopathogenic fungi, which is a disease-causing in fruits and vegetables during postharvest duration, is examined.

Keywords: Mentha piperita L., essential oil, antifungal, fruit, vegetable, storage.

INTRODUCTION

Plant essential oils are oil-like mixture that a liquid form at room temperature, can crystallize, pungent odor and can be kept in a water vapor, which can be obtained from plants and herbal medicines in various ways. Essential oils are found in the plant's rhizome, stek, bark, leaves, fruit and flowers. Most of the essential oils in the plants are not accumulated in the plant's stem or their natural habitat because of they are quickly disintegrated in the nature. (Altındal and Altındal, 2017).

Essential oils are composed of terpenes such as pinen, myrcene, terpinen, limonen, cymene and phellandrene; asyclic monoterpene alcohol (geraniol, linalool) and monosyclic alcohols (menthol, 4-carvomenthenol, terpineol, carveol, borneol), terpenoids (oksijenated hydrocarbons); aliphatic aldehydes (citral, citronellal, perilaldehiyde); aromatic phenols (carvacrol, thymol, safrol, eugenol); biocyclic alcohol (verbenol); monocyclic ketons (menthone, pulefon, carvon); bicyclic monoterpene ketons (thujone, verbenon, fenkondan), acids (citronellic acid, cinnamic acid) and esters (linalil acetate). Some of the essential oils include oxides such as 1,8-cineole, sulfur containing compounds, methyl anthranilate, coumarins, zingiberene, curcumen, farnesol, sesquiphellandrene, turmeron and nerolidol. Most essential oils have strong biological activity and are responsible for bitter taste and toxic properties (El-Mohamedy, 2017).

The effect grade of essential oils shows variability depending on the type and amount of active ingredients. Although limited acquired knowledge on mechanisms of action, it is suggested that the effect is related to the lipophilic properties and chemical structures of the oils. The composition and amount of essential oils varies depending on the type of plant, the organ obtained, the mode of production, the geographical structure of the area where it is grown and climatic factors (Bayaz, 2014)

Phytopathogenic fungi have been found to be more effective in the formation of plant diseases than other plant pathogens and that various diseases in about 8000 plant species have been derived from these pathogens. It is also stated that in some parts of the world the reason of human deaths due to food scarcity are pathogenic fungi on plants. Today, excessive use of synthetic chemical fungicides poses a risk for both humans and plant flora and fauna. In addition, the random use of chemical fungicides causes the plant pathogenic fungi species to become resistant to these chemicals. Essential

oils found in plants increase the resistance to disease and damage by strengthening the defense mechanism of the plant by transferring chemical substances to the cells. Apart from that, it participates in the structure of hormones in plant cells and plays a role in transferring information between the cells and the defense mechanism of the plant. Most essential oils have a broad spectrum of action against plant pathogenic fungi and have traditionally been used for a long time in the preservation of stored products (El-Mohamedy, 2017).

It is also determined that essential oils are effective on yeast, molds (*Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus parasiticus*) and bacteria which cause food spoilage and poisoning due to antibiotic and anticeptic properties such as *Listeria monocytogenes*, *Enterococcus faecalis*, *Staphylococcus* spp., *Micrococcus* spp., *Bacillus* spp., *Campylobacter jejuni*, *Vibrio parahaemolyticus*, *Pseudomonas fluorescens*, *Shigella* spp., *Escherichia coli* (Evren and Tekgüler, 2011).

Mint essential oil contains menthol, menthone, mentholuras, limonene, b-pinene, methyl acetate, germacrene-d, trans-sabinene hydrate, isomenthone, 1,8-cineole, pulegone (Altındal and Altındal, 2017). Mint Essential Oil has been found to have very strong antimycotic activity against *Candida albicans* and dermatophytes. It is determined that *Mentha arvensis* L., and *Mentha piperita* L. showed strong antifungal activity against mycotoxin producing *Aspergillus flavus* and *A. parasiticus* (El-Mohamedy, 2017).

In this review, studies that intended preventive effects of mint essential oil on the development of phytopathogenic fungi causing postharvest losses on fruits and vegetables have been examined and the composition of mint oil, mechanism of action of mint oil for prevention of fungi has been presented.

The Plant Families that Contain Essential Oil

From the plant, family contains plants containing essential oils of Apiaceae, Asteraceae, Brassicaceae, Chenopodiaceae, Compositaceae, Cupressaceae, Iridaceae, Lamiaceae, Lauraceae, Myrtaceae, Pineaceae, Poaceae, Rosaceae, Rutaceae and Zingiberaceae (Evren and Tekgüler, 2011).

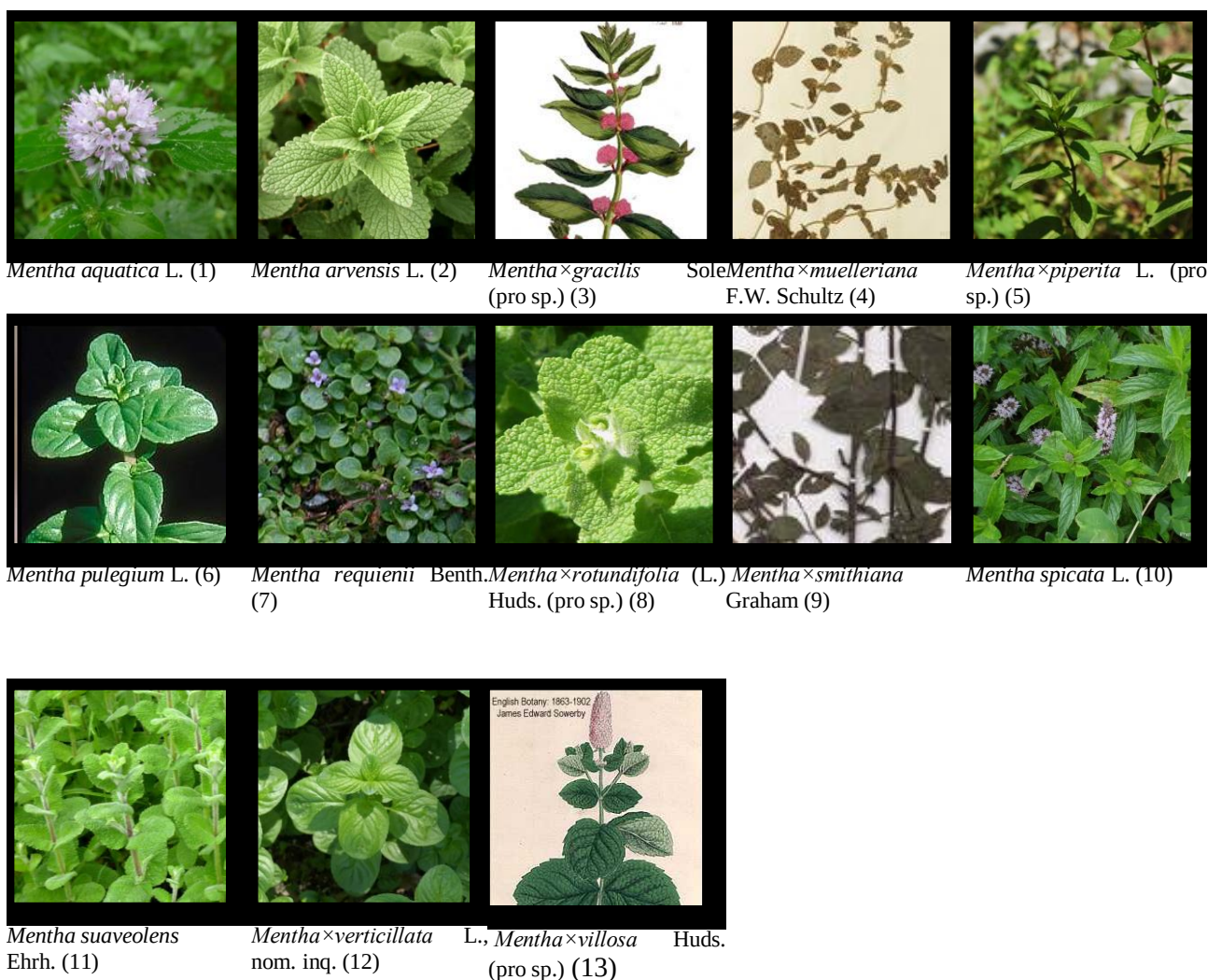
Lamiaceae Family

Most of the plants in the Lamiaceae family are used for medical or nutritional purposes. In addition, these plants have an economic importance as an ornamental plant. Essential oils found in plants belonging to Lamiaceae family have very high biological activity and have remarkable antimicrobial potential. The biological activity of essential oils often changes in direct proportion to the chemical composition of the oil and the difference between the activity levels of the oils is arises the different chemical composition (El-Mohamedy, 2017).

Plants belonging to Lamiaceae family are important in economical aspects due to different chemical compounds such as essential oils found in their constituents, as well as having an important place in the floristic richness in Turkey. Within the Lamiaceae family there are 250 genera and 7000 species. The natural distribution areas of plants belonging to this family are Australia, South West Asia and South America, especially Mediterranean countries (Yıldırım, 2014). Turkey is one of the important gene centers of the Lamiaceae family, in our country there are about 45 genera and 574 species belonging to this family. This family that the rate of endemism in our country, which is about 44.5%, in terms of the number of taxa that are included in Turkey is the third richest family (Kahraman et al. 2009). The *Mentha piperita* studied in this study is also within the Lamiaceae family and their classification of mint species is given in Table 1 and their plant characteristics in Figure 1.

Table 1. Classification of mint (USDA, 2018).

Kingdom	<u>Plantae</u>
Subkingdom	<u>Tracheobionta</u>
Superdivision	<u>Spermatophyta</u>
Division	<u>Magnoliophyta</u>
Class	<u>Magnoliopsida</u>
Subclass	<u>Asteridae</u>
Order	<u>Lamiales</u>
Family	<u>Lamiaceae</u>
Genus	<u>Mentha L.</u>
Species	<u>Mentha aquatica L.</u> <u>Mentha arvensis L.</u> <u>Mentha ×gracilis Sole (pro sp.)</u> <u>Mentha ×muelleriana F.W. Schultz</u> <u>Mentha ×piperita L. (pro sp.)</u> <u>Mentha pulegium L.</u> <u>Mentha requienii Benth.</u> <u>Mentha ×rotundifolia (L.) Huds. (pro sp.)</u> <u>Mentha ×smithiana Graham</u> <u>Mentha spicata L.</u> <u>Mentha suaveolens Ehrh.</u> <u>Mentha ×verticillata L., nom. inq.</u> <u>Mentha ×villosa Huds. (pro sp.)</u>


Figure 1. Varieties of mint.

Peppermint (*Mentha piperita* L.) Essential Oil

Essential oils are natural raw materials used in perfume, cosmetics, aromatherapy, phototherapy, spices and nutrition. Peppermint oil is one of the essential oils widely used because of the menthone and menthol it contains. It is used as sweetener and fragrance in foods as well as leaves and flowers have medicinal properties. Peppermint has an economically important place for foods and industrial cosmetic products with its taste, odor and therapeutic properties. It has also been determined that mint oil has prevented the development of microorganisms on various crops in recent years. In previously studies; antiviral, antibacterial, antifungal, insecticide, larvicidal, antibiofilm formation, radioprotector, antioedema, analgesic and antioxidant effects of peppermint oil were determined and it was found that it was used as mosquito repellent and had no toxic effect for human health. In addition, it was determined that *M. piperita* essential oil has been shown to inhibit the growth of radial fungi and aflatoxin production of *Aspergillus* species (Freire, et al. 2012; Sharkhiz et al. 2012).

Although the composition of peppermint essential oil varies depending on the season and variety, menthon, menthol and carvone were found to be the most important components of this oil. (Özgüven and Kırıcı, 1999). Behnam et al. (2006) indicated that the most important components of *M. piperita* essential oil were Menthanol (36.24%) and menthon (32.42%); whereas Moghaddam et al. (2013) determined that peppermint essential oil has thirty five components (Table 2) and the important components are menthone (30.33%), menthol (25.16%), menthofuran (6.47%), β -phellandrene (5.59%), and isomenthone 4.74%), menthol acetate (4.61%), pulegone (4.39%), β -caryophyllene (3.05%), neomentol (2.83%) and 1,8-cinine (2.15%). Sellamuthu et al. (2013) determined that the ratio of menthol which is the major component of mint oil as 46.45% using with gas chromatography / mass spectrometry analysis.

Table 2. Components found in *M.piperita* essential oil.

No.	Component	Percentage	No.	Component	Percentage
1	-Thujene	0.05	19	Menthol	25.16
2	-Pinene	0.85	20	Isomenthol	0.24
3	Sabinene	0.62	21	-Terpineol	0.36
4	-Pinene	1.26	22	Pulegone	4.39
5	3 Octanol	0.05	23	Piperitone	0.48
6	Myrcene	0.17	24	Neomenthol acetate	0.28
7	-Terpinene	0.09	25	Menthol acetate	4.61
8	p-Cymene	0.14	26	Isomenthol acetate	0.14
9	1,8-Cineole	2.15	27	-Bourbonene	4.61
10	-Phellandrene	5.59	28	-Elemene	0.15
11	(Z)- α -ocimene	0.02	29	-Caryophyllene	3.05
12	-Terpinene	0.17	30	(Z)- α -farnesene	0.39
13	TransSabinene hydrate	1.53	31	-Humulene	0.13
14	Linalool	0.14	32	Germacrene D	1.87
15	Menthone	30.63	33	Germacrene A	0.4
16	Isomenthone	4.74	34	Caryophyllen oxide	0.19
17	Menthofuran	6.47	35	Viridiflorol	0.4
18	Neomenthol	2.83		Toplam	99.97

Peppermint Oil on Control of Post-Harvest Pathogenic Fungi

The postharvest life of fruits and vegetables varies depending on the species, however, is generally short. Surface disinfection is very important for prolonging their postharvest life. In the past years different chemical substances were used for postharvest surface disinfection of fruits and

vegetables. However, due to strict regulations on minimum pesticide residue levels required by the countries importing fresh fruit and vegetables, increased resistance of fungus pathogens to synthetic fungus drugs, concerns about the destruction of fungicide wastes, studies have been carried out to determine the natural fungicides that alternative to synthetic fungicidal applications during the post-harvest period (Sellamuthu et al. 2013). One of the methods applied for this purpose is the use of plant essential oils.

Since plant essential oils naturally contain antimicrobial substances, they have gained considerable popularity and scientific interest in recent times (Tabassum and Widyasagar, 2013). One of these products, mint oil, has been used in recent years to increase the postharvest life of fruit and vegetable products. Peppermint essential oil has been reported to have antimicrobial, antioxidant, radical scavenger and cytotoxic activity (Güllüce et al. 2007). Since the essential oil activity used varies according to the characteristics of fruits and vegetables, storage period and disease agent, the method of application is very important in increasing the antifungal effect of essential oil. Also, as the antifungal activity of essential oil can be reduced depending on the length of the storage period, repeated applications in long-term storage should be preferred (Lopez Reyes et al. 2013).

In different studies, the effects of mint essential oil on different fruits and vegetables were investigated. It has determined that, *Mentha piperita* oil at the doses of 150, 200 and 250 µg mL⁻¹ was effect on inhibition of conidial germination and mycelial growth of *Colletotrichum gloeosporioides* that isolated as *in vitro* from papaya (*Carica papaya* L.) (Barrera-Necha et al. 2008).

It has been found that *M. piperita* oil shows good activity against *Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus niger*, *Aspergillus ochraceus*, *Colletotrichum gloeosporioides*, *Colletotrichum musae*, *Fusarium oxysporum* and *Fusarium semitectum* which are the main cause of deterioration after harvest. It was also determined that mint oil at a concentration of 0.2% was controlled by *A. glaucus* and *Aspergillus musae* at 90% and 98%, respectively, and completely destroyed all other fungi. In addition, it has been found that this antifungal effect of mint oil is due to menthon, neomenthol, menthol and carvone active substances (Freire, et al., 2012). In the study of the antifungal effect of *M. piperita* L. essential oil on the development of *Aspergillus niger* in comparison with synthetic menthol (38.33%), menthon (21.45%) and menthyl acetate (12.43%) were found to be the active ingredients of mint oil. It has been found that 1 and ½ dilutions of *M.piperita* oil exhibit stronger antifungal activity than the antibiotic of entamycin (8 mg / L) on the development of *A. niger* (Moghtader, 2013).

In a study evaluating that the antifungal potential of methanol, ethanol, water, acetone, chloroform and hexane active substances in leaves and stems of *Mentha piperita* against phytopathogenic fungi such as *Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus oryzae*; methanol extract of *Mentha piperita* leaf was found more effective than hexane, ethanol, water, chloroform and acetone to preventing fungi (Ali et al. 2017).

In the research that studying of antifungicidal effect of *M.piperita* oil against to *Dreschlera spicifera*, *Fusarium oxysporum* f.sp.ciceris and *Macrophomina phaseolina* fungi, five different concentrations of mint oil (100, 200, 400, 800, and 1600 ppm) used, but these concentrations were found not to have fungicidal activity (Moghaddam et al. 2013). On the other hand, it has been determined that *Phytophthora drechleri* is completely reduced in 0.4% (v/v) concentration of mint oil and this ratio is minimal inhibitory and minimal fungicidal concentration (Mohammadi et al. 2015).

In a similar study, it was found that peppermint oil has showed strong antifungicidal effect to *Alternaria alternaria* (38.16%), *Fusarium baseicum* (35.24%), *Penicillium* spp. (34.10%), *Fusarium oxysporum* (33.44%) and *Aspergillus fumigates* (30.08%). In addition, the maximum and minimum inhibitory concentrations for yeast maya (Ertürk et al. 2010) and fungi species were determined to be 10.22-38.16 and 0.50-10.0 µg/mL, respectively (Desam et al. 2017).

In the study that evaluated *in vitro* activity of *Saccharomyces cerevisiae*, *Candida tenuis* and *Saccharomyces cerevisiae* mixture with peppermint oil to prevent fungus growth of *Botrytis cinerea*, *Rhizopus stolonifer* ve *Alternaria alternate* which are caused fruit decay of tomato, it was found that

peppermint essential oil was showed inhibiting effect to fungus growth that evaluating pathogenic fungi. The application of the carnauba wax formula containing *S. cerevisiae* or *S. cerevisiae* together with mint oil (1%) under storage conditions are reducing the incidence of gray mold, soft rot and black rot, thus the effect of pathogenic fungus causing 100% decay of tomato fruit under artificial inoculation conditions was reduced significantly (Abd-Alla et al. 2009).

The peppermint oil (*Mentha piperita*) was applied at concentrations of 250, 500 and 1000 µg / cm³ to prevent the development of *Trichoderma harzianum* fungus (mycelial growth and sporulation) on mushroom (*Agaricus bisporus*) and consequently fungus sporulation decreased at the range 50.2% after 6 days from inoculation of growth media (Gorski et al. 2010).

When peppermint oil at 100, 300, 500 and 1000 ppm doses were applied to the organically grown Valencia oranges that is inoculated or non-inoculated with green mold to control green mold and to increase post-harvest fruit quality, mint oil at concentration of 1000 ppm has reduced decay rate (27%) compared to control (100%) in inoculated groups (Fatemi et al. 2011).

The fungicidal and fungistatic activity of mint oil against *Alternaria solani* which is an early blight agent of tomato is examined in vitro, and it was determined that the concentration of 2% provided complete inhibition and the concentrations of 4% and 6% showed fungicidal effect (Ragab et al. 2016).

There are studies to determine the effects of essential oils on the qualitative and quantitative characteristics of products after harvest. Anna variety apple fruit (*Malus Domestica* Borkh.) was stored for 3 weeks after treated with mint essential oil at the doses of 1%, 2.5% and 5% doses. In apples treated with peppermint oil both pectinase activity and weight loss decreased, while the fruit firmness increased (Amin, 2016).

CONCLUSION

Because *Mentha piperita* oil provided surface disinfection of fruit and vegetables, so it has caused the increase of the storage life of products.. Also, with the use of this oil, both visual and biochemical quality of product is rised during the postharvest storage. But, the studies related to using peppermint oil for different product is limited. Therefore, it is necessary that more studies needs to be done in the future.

REFERENCES

1. <https://www.pfaf.org/user/Plant.aspx?LatinName=Mentha+aquatica>. Date of access: 16/07/2018.
 2. <http://www.lxcoexp.com/san-pham/178-mentha-arvensis-l.html>. Date of access: 16/07/2018.
 3. <https://nas.er.usgs.gov/queries/greatlakes/FactSheet.aspx?SpeciesID=2698&Potential=N&Type=0&HUC Number DGreatLakes>, Date of access: 16/07/2018.
 4. <http://plants.jstor.org/stable/10.5555/al.ap.specimen.k000494766>. Date of access: 16/07/2018.
 5. <https://www.uniprot.org/taxonomy/34256>. Date of access: 16/07/2018.
 6. <https://florabase.dpaw.wa.gov.au/browse/profile/6883>. Date of access: 2018.
 7. <http://www.uniprot.org/taxonomy/294740>. Date of access: 16/07/2018.
 8. Glasson, H.A., Cronquist, A. Manual of vascular plants of northeastern United States and adjacent Canada.
 9. <https://www.gbif.org/species/2927185>. Date of access: 16/07/2018.
 10. <http://www.terrain.net.nz/friends-of-te-henui-group/weeds/mentha-spicata-l-subsp-spicata-mint.html>, Date of access: 16/07/2018.
 11. https://www.gardensonline.com.au/GardenShed/PlantFinder/Show_902.aspx. Date of access: 16/07/2018.
 12. <http://lurig.altervista.org/flora/taxa/floranam.php?genere=Mentha>, Date of access: 16/07/2018.
 13. <http://wisflora.herbarium.wisc.edu/taxa/index.php?taxon=13140>. Date of access: 16/07/2018.
- Abd-Alla, M., El-Mougy, N., El-Gamal, N. 2009. Formulation of essential oils and yeast, for controllign postharvest decay of tomato fruits. Plant Pathol.Bull., 18, 23-33.
- Ali, J., Hussain, A., Rehman, S., Khan, F., Sher, M. 2017. Antifungal potential of *Mentha piperita* leaves and stem extracts against phytopathogenic fungi. Science Arena Publications Specialty Journal of Biological Sciences, 3(4), 38-43.
- Altındal, D., Altındal, N. 2017. Plant Volatile Compounds in Growth. D. Choudhary, A. Sharma, P. Agarwal, A. Varma, N. Tuteja içinde, Volatiles and Food Security, Role of Volatiles in Agro-ecosystems (s. 1-15).
- Amin, A. 2016. Determination of some essential oils effects on the quality traits of the Egyptian Anna apple fruit during its shelf life. Journal of Horticultural Science & Ornamental Plants, 8(1), 35-45.

- Barrera-Necha, L., Bautista-Banos, S., Flores-Moctezuma, H., Estudillo, A. 2008. Efficacy of essential oils on the conidial germination, growth of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc and Control of postharvest diseases in Papaya (*Carica papaya* L.). *Plant Pathology Journal*, 7, 174-178.
- Bayaz, M. 2014. Esansiyel Yağlar: Antimikrobiyal, Antioksidan ve Antimutajenik Aktiviteleri. *Akademik Gıda*, 12(3), 45-53.
- Behnam, S., Farzaneh, M., Ahmadzadeh, M., Tehrani, A. 2006. Composition and antifungal activity of essential oils of *Mentha piperita* and *Lavandula angustifolia* on post-harvest phytopathogens. *Commun Agric Appl Biol Sci*, 71(3), 1321-6.
- Desam, N., Al-Rajab, A., Sharma, M., Mylabathula, M., Gowkanapalli, R., Albratty, M. 2017 (In press). Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha x Piperita* L. (peppermint) essential oils. *Science*.
- El-Mohamedy, R. 2017. Plant essential oils for controlling plant pathogenic fungi. D. Choudhary, A. Sharma, P. Agarwal, A. Varma, & N. Tuteja içinde, *Volatiles and Food Security, Role of Volatiles in Agro-ecosystems* (s. 171-199).
- Ertürk, R., Çelik, C., Kaygusuz, R., Aydın, H. 2010. Ticari olarak satılan kekik ve nane uçucu yağlarının antimikrobiyal aktiviteleri. *Cumhuriyet Tıp Derg.*, 32, 281-286.
- Evren, M., Tekgüler, B. 2011. Uçucu yağların antimikrobiyal özellikleri. *Elektronik Mikrobiyoloji dergisi TR*, 9(3), 28-40.
- Fatemi, S., Jafarpour, M., Eghbalsaiid, S., Rezapour, A., Borji, H. 2011. Effect of essential oils of *Thymus vulgaris* and *Mentha piperita* on the control of green mould and postharvest quality of *Citrus sinensis* cv. Valencia. *African Journal of Biotechnology*, 10(66), 14932-36.
- Freire, M., Jham, G., Dhingra, O., Jardim, C., Barcelos, R., Valente, V. 2012. Composition, antifungal activity and main fungitoxic components of the essential oil of *Mentha piperita*. *Journal of Food Safety*, 32, 29-36.
- Gorski, R., Sobielralski, K., Siwulski, M., Gora, K. 2010. Effect of selected natural essential oils on in vitro development of fungus *Trichoderma harzianum* found in common mushroom (*Agaricus bisporus*) cultivation. *Ecological Chemistry and Engineering Science*, 17(2).
- Güllüce, M., Shain, F., Sokmen, M., Özer, H., Daferera, D., Sökmen, A. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *mentha longifolia* L. spp. *longifolia*. *Food Chem.*, 103, 1449-1456.
- Kahraman, A., Celep, F., Doğan, M. 2009. Morphology, Anatomy and Palynology of *Salvia indica* L. (Labiatae). *World Applied Sciences Journal*, 6(2), 289-296.
- Lopez Reyes, J., Spadaro, D., Prella, A., Gullino, M. 2013. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo. *Journal of Food Protection*, 76(4), 631-639.
- Moghaddam, M., Pourbaige, M., Tabar, H., Farhadi, N., Hosseini, S. 2013. Composition and Antifungal Activity of Peppermint (*Mentha piperita*) Essential Oil from Iran. *Journal of Essential Oil Bearing Plants*, 16(4), 506-512.
- Moghtader, M. 2013. In vitro antifungal effects of the essential oil of *Mentha piperita* L. and its comparison with synthetic menthol on *Aspergillus niger*. *African Journal of Plant Science*, 7(11), 521-527.
- Mohammadi, A., Hashemi, M., Hosseini, S. 2015. Comparison of antifungal activities of various essential oils on the *Phytophthora drechsleri*, the causal agent of fruit decay. *Iran J. Microbiol.*, 7(1), 31-37.
- Özgüven, M., Kırıcı, S. 1999. Research on yield, essential oil, contents and components of mint (*Mentha*) species in different ecologies. *Türk J. Agric For*, 23, 465-472.
- Ragab, M., Ashour, A., Abdel-Kader, M., El-Mougy, N., Abdel-aziz, A. 2016. Fungicidal and fungistatic activity of some plant essential oils against *Alternaria solani* the causal of tomato early blight. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7(4), 998.
- Sellamuthu, P., Sivakumar, D., Soundy, P. 2013. Antifungal activity and chemical composition of thyme, peppermint and citronella oils in vapor phase against avocado and peach postharvest pathogens. *Journal of Food Safety*, 33(1).
- Sharkhiz, M., Motamedi, M., Zomorodian, K., Pakshir, K., Miri, R., Hemyari, K. 2012. Chemical Composition, Antifungal and Antibiofilm Activities of the Essential Oil of *Mentha piperita* L. *International Scholarly Research Network ISRN Pharmaceuticals*, 6 pages.
- Tabassum, N., Widyasagar, G. 2013. Antifungal investigations on plant essential oils. A review. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(2).
- USDA. 2018. <https://plants.usda.gov/java/ClassificationServlet?source=display&classid=MENTH>.
- Yıldırım, E. 2014. Melisa, adaçayı ve nane yağlarının bilimsel olarak incelenmesi, piyasa analizi ve kalite tayini. Erciyes Üniversitesi Eczacılık Fakültesi, Basılmamış Yüksek Lisans Tezi.

ANALYSIS OF FACTORS AFFECTING CONSUMPTION OF MEDICAL AND AROMATIC PLANTS IN URBAN AREA OF IZMIR PROVINCE

Hülya ARSLAN¹, Sait ENGİNDENİZ², Bahar AYDIN CAN³, Gökhan ÇINAR⁴

¹Forest General Directorate, Izmir Forest District Directorate, Izmir, Turkey

²Ege University, Faculty of Agriculture, Department of Agricultural Economics, Izmir, Turkey

³Kocaeli University, Arslanbey Vocational School, Kocaeli, Turkey

⁴Adnan Menderes University, Faculty of Agriculture, Department of Agricultural Economics, Aydın, Turkey

Corresponding author email: sait.engindeniz@ege.edu.tr

Abstract

The purpose of this study is to analyze consumer structure for medicinal and aromatic plants, consumer trends and preferences in urban area of Izmir province. For this aim, 96 surveys by proportional sampling have been carried out with members of households in center of Karşıyaka district of Izmir. The criteria for consumption of medicinal and aromatic plants were analyzed through Fuzzy Pairwise Comparison. According to results of the study, a large part of consumers, medical and aromatic plants does not recognize under the name. Consumer purchase medicinal and aromatic plants mostly from aktars and supermarkets. The most bought plants are mint, thyme and linden. The main criteria for consumption and purchase of medicinal and aromatic plants are price and packing. Consumers should be informed on the importance and benefits of medicinal and aromatic plants, their awareness should be raised in regard to quality standards of products and they should be guided into opting for packaged and branded products.

Keywords: medical and aromatic plant, consumer analysis, consumer preferences, fuzzy pairwise comparison.

INTRODUCTION

The demand for medicinal plants is currently increasing in both developed and developing countries for various reasons. Medicinal and aromatic plants can be processed to become essential oils through distillation, however, their cut flower marketing and the plants parts are in demand (Ghorbanpour et al., 2017).

Medicinal and aromatic plants are used as ground cover in agriculture, as decorative plants in parks and gardens, in erosion control, borders, parterre and rock gardens in addition to being used for food, drug and treatment purposes. In addition, medicinal plants are also used in areas such as nutrition, cosmetic, body care, incense or religious ceremonies, whereas aromatic plants are widely used in food, cosmetic and perfume sectors due to their nice scents and tastes (Kılıç et al., 2017).

In Turkey, production of medicinal and aromatic plants aims to enhance the living standards of forest villagers through employment in production areas; decrease the damage made by villagers on the natural resources in forests; sustain cultivation and production of rare and endangered medicinal and aromatic plants by limiting their production even in the face of high domestic and foreign demand; conserve the biological and genetic diversity of important natural resources by limiting production; improve the economic value of medicinal and aromatic plants by offering only high quality produce for export (Veziroğlu and Gültekin, 2018).

In recent years, a lot of studies have been made on the production technique, place of use, marketing and economic aspects of medicinal and aromatic plants in Turkey (Özkan et al., 2002; Artukoğlu et al., 2002; Artukoğlu and Uzmay, 2003; Özgüven et al., 2005; Bayramoğlu et al., 2009; Alpınar, 2010; Bayram et al. 2010; Toksoy et al., 2010; Faydaoğlu and Sürücüoğlu, 2011; Aydın et al., 2014; Çelik, 2014; Arslan et al., 2015; Kırıcı, 2015; Akbulut and Özkan, 2016; Bayraktar et al., 2017). But, there are limited research on consumption of medicinal and aromatic plants (Kızıl and Tonçer, 2005; Korkmaz and Fakir, 2009; Dicle, 2010; Özdemir et al., 2014; Kızıloğlu et al., 2017; Veziroğlu and Gültekin, 2018).

The purpose of this study is to analyze consumer structure for medicinal and aromatic plants, consumer trends and preferences in Karşıyaka district of İzmir province.

MATERIAL AND METHOD

The data was collected from the households in Karşıyaka district of İzmir. There are a total of 117,901 households in Karşıyaka district according to the data of the year 2013 (TurkStat, 2014). The sample size was calculated as 96 households using the following proportional sampling formula (Newbold, 1995). At 95% confidence level and 10% error level with $p = 0.5$ and $q = 0.5$ is used for getting the maximum sample size.

$$n = \frac{Np(1-p)}{(N-1)\sigma_{px}^2 + p(1-p)}$$

In this formula;

n = Sample size

N = Total number of households

p = Proportion for the households who consume medicinal and aromatic plants

σ_{px}^2 = Variance

In the determination of the households to be interviewed, it was planned to conduct in six streets in each neighborhood. For this purpose, 16 of the 27 neighborhood in the district of Karşıyaka were randomly selected from the neighborhood list.

In the analysis of the research data, firstly the demographic structure of the households were revealed. Then the level of knowledge, information resources, attitudes and behaviors towards purchasing and consumption of medicinal and aromatic plants were analyzed. At this stage, arithmetic mean and percentage calculations were made, the results were arranged and interpreted in schedules.

In this study, the criteria for consumption and purchase of medicinal and aromatic plants were analyzed through Fuzzy Pairwise Comparison Method. Five different criteria were presented to consumers for determining the consumption choices. These criteria were price, packing, brand, reliability, health and nutritive value. Method steps may be summarized as follows (Zadeh, 1983).

First, pairwise comparisons were presented to indicate individual preferences. The total distance in comparison is follow equal.

If $G_{KH}=0.5$ then $K \approx H$; if $G_{KH}>0.5$ then $K>H$ and if $G_{KH}<0.5$ then $K<H$.

The number of paired comparisons of the objectives (C) were determined as $C = [(Z.(Z-1))/2]$. Z refers to preferred number of objectives in the formula.

In this study, 10 comparisons of five different criteria were presented to each individual. For each pairwise comparison, g_{cr} preference was obtained. Measurement of the preference degree of r according to c can be expressed as $g_{cr}=1-g_{rc}$. Then, fuzzy preference matrix was as follow generated as follow;

$$G_{cr} = \begin{cases} 0 & \text{if } c = r \quad \forall c, r = 1, \dots, n \\ g_{cr} & \text{if } c \neq r \quad \forall c, r = 1, \dots, n \end{cases}$$

In this study, 5x5 fuzzy preference matrix was created for each individual as follow;

$$G = \begin{vmatrix} 0 & g_{12} & g_{13} & . & g_{1r} \\ g_{21} & 0 & . & . & . \\ g_{31} & g_{32} & 0 & . & . \\ . & . & . & 0 & . \\ g_{c1} & . & . & . & 0 \end{vmatrix}$$

Seperately preferred density of each objective(μ_j) was obtained using the following equation;

$$\mu_j = 1 - (\sum_{c=1}^n G_{cr}^2 / (n - 1))^{1/2}$$

The value of μ_j ranges between 0 and 1. The purpose of the comparison was determined whether they are equally important using Friedman Test. Furthermore, Kendall's coefficient of concordance for ranks was used.

RESULTS AND DISCUSSION

Demographic characteristics of households are presented in Table 1. 46.88% of members of household are men, 53.12% of members of household are women. Average age of members households is 42.52. 44.79% of members of households had completed university and more than 70% of them work. Majority of members of household (56.25%) are married. The place where they live most is the metropolitan and the city center. The average household size is 3.07. The monthly income of majortiy of the households (89.58%) is 3000 TL or more.

Table 1. Demographic characteristics of households

Characteristics		Number of households	%
Gender of member	Male	45	46.88
	Female	51	53.12
Age of member	18-29	19	19.79
	30-41	22	22.92
	41-52	38	39.58
	≥ 53	17	17.71
Education of member	Elementary school	12	12.50
	Secondary school	14	14.58
	High school	27	28.13
	University	43	44.79
Marital status of member	Single	28	29.17
	Maried	54	56.25
	Widowed	8	8.33
	Divorced	6	6.25
Employment status of member	Employed	68	70.83
	Unemployed	28	29.17
The most living place	Metropolitan	49	51.04
	City center	30	31.25
	District center	13	13.54
	Town/village	4	4.17
Average household size		3.07	-
Total monthly income of household (TL)	<3000	10	10.42
	3000-3999	35	36.46
	≥ 4000	51	53.12

Their knowledge degree about medicinal and aromatic plants was asked to members of household. This question responded as 40.63% of members of households low knowledgeable,

39.58% of members of household knowledgeable and 19.79% of members of household unknowledgeable (Table 2).

Table 2. Knowledge level related to medicinal and aromatic plants of households.

Knowledge level	Number of households	%
Unknowledgeable	19	19.79
Low knowledgeable	39	40.63
Knowledgeable	38	39.58
Total	96	100.00

The information sources for medicinal and aromatic plants of households were neighbourhood (61.46%), television programs (60.41%) and internet (58.33%) (Table 3).

Table 3. Information sources related to medicinal and aromatic plants of households.

Information sources	Number of households (*)	%
Neighbourhood	59	61.46
Television programs	58	60.41
Internet	56	58.33
Newspapers, magazines etc.	36	37.50
Doctor suggestions	27	28.13
Advertisement	18	18.75
Radio programs	12	12.50
Sales representative	8	8.33

(*) Household members indicated more than one source.

Medicinal and aromatic plant consumption aims of households are presented in Table 4. Accordingly, the most using aims are general health, treatment and pleasure.

Table 4. Medicinal and aromatic plant consumption aims of households.

Consumption aims	Number of households	%
General healthy	24	25.00
Treatment	8	8.33
Pleasure	20	20.83
General healthy and treatment	7	7.29
General healthy and pleasure	21	21.88
General healthy, treatment and pleasure	14	14.59
Cosmetic	2	2.08
Total	96	100.00

The medicinal and aromatic plant consumption preferences of households are given in Table 5. 37.50% of the households consume these products by mixing with the infusion. 29.17% and 15.63% of households prefer to consume with decoction and mixing food, respectively.

Table 5. Consumption preferences for medicinal and aromatic plants of households.

Consumption preference	Number of households	%
Infusion and mixing of food	36	37.50
Decoction	28	29.17
Mixing food	15	15.63
Infusion, mixing and oiling	11	11.46
Pomade	4	4.16
Oil	2	2.08
Total	96	100.00

Frequency of purchasing for medicinal and aromatic plants of households is presented in Table 6. According to table, 33.33% of the households purchasing seldom, 33.33% of the households purchasing sometimes, 29.17% of the households purchasing frequently, and 4.17% of the households purchasing always.

Table 6. Purchasing frequency for medicinal and aromatic plants of households.

Purchasing frequency	Number of households	%
Seldom	32	33.33
Sometimes	32	33.33
Frequently	28	29.17
Always	4	4.17
Total	96	100.00

Households prefer to purchase medicinal and aromatic plants mostly in both collected from the nature and processed packaged form (56.25%). It is also seen that some of the households prefer to purchase only collected from the nature form (14.58%) and only processed and packaged form (29.17%) (Table 7).

Table 7. Purchasing preferences for medicinal and aromatic plants of households.

Purchasing preference	Number of household	%
Collected from the nature	14	14.58
Processed packaged	28	29.17
Both	54	56.25
Total	96	100.00

The most purchased places of medicinal and aromatic plants of households are herbalists with 35.42%. Subsequently, supermarkets / hypermarkets with 29.17% and neighborhood markets with 22.91% are the most preferred purchasing places (Table 8).

Table 8. Places of purchase for medicinal and aromatic plants of households.

Places of purchase	Number of households	%
Herbalist	34	35.42
Supermarkets / hypermarkets	28	29.17
Local markets	22	22.91
Pharmacy	8	8.33
Wholesaler	4	4.17
Total	96	100.00

Mint is the most purchased plant by the household. It is followed by, linden, thyme, sage, saleb, fennel flower, stinging nettle. The least purchased plants are; mares tail, rock rose, mistletoe and yarrow (Table 9).

As shown in Table 10, 56.25% of households stated that prices are suitable, 19.79% of households did not have an opinion, 16.67% of households prices are high and 7.29% of households prices are low.

In this study, Fuzzy Pairwise Comparison Method was used for determining the consumption and purchase choices of consumers. Five different criteria were presented to consumers. These criteria were price, packing, brand, reliability, health and nutritive value.

Results related to choices of consumers are presented in Table 11. The main criteria for consumption and purchase of medicinal and aromatic plants are price and packing. According to Friedman test, there is a statistically significant difference for preferences ($p < 0.01$). Kendall's W value was determined as 0.375.

Table 9. Purchased medicinal and aromatic plants by households.

Plants	Number of purchaser households	%	Number of non-purchaser households	%
Centaury	39	40.62	57	59.38
Tyme	80	83.33	16	16.67
Sage	68	70.83	28	29.17
Linden	83	86.46	13	13.54
Mint	89	92.71	7	7.29
Bay leaf	42	43.75	54	56.25
Mallow	21	21.87	75	78.13
Rose hip	55	57.29	41	42.71
Anise	15	15.63	81	84.37
Fennel	30	31.25	66	68.75
Camomile	33	34.38	63	65.62
Saleb	61	63.54	35	36.46
Rosemary	24	25.00	72	75.00
Lemon balm	21	21.88	75	78.12
Yarrow	7	7.29	89	92.71
Mistletoe	6	6.25	90	93.75
Folium quince	16	16.67	80	83.33
Fennel flower	62	64.58	34	35.42
Flaxseed	41	42.71	55	57.29
Spanish lavender	16	16.67	80	83.33
Mares tail	3	3.13	93	96.87
Stinging nettle	37	38.54	59	61.46
Basil	29	30.21	67	69.79
Rock rose	5	5.21	91	94.79
Sumach	54	56.25	42	43.75

Table 10. Opinions on prices of medicinal and aromatic plants of households.

Opinion	Number of households	%
No opinion	19	19.79
Low	7	7.29
Suitable	54	56.25
High	16	16.67
Total	96	100.00

Table 11. Criteria preferences for medicinal and aromatic plants consumption and purchase of households.

Criteria	Mean	Standart error	Min.	Max.
Price	0.588	0.154	0.165	0.900
Packing	0.549	0.129	0.100	0.900
Brand	0.509	0.112	0.286	0.800
Healty and nutritive value	0.349	0.126	0.100	0.582
Reliability	0.344	0.129	0.100	0.629

CONCLUSION

According to results of this study, these plants are generally known by households, but not as medicinal and aromatic plants. Influential factors on consumption of the medicinal and aromatic plants are neighbourhood, experiences from the past, visual and written materials. Households consume these products by mixing with the infusion. It can be said that are purchasing habits of households for medicinal and aromatic plants. The belief that the effect is beneficial in terms of human health when the products are unprocessed, collected from nature and consumed is widespread, and hygiene is the most important choice for it is consumed as processed and packed. Households prefer to purchase medicinal and aromatic plants mostly in both collected from the nature and processed packaged form. The most purchased places of medicinal and aromatic plants of households are herbalists. Mint is the most purchased plant by the household. Most of households stated that

prices are suitable. The main criteria for consumption and purchase of medicinal and aromatic plants are price and packing.

These products are mostly used for general health purposes. There are also preferred for treatment and pleasure. It is often seen that households are aiming to take advantage of the protective and immune system booster properties of natural plants. In recent years, consciousness towards increasing human health and quality of life and accompanying preventive and protective treatment have been among the aims of preferring medicinal and aromatic plants. The market volumes of medicinal and aromatic plants continue to increase parallel to their use in different areas and fields of industry. Local consumption and foreign trade data should be taken into consideration for medicinal and aromatic plants for determining how much should be collected from which plant in nature and how much should be cultivated. The cultivation of plants should be adopted by the farmers, the products grown by organization on the basis of cooperatives should be processed and marketed to the consumers as packaged. Herbalist who should actively participate in the marketing and who should make this profession should be legally determined. It will also be of great benefit to inform consumers about the correct and proper use of these plants through visual and written communication tools, to inform consumers, to raise awareness, and to their characteristics.

REFERENCES

- Akbulut, S., Özkan, Z.C., 2016. Herbalist-customer profile in medicinal and aromatic herbs trade: a case study of Kahramanmaraş, Turkey, Kastamonu Univ., Journal of Forestry Faculty, 16 (1): 246-252.
- Alpınar, K., 2010. Collection of medicinal plants used in public, Treatment Symposium with Plants, 5-6 June, İstanbul, Turkey. p.19-28.
- Arslan, N., Baydar, H., Kızıl, S., Karık, Ü., Şekercioğlu, N., Gümüştü, A., 2015. Changes in the production of medicinal plants and new seeking. The Chamber of Agricultural Engineers VIII. Technical Congress, 12-16 January, Ankara, Turkey. p. 483-507.
- Artukoğlu, M. M., Uzmay, A., Olgun, A., 2002. An evaluation of medicinal and aromatic plant trade in the World, in the EU and in Turkey. Agro-Food Industry Hi-Tech, 13(6):19-22.
- Artukoğlu, M. M., Uzmay, A., 2003. A research on domestic and foreign trade of medicinal and aromatic plants. Izmir Chamber of Commerce Publications, Izmir, Turkey. p. 42.
- Aydın, E., Yurum, Ç., Kevseroğlu, K., Seyis, F., 2014. Risk status of important medicinal and aromatic plants collected intensive from the nature and displaying a high market share, The Second Symposium of Medicinal and Aromatic Plants, 23–25 September, Yalova, Turkey. p. 281-286.
- Bayraktar, Ö.V., Öztürk, G., Arslan, D., 2017. Evaluation of the developments in production and marketing of some medicinal and aromatic plants in Turkey, Journal of Field Crops Central Research Institute, 26(2): 216-229.
- Bayram, E., Kırıcı, E., Tansi, S., Yılmaz, G., Arabacı, O., Kızıl, S., Telci, İ., 2010. Possibilities for increasing production of medicinal and aromatic plants, The Chamber of Agricultural Engineers VII. Technical Congress, 11-15 January, Ankara. p. 437-457.
- Bayramoğlu M., Toksoy, D., Şen, G., 2009. Medicinal plant trade in Turkey. II. Congress on Forestry Sociological Issues, 19-21 February, Isparta, Turkey. p.89-98.
- Çelik, Y., 2014. A research on the socio-economic structure of the herbalists who sell medical and aromatic plants in Konya province, Turkish Journal of Agricultural and Natural Sciences, 1(3):369-376.
- Dicle, M., 2010. A research on the determination of consumer behaviors of medical plants in the province of Bornova, Izmir, Master thesis, Ege University Institute of Graduate Studies in Science and Engineering, Izmir, Turkey. p. 89.
- Faydaoğlu, E., Sürücüoğlu, M.S., 2011. History of the use of medicinal and aromatic plants and their economic importance, Kastamonu Univ., Journal of Forestry Faculty, 11(1):52–67.
- Ghorbanpour, M., Hadian, J., Nikabadi, S., Varma, A., 2017. Importance of medicinal and aromatic plants in human life, In: Ghorbanpour M., Varma A. (eds) Medicinal Plants and Environmental Challenges. Springer, Cham. p.1-23.
- Kılıç, Ö., Özdemir, F.A., Yıldırım, Ş., 2017. Some aromatic and medicinal plants from Bingöl-Turkey, International Journal of Secondary Metabolite, 4(3):244-248.
- Kırıcı, S., 2015. General situation of medical and aromatic plants in Turkey. Journal of Turkey Seed Association, 15:4-11.
- Kızıl, S., Tonçer, Ö., 2005. The medicinal plants market and consumption of its potency in Diyarbakır, Turkey VI. Field Crops Congress, 5-9 September 2005, Antalya.
- Kızıloğlu, R., Kızılasslan, H., Eren, H.Z., 2017. Investigating consumer behaviors towards plants used for medicinal and aromatic purposes: The Case Of Kahramanmaraş City, Journal of Agricultural Faculty of Gaziosmanpaşa University, 34(3):27-35.
- Korkmaz M., Fakir, H., 2009. Determination of the ultimate consumer characteristics of nonwood forest products, Süleyman Demirel University, Journal of Forestry Faculty, 2(1):10-20.

- Newbold, P.,1995. Statistics for business and economics, Prentice-Hall, New Jersey. p.887.
- Özdemir, Y., Arslan, D., Özkan, M., 2014. Risk assessment of the medicinal and aromatic plants sector in terms of consumer security and food reliability in Turkey, II. Medical and Aromatic Plants Symposium, 23-25 September, Yalova, Turkey. p.157.
- Özgüven, M., Sekin, S., Gürbüz, B., Şekeroğlu, N., Ayanoğlu, F., Erken, S., 2005. Manufacture and trade of tobacco, medicinal and aromatic plants, The Chamber of Agricultural Engineers VI. Technical Congress, 3-7 January, Ankara, Turkey. p.481-501.
- Özkan, Z.C., Merev, N., Terzioğlu, S., Üçler, A.Ö., Gümüş, C., Toksoy, D., 2002. Recognition, training and evaluation of natural medical plants of Gümüşhane region, Project Final Report, Governorship of Gümüşhane, Gümüşhane, Turkey. p.102.
- Toksoy, D., Bayramoğlu, M., Hacisalihoğlu, S., 2010. Usage and the economic potential of the medicinal plants in Eastern Black Sea Region of Turkey, Journal of Environmental Biology, 31(5):623-628.
- TurkStat., 2014. Address Based Population Census Results -2013, Ankara, Turkey.
- Veziroğlu, P., Gültekin, U., 2018. Measuring perception and consumption level of agricultural engineering students for medicinal and aromatic plants: a case study for Cukurova University. Journal of Innovative Agriculture, 5(1):1-4.
- Zadeh, L. A., 1983. The role of fuzzy logic in the management of uncertainty in expert systems. Fuzzy sets and systems,11(1-3): 199-227.

CHEMICAL COMPOSITION OF SUMMER SAVORY (*Satureja hortensis*) ESSENTIAL OILS FROM EASTERN ANATOLIA REGION, TURKEY

Furkan ÇOBAN¹, Hakan ÖZER¹, Ufuk ATMACA²

¹Ataturk University, Faculty of Agriculture, Field Crop Departments 25040 Erzurum, Turkey

²Ataturk University, Oltu Vocational School, 25400 Erzurum, Turkey

Corresponding author email: furkan.coban@atauni.edu.tr

Abstract

Satureja hortensis, belonging to the family Lamiaceae, is an annual herbaceous plant, and have been widely used in foods as a flavour component and traditional medicine. Essential oil composition of *Satureja hortensis* can vary largely with different ecological conditions. Therefore, the present work reports the different chemical composition of essential oil from *Satureja hortensis* collected from Erzurum province, Turkey. The chemical composition of hydrodistilled essential oil of *Satureja hortensis* was analyzed by GC-MS. Thirty components representing 99.27 % of the oil were identified in the essential oil of aerial parts of *S. hortensis*. The main constituents of the oil were carvacrol (75.43%), γ -terpinene (5.41%), *p*-cymene (4.29%), and β -caryophyllene (3.04%). The results showed considerable differences compared to other studies on essential oil composition.

Keywords: Lamiaceae, *Satureja hortensis*, GC-MS, carvacrol

INTRODUCTION

Summer savory (*Satureja hortensis* L.) is medicinal and aromatic plant belonging to the family Lamiaceae, which its 50 species grow around the world. Within the 15 *Satureja* species growing in Turkey (Davis, 1982; Tumen et al., 1998).

Satureja hortensis, native to across southern and southeastern Europe, North Africa, Central and Middle East Asia (Govaerts et al., 2018), is an annual herb with slender, lightly hairy stems, elongate leaf, light purple and white flowers, and 30-35 cm high. It is extensively cultivated in Germany, France, Hungary and Spain for the production of essential oil (EO) (Omidbaigi and Hejazi 2004). Generally, colorless or yellowish color and very pungent EO is obtained from the leaves and flowers of *Satureja hortensis*.

The major components of *Satureja hortensis* L. EO include phenols, carvacrol, and thymol, as well as *p*-cymene, β -caryophyllene, γ -terpinene and other terpenes (Baser et al., 2004; Pfefferkorn et al., 2008; Alizadeh et al., 2010; Wesolowska et al., 2015; Lesjak et al., 2016; El-Leithy et al., 2017; Sharifi et al., 2018). The plant is used traditional medicine as a cure for diverse ailments such as indigestion, nausea, cramps, diarrhea (Ramezani et al., 2016) and shows anti-inflammatory (Hajhashemi et al., 2002), antibacterial (Deans et al., 1989; Karami-Osboo et al., 2010; Kotan et al., 2013; Afshar and Sedaghat 2016; Sharifi et al., 2018; Rasaee et al., 2018), antifungal (Gumus, 2010; Farzaneh et al., 2015; Sharifzadeh et al., 2016), antioxidant (Plander et al., 2012; Shojaee-Aliabadi et al., 2013; Moghadam, 2015; Najafian and Zahedifar 2015; Samsonowicz and Regulska, 2016; Yaldiz and Camlica, 2017) and insecticidal effects (Pavela et al., 2008; Tozlu et al., 2011).

The the results of these studies demonstrated that yield and chemical composition of EO's obtained from *Satureja hortensis* could vary according to climatic conditions and regions. In this study, we report on the content of the *Satureja hortensis* with a different composition than the samples examined earlier in the region.

MATERIAL AND METHODS

The aerial parts of *Satureja hortensis* plants were collected during the flowering season at an altitude of 1790 m on August 2016 from the Narman, Erzurum, Turkey, which is located at Irano-Turanian (East Anatolia) floristic regions (Fig. 1).

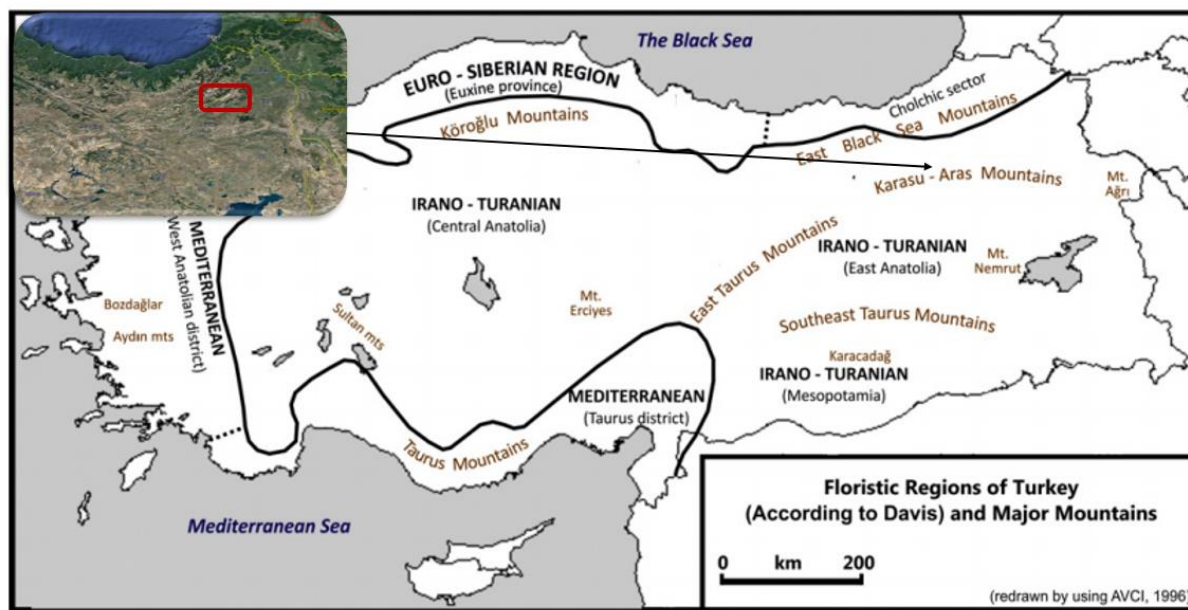


Fig. 1. Floristic regions and Major Mountains of Turkey (Avci 1996).

The taxonomic identification of plant material was confirmed by a senior plant taxonomist, Ali Kandemir, in Department of Biology, Erzincan University, Erzincan, Turkey. Plant material collected was dried in shadow and ground in a grinder with a 2 mm in diameter mesh.

The aerial parts of air-dried plant samples were subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours. The obtained essential oils were separated from water, dried over anhydrous sodium sulfate and after filtration, stored at +4 °C until tested and analyzed. The yields (1.40 % v/w) were averaged over three experiments, and calculated on relying on the dry weight of the plant material.

The GC analysis of the essential oil was performed using a Thermofinnigan Trace GC/A1300, (E.I) equipped with a SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 µm). Helium was the carrier gas, at a flow rate of 1 mL/min. Injector temperature was set at 220 °C. The programme used was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually and in the splitless mode. Quantitative data were obtained from FID area percentage data.

Also for GC-MS analyses of the EO's were carried out using a Thermofinnigan Trace GC/Trace DSQ /A1300, (E.I Quadrupole) (Thermo Finnigan, CA, USA) equipped with a SGE-BPX5 MS capillary column (Scientific Instrument Services Inc., NJ, USA) (30 m x 0.25 mm i.d., 0.25 µm). For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Helium, at a flow rate of 1 mL/min, was the carrier gas. Injection and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The programme used was 50-150 °C at a rate of 3 °C/min, held isothermal for 10 minutes and finally raised to 250 °C at 10 °C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 µL were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system and literature data. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2007).

RESULTS AND DISCUSSION

The essential oil yield (v/w, on dried mass basis) of *Satureja hortensis* was 1.40%, similar to those reported previously for Summer savory (Héjja et al., 2002; Sefidkon et al., 2006; Adiguzel et al., 2007; Hadian et al., 2010).

The results of the analysis of *S. hortensis* essential oils are given in Table 1. In summer savory EO, total of 30 compounds were identified, representing 99.27%. The oil consisted nearly particularly of monocyclic monoterpenes, with two main compounds carvacrol (75.43%) and γ -terpinene (5.46%) amounting up to 80.84%. The other prominent components of the oil were p-cymene (4.29%), β -caryophyllene (3.04%), β -bisabolene (2.30) and α -pinene (1.66) (Table 1).

Table 1. Chemical composition of the essential oil of *Satureja hortensis* growing in Eastern Anatolia Region of Turkey.

Constituents	RI*	Composition (%)	Method of identification
α -thujene	924	0.14	GC-MS- RI
α -pinene	932	1.66	GC-MS- RI
β -pinene	974	0.88	GC-MS- RI
myrecene	988	0.55	GC-MS- RI
α -terpinene	1014	0.65	GC-MS- RI
p-cymene	1020	4.29	GC-MS- RI
γ -terpinene	1054	5.41	GC-MS- RI
terpinolene	1086	0.10	GC-MS- RI
camphor	1141	0.32	GC-MS- RI
terpinen-4-ol	1174	0.99	GC-MS- RI
carvacrol methyl ether	1241	0.47	GC-MS- RI
thymol	1289	0.20	GC-MS- RI
carvacrol	1298	75.43	GC-MS- RI
thymol acetate	1349	0.15	MS- RI
α -copaene	1374	0.10	GC-MS- RI
β -bourbonene	1387	0.11	GC-MS- RI
β -caryophyllene	1417	3.04	GC-MS- RI
β -gurjunene	1431	0.10	GC-MS- RI
aromadendrene	1439	0.08	GC-MS- RI
cis- β -farnesene	1440	0.03	GC-MS- RI
α -humulene	1452	0.16	GC-MS- RI
γ -muurolene	1478	0.29	GC-MS- RI
germacrene D	1484	0.15	GC-MS- RI
vidiriflorene	1496	0.22	GC-MS- RI
α -muurolene	1500	0.16	GC-MS- RI
β -bisabolene	1505	2.30	GC-MS- RI
δ -Cadinene	1522	0.53	GC-MS- RI
α -Cadinene	1537	0.19	GC-MS- RI
spathulenol	1577	0.07	GC-MS- RI
caryophyllene oxide	1582	0.50	GC-MS- RI
Total		99.27	

*RI, retention index: Compounds listed in order of elution from a BPX5 MS column.

Previous research showed that carvacrol and γ -terpinene were usually the most ample constituents of *Satureja hortensis* EO's and our results are in agreement with the literature reports on the EO's of summer savory (Baser et al., 2004; Novak et al., 2006; Mihajilov-Krstev et al., 2009; Lesjak et al., 2016). As a difference γ -terpinene was found to be quite lower than Baser et al., 2004; Novak et al., 2006; Mihajilov-Krstev et al., 2009; Lesjak et al., 2016 studies but contrary to these studies carvacrol was found high amounts in our research. In addition, the material collected from the Eastern Anatolia region of the Turkey consist of thymol (40.54%) as the major component of the EO Adiguzel et al., (2007) reports, while in our research, the major component was carvacrol. These discrepancy may explained with its climatically and genetically factors. Because of this, yield and chemical composition of essential oil can vary among the populations of the same species from different localities. Eventually, our results are generally consistent with other literature but *Satureja hortensis* essential oil studies made in the same region showed considerable quantitative differences with respect to composition studies. This result shows that the chemical composition of *Satureja*

hortensis essential oil will show significant changes even in a narrow region. As a matter of fact, Davis (1982) reported that *Satureja hortensis* has a very substantial variation.

CONCLUSION

S. hortensis shows significant differences in phenological and morphological characteristics due to its existence in different environmental conditions, and this situation can also influence the essential oil composition of *S. hortensis*. The results from the present study supports the concept that *S. hortensis* can indicate considerable variations in essential oil composition due to varying ecological conditions. This information can be highly beneficial for essential oil industry and for researchers who want standard quality in aromatic plants.

REFERENCES

- Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publishing, Carol Stream, IL.
- Adiguzel, A., Ozer, H., Kilic, H., Cetin, B., 2007. Screening of antimicrobial activity of essential oil and methanol extract of *Satureja hortensis* on foodborne bacteria and fungi. Czech Journal of food sciences, 25 (2): 81.
- Afshar, P., Sedaghat, S., 2016. Bio-synthesis of silver nanoparticles using water extract of *Satureja hortensis* L and evaluation of the antibacterial properties. Current Nanoscience, 12 (1): 90-93.
- Alizadeh, A., Khoshkhui, M., Javidnia, K., Firuzi, O., Tafazoli, E., Khalighi, A., 2010. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran. Journal of Medicinal Plants Research, 4 (1): 033-040.
- Avci, M., 1996. The floristic regions of Turkey and a geographical approach for Anatolian diagonal. Review of the Department of Geography University of Istanbul, 3: 59-91.
- Baser, K.H.C., Ozek, T., Kirimer, N., Tumen, G., 2004. A comparative study of the essential oils of wild and cultivated *Satureja hortensis* L. Journal of Essential Oil Research, 16 (5): 422-424.
- Davis, P.H., 1982. Flora of Turkey and the East Aegean Islands, Vol 7. Edinburg University Press, Edinburgh, 319.
- Deans, S.G., Svoboda, K.P., 1989. Antibacterial activity of summer savory (*Satureja hortensis* L.) essential oil and its constituents. Journal of Horticultural Science, 64: 205-210.
- EL-Leithy, A. S., EL-Hanafy, S. H., Khatib, M. E., Ahmed, S. S., EL-Sayed, A. A. A., 2017. Effect of Nitrogen Fertilization Rates, Plant Spacing and Their Interaction on Essential Oil Percentage and Total Flavonoid Content of Summer Savory (*Satureja hortensis* L.) Plant. Egyptian Journal of Chemistry, 60 (5):805-816.
- Farzaneh, M., Kiani, H., Sharifi, R., Reisi, M., Hadian, J., 2015. Chemical composition and antifungal effects of three species of *Satureja* (*S. hortensis*, *S. spicigera*, and *S. khuzistanica*) essential oils on the main pathogens of strawberry fruit. Postharvest Biology and Technology, 109: 145-151.
- Govaerts, R., Dransfield, J., Zona, S., Hodel, D.R., Henderson, A., 2018. World Checklist of Lamiaceae. Facilitated by the Royal Botanic Gardens, Kew.
- Gumus, T., 2010. Determination of the changes of antifungal properties of *Satureja hortensis*, *Thymus vulgaris* and *Thymbra spicata* exposed to gamma irradiation. Radiation Physics and Chemistry, 79 (1): 109-114.
- Hadian, J., Ebrahimi, S.N., Salehi, P., 2010. Variability of morphological and phytochemical characteristics among *Satureja hortensis* L. accessions of Iran. Industrial Crops and Products, 32 (1): 62-69.
- Hajhashemi, V., Ghannadi, A., Pezeshkian, S. K., 2002. Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil. Journal of ethnopharmacology, 82 (2-3): 83-87.
- Hejja, M., Bernath, J., Szentgyörgyi, E., 2001. Comparative investigation of *Satureja hortensis* of different origin. In International Conference on Medicinal and Aromatic Plants. Possibilities and Limitations of Medicinal and Aromatic Plant 576: 65-68.
- Karami-Osboo, R., Khodaverdi, M., Ali-Akbari, F., 2010. Antibacterial effect of effective compounds of *Satureja hortensis* and *Thymus vulgaris* essential oils against *Erwinia amylovora*. Journal of Agricultural Science and Technology, 12: 35-45.
- Kotan, R., Dadasoğlu, F., Karagoz, K., Cakir, A., Ozer, H., Kordali, S., Cakmakci, R., Dikbas, N., 2013. Antibacterial activity of the essential oil and extracts of *Satureja hortensis* against plant pathogenic bacteria and their potential use as seed disinfectants. Scientia Horticulturae, 153: 34-41.
- Ladan Moghadam, A.R., 2015. Antioxidant Activity and Essential Oil Evaluation of *Satureja hortensis* L.(Lamiaceae) from Iran. Journal of Essential Oil Bearing Plants, 18(2): 455-459.
- Lesjak, M., Simin, N., Orcic, D., Franciskovic, M., Knezevic, P., Beara, I., Mimica-Dukic, N., 2016. Binary and tertiary mixtures of *Satureja hortensis* and *Origanum vulgare* essential oils as potent antimicrobial agents against *Helicobacter pylori*. Phytotherapy research, 30 (3): 476-484.
- Mihajilov-Krstev, T., Radnović, D., Kitić, D., Zlatković, B., Ristić, M., Branković, S., 2009. Chemical composition and antimicrobial activity of *Satureja hortensis* L. essential oil. Open Life Sciences, 4 (3): 411-416.

- Najafian, S., Zahedifar, M., 2015. Antioxidant activity and essential oil composition of *Satureja hortensis* L. as influenced by sulfur fertilizer. *Journal of the Science of Food and Agriculture*, 95 (12): 2404-2408.
- Novak, J., Bahoo, L., Mitteregger, U., Franz, C., 2006. Composition of individual essential oil glands of savory (*Satureja hortensis* L., Lamiaceae) from Syria. *Flavour and Fragrance Journal*, 21 (4): 731-734.
- Omidbaigi, R., Hejazi, M., 2004. Essential oil content and composition of *Satureja hortensis* of two different origins. *J. Essent. Oil Bearings. Plants*, 7 (2): 175-178.
- Pavela, R., Sajfirtová, M., Sovová, H., Bárnét, M., 2008. The insecticidal activity of *Satureja hortensis* L. extracts obtained by supercritical fluid extraction and traditional extraction techniques. *Applied entomology and zoology*, 43 (3): 377-382.
- Pfefferkorn, A., Krüger, H., Pank, F., 2008. Chemical composition of *Satureja hortensis* L essential oils depending on ontogenetic stage and season. *Journal of Essential Oil Research*, 20 (4): 303-305.
- Plánder, S., Gontaru, L., Blazics, B., Veres, K., Kéry, Á., Kareth, S., Simándi, B., 2012. Major antioxidant constituents from *Satureja hortensis* L. extracts obtained with different solvents. *European journal of lipid science and technology*, 114(7): 772-779.
- Ramezani, M., Ehtesham-Gharaee, M., Khazaie, M., & Behravan, J., 2016. *Satureja hortensis* L. Methanolic Extract and Essential Oil Exhibit Antitumor Activity. *Journal of Essential Oil Bearing Plants*, 19 (1): 148-154.
- Rasaee, I., Ghannadnia, M., Baghshahi, S., 2018. Biosynthesis of silver nanoparticles using leaf extract of *Satureja hortensis* treated with NaCl and its antibacterial properties. *Microporous and Mesoporous Materials*, 264: 240-247.
- Samsonowicz, M., Regulska, E., 2016. Evaluation of influence of selected metal cations on antioxidant activity of extracts from savory (*Satureja hortensis*). *Chemical Papers*, 70 (6): 811-819.
- Sefidkon, F., Abbasi, K., Khaniki, G.B., 2006. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. *Food chemistry*, 99 (1): 19-23.
- Sharifi, A., Mohammadzadeh, A., Zahraei Salehi, T., Mahmoodi, P. (2018). Antibacterial, antibiofilm and antiquorum sensing effects of *Thymus daenensis* and *Satureja hortensis* essential oils against *Staphylococcus aureus* isolates. *Journal of applied microbiology*, 124 (2): 379-388.
- Sharifzadeh, A., Khosravi, A.R., Ahmadian, S., 2016. Chemical composition and antifungal activity of *Satureja hortensis* L. essential oil against planktonic and biofilm growth of *Candida albicans* isolates from buccal lesions of HIV+ individuals. *Microbial pathogenesis*, 96: 1-9.
- Shojaee-Aliabadi, S., Hosseini, H., Mohammadifar, M.A., Mohammadi, A., Ghasemlou, M., Ojagh, S.M., Hosseini, S.M., Khaksar, R., 2013. Characterization of antioxidant-antimicrobial κ-carrageenan films containing *Satureja hortensis* essential oil. *International journal of biological macromolecules*, 52: 116-124.
- Tozlu, E., Cakir, A., Kordali, S., Tozlu, G., Ozer, H., Akcin, T.A., 2011. Chemical compositions and insecticidal effects of essential oils isolated from *Achillea gypsicola*, *Satureja hortensis*, *Origanum acutidens* and *Hypericum scabrum* against broadbean weevil (*Bruchus dentipes*). *Scientia Horticulturae*, 130 (1): 9-17.
- Tumen, G., Kirimer, N., Ermin, N., Baser, K.H.C., 1998. The essential oil of new *Satureja* species for Turkey, *S. pilosa* and *S. icaria*. *J. Essent Oil Res.* 10: 524-526.
- Wesolowska, A., Grzeszczuk, M., Jadczyk, D., 2015. Influence of distillation method on the content and composition of essential oil isolated from Summer Savory (*Satureja hortensis* L.). *Journal of Essential Oil Bearing Plants*, 18 (1): 215-221.
- Yaldiz, G., Camlica, M., 2017. Antioxidant Activities of *Satureja hortensis* L. Essential Oil during the Flowering Period. *Indian Journal of Pharmaceutical Education and Research*, 51 (3): 258-261.

ORGANIC PRODUCTION OPPORTUNITIES WITH NEWLY DEVELOPED BIOLOGICAL FERTILIZERS IN TURKISH TEA CLONE PAZAR-20

Ramazan ÇAKMAKÇI¹, Atefeh VARMAZYARI², Erdim ERDURAN²

¹Çanakkale University, Faculty of Agriculture, Department of Field Crops, Çanakkale, Turkey

²Atatürk University, Faculty of Agriculture, Department of Agronomy, Erzurum, Turkey,

Corresponding author email: rcakmakci@comu.edu.tr

Abstract

The objective of this study was to evaluate possible effects of mineral NPK fertilizer (800 kg ha⁻¹ a compound fertilizer 25:5:10%), and N₂-fixing and P-solubilizing bacteria based four bio-fertilizers formulations in dual and three strains combinations (BF1: *Bacillus megaterium* RC3D+ *Pseudomonas fluorescens* RC512, BF2: *Bacillus subtilis* RC521+ *Pseudomonas fluorescens* RC77; BF3: *Pantoea agglomerans* RC58 + *Bacillus megaterium* RC07 + *Pseudomonas fluorescens* RC77; BF4: *Bacillus megaterium* RC3D + *Bacillus subtilis* RC63+ *Pseudomonas fluorescens* RC512) on the growth, yield, and nutrient uptake in tea under acidic field conditions of Rize in three years. On average of three years, inoculation of tea with BF1, BF2, BF3 and BF4 bio-fertilizer gave increases over control respectively of by 11.2, 13.4, 20.4 and 17.5% in fresh leaf yield; by 10.1, 13.1, 15.5 and 12.7 in dry leaf yield, by 12.2, 14.2, 24.9 and 21.0% in leaf area and by 7.2, 7.9, 11.2 and 11.1% in chlorophyll contents, NPK applications, however, increased fresh leaf yield up to 25.4%, dry leaf yield by 22.3, leaf area by 27.7% and chlorophyll contents by 12.2%. The inoculation with the multi-traits rhizobacterial formulations and NPK fertilizer enhanced N, P, K, Ca, Mg, S, Fe, Cu and Mn uptake by tea plants, whereas only the BF4 and NPK increased the Mo content of tea leaf. Biological fertilizer applications gave approximately equal results to the conventional production system in which mineral fertilizer was used and did not cause a significant decrease in tea growth, nutrition and yield. These formulations tested in this study has a potential to be used as a bio-fertilizer in sustainable and organic tea production.

Keywords: Tea plant, organic farming, bio-fertilizers, mixed inoculations

INTRODUCTION

Tea, a well-known important ancient beverage crop, is consumed as a drink and cultivated all over the world for its commercial value and beneficial effects. Tea (*Camellia sinensis* L.) is consumed by over two-thirds of the world's population. It has an attractive aroma, good taste, health-promoting effects, and these benefits together make it one of the oldest and most popular drinks in the world (Kottur et al., 2011). Tea the most important plant of Turkey is used in the traditional preparation of its national food and is planted widely on acidic soils. Currently, Turkey is one of the most important tea producing countries, ranking 5th places in the world after China, India, Kenya and Sri Lanka with annually 200.000 tons of dry tea production. Turkey's tea growing area is only located at the very mountainous eastern end of the Black Sea coast, centered on the town of Rize and Trabzon. The soils of tea land in Turkey are also generally acidic condition. Tea gardens are usually grown as a monoculture and receive considerable amounts of fertilization, root exudates and leaf litter (Çakmakçı et al., 2010).

Tea is a perennial leaf crop which requires more nitrogen than most other crops and N application significantly increases both the yield and quality of tea (Han et al., 2008; Çakmakçı 2016). In the cultivation of tea, compared with other crops, large amounts of N have been applied as fertilizer, resulting in an increase in the nitrate (NO₃)-N concentrations in the water systems (Hirono et al., 2009). Many studies showed that excess amounts of mineral fertilizer application can cause tea orchard soil acidification (Han et al., 2008), groundwater and surface water pollution (Liu et al., 2012), affect nitrification rates (Xue et al., 2006), contribute in low N use efficiency and also cause serious environmental pollution (Han et al., 2008), and was responsible for altering the soil quality and soil microbial populations (Nath et al., 2013; Bhatt et al., 2012). Soil pH in tea orchards generally continues to decrease with the increase in cultivation age. Soil becomes strongly acidified following

the planting of tea and soil pH generally continues to decrease with the increase in stand age and tea productivity. Long-term tea cultivation resulted in the accumulation of soil nutrition content. Also, phosphorus-deficiency is frequently observed in tea plantations and inhibits photosynthesis in tea (Salehi and Hajiboland, 2008). Phosphorus (P) is one of the main limiting factors to the productivity of tea plants and P utilization efficiency is very low in the soil due to its low solubility and mobility. Undoubtedly, excess use of chemical fertilizers has resulted in several problems including persistence of chemicals in plant products and adverse impact on the soil environment, which in turn leads to loss in productivity (Chakraborty et al., 2012). The intensive use of chemical fertilizers creates a highly selective environment and adversely affects microbial diversity. Tea orchard ecosystems are very particular compared with other agriculture ecosystems. Long-term heavy N application, root exudates and leaf litter can result in the decline of pH and the accumulation of Al toxicity and antimicrobial substances. Also, chemical farming practice has brought drastic changes in soil ecology, crop productivity and quality, in tea farming system. To combat such adverse consequences and regain of lost resources, organic farming practice and utilization of PGPB as biofertilizers has emerged as an alternative for providing plant nutrients to increase plant yield and quality in sustainable agroecosystems.

The soil microbial community plays an important role in the decomposition of soil organic matter and elemental cycling. Microbial diversity has also been recommended as a biological indicator of soil quality. The fact that microbes are essential for the entire tea ecosystem since they perform numerous important functions like maintenance of biogeochemical cycles, promotion of plant growth; and inhibition of destructive tea pest and pathogens. Growth promoting substances are produced in large quantities by the beneficial microorganisms that influence indirectly on the overall morphology of the plants. Also soil microorganisms are reported to influence the physical, chemical and biological properties of soil either directly or indirectly. Soil quality declined and tea growth was inhibited under long-term continuous cropping tea orchard systems. While the number of bacteria decreases over time in the conventional system, it increases with time in the organic system.

In this regard the use of PGPR is seen as an important approach in agriculture. The use of beneficial bacteria as agricultural inputs for increasing crop production needs the selection of competent rhizobacteria with plant growth promoting attributes. PGPR are reported to influence the growth, yield, and nutrient uptake by an array of mechanisms. The use of PGPR may have more important in plants that their leaves used. As well-known only leaves are used in processing in tea and therefore these kinds of bacteria can be more important in sustainable and organic tea production. However, information about the characteristics of composition and diversity of the bacterial community in tea plantations soil ecosystems is scarce. Also, information on use of bio-fertilizers in tea is very scanty and there is no systematic study to see whether these can be used in tea field with advantage (Çakmakçı et al., 2011; Saikia et al., 2011). Therefore, this study was conducted in order to investigate the effect of different bacteria-based biofertilizer formulations on the growth, leaf yield, nutrient uptake, and chlorophyll and anthocyanin contents in the leaves of tea.

MATERIAL AND METHODS

The objective of this study was to evaluate possible effects of mineral NPK fertilizer (800 kg ha⁻¹ year⁻¹ in compound NPK 25-5-10% fertilizers) and indole acetic acid (IAA)-producing, N₂-fixing and P-solubilizing bacteria-based four bio-formulations in dual and three strains combinations combinations (BF1: *Bacillus megaterium* RC3D+ *Pseudomonas fluorescens* RC512, BF2: *Bacillus subtilis* RC521+ *Pseudomonas fluorescens* RC77; BF3: *Pantoea agglomerans* RC58 + *Bacillus megaterium* RC07 + *Pseudomonas fluorescens* RC77; BF4: *Bacillus megaterium* RC3D + *Bacillus subtilis* RC63 + *Pseudomonas fluorescens* RC512) on growth, yield, chlorophyll (SPAD) and anthocyanin (ACI) content, leaf area and N,P, and K content in 12 years old Fener-3 tea clone under field soil conditions, by conducting field experiments over three years in the Hayrat Trial Station of the Atatürk Tea and Horticultural Research Institute in Rize. Sources and some biochemical characteristics of the bacterial strains used in the bio-formulations are given in Table 1. The

experiment was arranged as a completely randomized design with six treatments and three replicates (each having six tea bushes). For this experiment, pure cultures were grown in 50% strength tryptic soy broth on a rotary shaker (120 rpm; 25 °C) for 3 days. Bacteria were then harvested by centrifugation (ca. 3000 x g for 10 min), washed and re-suspended in 10 mM sterile phosphate buffer, pH 7 to a density of 10^9 cfu ml⁻¹ for the bacterial strains. For triple inoculation, equal volume (10^9 cfu ml⁻¹ of each inoculant) of three cultures were mixed and then used for tea bushes. Bacterial suspension was injected into each uniform twelve-year old saplings root zone at the time of fertilizer application.

Table 1. Sources, N₂-fixation, P-solubilization, and IAA-production ability of the bacterial strains tested.

Formulations	Bacterial strains	Sources	Nitrogenase activity (nmol C ₂ H ₄ , 10 ⁷ cfu h ⁻¹)	P-solubilization (µg P ml ⁻¹ d ⁻¹)	IAA production (µg mL ⁻¹ (OD ₆₀₀ Unit ⁻¹))
BF1	<i>Bacillus megaterium</i> RC3D	Rye	0.76.7± 0.17	64.7 ± 2.8	27.8 ± 2.5
	<i>Pseudomonas fluorescens</i> RC512	Grapevine	0.78 ± 0.13	40.6 ± 1.5	31.6 ± 2.3
BF2	<i>Bacillus subtilis</i> RC521	Wild red raspberries	0.86 ± 0.14	124.7 ± 6.18	41.4 ± 2.5
	<i>Pseudomonas fluorescens</i> RC77	Wild red raspberries	0.79 ± 0.15	39.4 ± 1.7	32.4 ± 2.9
BF3	<i>Pantoea agglomerans</i> RC58	Grapevine	0.84 ± 0.21	48.6 ± 1.87	ND
	<i>Bacillus megaterium</i> RC07	Wheat	0.18 ± 0.04	36.4 ± 1.5	25.3 ± 1.7
	<i>Pseudomonas fluorescens</i> RC77	Wild red raspberries	0.79 ± 0.15	39.4 ± 1.7	32.4 ± 2.9
BF4	<i>Bacillus megaterium</i> RC3D	Rye	0.76.7± 0.17	64.7 ± 2.8	27.8 ± 2.5
	<i>Bacillus subtilis</i> RC63	Wild red raspberries	0.74 ± 0.17	34.6 ± 0.08	29.7 ± 1.9
	<i>Pseudomonas fluorescens</i> RC512	Grapevine	0.78 ± 0.13	40.6 ± 1.5	31.6 ± 2.3

Tea leaves were picked three times between spring and fall of each year. The tea flush (two leaves and one bud) were harvested every three years during three consecutive shooting periods, in the second week of May (first flush), in the third week of July (second flush) and in the third week of September (third flush). Leaf samples were oven-dried at 68 °C for 48 h and ground to pass 1 mm. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Königswinter, Germany) were used to determine total N the tea leaves. After extraction methods, macro- and micro-elements were determined with an inductively Coupled Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham MA, USA). Chlorophyll contents and anthocyanin content index (ACI) of the top two and third leaves were measured using a chlorophyll meter (SPAD-502, Minolta, Japan) and anthocyanin content meter (ACM-200 plus Anthocyanin Content Meter, Opti-Sciences, Inc. USA).

RESULTS AND DISCUSSION

Three years of field trials showed that treatments including bacterial formulations and fertilizer application significantly affected the parameters investigated compared to the control in tea depending on the years, bacterial formulations and growth parameter evaluated (Table 2). In the first year, inoculation with BF1, BF2, BF3, and BF4 increased fresh leaf weight over control respectively by 15.1, 18.2, 22.9, and 19.3%, dry leaf weight by 9.2, 13.9, 15.6, and 12.2%. Mineral fertilizer applications, however, corresponded to increases fresh and dry leaf weight respectively of 26.4% and 23.1%. Second year dual inoculation and mixture of three bacteria gave increases fresh weight by 11.5-24.3%, and dry leaf weight by 12.8-18.1% over control as compared with 28.9 and 23.9% fresh and dry leaf weight increases by NPK application. Three year's data suggest that bacterial formulations inoculation of tea bushes with BF1, BF2, BF3 and BF4 increased fresh leaf yield by 11.3, 13.5, 20.5 and 17.5% as compared to the control and dry leaf yield by 10.1, 13.1, 15.5 and 12.6%, respectively. NPK application increased fresh and dry leaf yield up to 25.2 and 22.3%.

Table 2. Effect of different combinations of bacteria and fertilizer applications on leaf yield at different harvesting periods of tea cv. Fener-3 in the field experiment during the 2013–2015 seasons.

Treatment*	First harvest**		Second harvest		Third harvest		Sum of three distinct harvests period	
	Fresh leaf weight (g/tea bushes)	Dry leaf weight (g/tea bushes)	Fresh leaf weight (g/tea bushes)	Dry leaf weight (g/tea bushes)	Fresh leaf weight (g/tea bushes)	Dry leaf weight (g/tea bushes)	Fresh leaf weight (g/tea bushes)	Dry leaf weight (g/tea bushes)
First year								
Control	258 b	128 b	278 b	141 b	159 b	79 b	694 b	347 b
NPK	332 a	159 a	347 a	169 a	198 a	99 a	877 a	427 a
BF1	298 a	136 a	322 a	153 a	179 a	90 a	799 a	379 a
BF2	304 a	141 a	329 a	161 a	188 a	94 a	820 a	395 a
BF3	307 a	137 a	344 a	163 a	202 a	101 a	853 a	401 a
BF4	310 a	139 a	326 a	154 a	192 a	96 a	828 a	389 a
Second year								
Control	273 d	130 c	259 d	125 c	181 d	88 c	713 d	343 c
NPK	358 ab	166 a	329 ab	152 ab	232 b	107 ab	919 ab	425 a
BF1	302 c	145 b	290 c	143 b	203 c	99 b	795 c	387 b
BF2	307 c	147 b	294 c	144 b	206 c	101 b	807 c	392 b
BF3	335 a	150 a	321 a	148 a	240 a	107 a	886 a	405 a
BF4	329 b	149 a	317 b	144 b	229 b	105 ab	875 b	398 ab
Third year								
Control	307 d	137 d	298 d	138 c	194 c	91 c	799 c	365 c
NPK	377 a	171 a	357 a	160 a	232 ab	107 a	967 a	438 a
BF1	325 c	150 c	316 c	141 b	220 b	99 b	861 b	396 b
BF2	329 c	150 c	320 c	152 b	227 b	105 ab	876 b	406 b
BF3	337 ab	154 ab	344 a	155 ab	237 a	104 ab	918 a	413 ab
BF4	335 bc	151 c	328 b	149 b	226 b	102 b	889 ab	402 b

*Control: without bacteria inoculation or mineral fertilizers; mineral NPK fertilizer (800 kg ha⁻¹ a compound fertilizer 25:5:10%), and N₂-fixing and P-solubilizing bacteria based four bio-fertilizers formulations in dual and three strains combinations ((BF1: *Bacillus megaterium* RC3D+ *Pseudomonas fluorescens* RC512, BF2: *Bacillus subtilis* RC521+ *Pseudomonas fluorescens* RC77; BF3: *Pantoea agglomerans* RC58 + *Bacillus megaterium* RC07 + *Pseudomonas fluorescens* RC77; BF4: *Bacillus megaterium* RC3D + *Bacillus subtilis* RC63 + *Pseudomonas fluorescens* RC512),

**Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.05$)

Table 3. Effects of The effect of different combinations of bacteria and fertilizer applications on the chlorophyll (SPAD) and anthocyanin (ACI) content, leaf area, N, P and K content in leaves of tea

Treatments	Chlorophyll contents (SPAD)	Anthocyanin (ACI) contents	Second leaf area (cm ²)	Third leaf area (cm ²)	N (%)	P (g/kg)	K (g/kg)
Second year							
Control	70.9 c	26.9 b	11.5 c	19.8 c	2.18 c	2.41 d	16.3 d
NPK	77.6 a	29.7 a	14.7 a	24.9 a	2.99 a	3.92 b	24.8 c
BF1	73.5 b	26.9 b	12.9 b	22.1 b	2.78 b	3.06 b	22.6 c
BF2	73.9 b	27.8 ab	13.0 b	22.4 b	2.82 b	3.12 c	22.4 c
BF3	77.4 a	29.4 a	14.4 a	24.2 a	3.06 a	4.48 a	27.6 b
BF4	75.8 ab	28.8 ab	14.3 a	24.0 ab	2.95 a	4.06 b	33.9 a
Third year							
Control	72.18 c	26.21 c	12.62 c	21.07 c	2.38 c	2.93 c	20.2 c
NPK	82.83 a	30.12 ab	16.56 a	26.85 a	2.98 ab	3.90 ab	26.0 ab
BF1	79.86 b	29.13 b	13.98 b	23.96 b	2.82 b	3.40 bc	24.6 b
BF2	80.48 b	29.46 b	14.18 b	24.63 b	2.80 b	3.43 bc	24.1 b
BF3	84.76 a	31.72 a	16.14 a	26.41 a	3.04 a	4.09 a	27.2 a
BF4	83.79 a	30.16 ab	15.27 ab	25.07 b	2.87 ab	3.77 ab	27.1 a

*Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($P \leq 0.05$)

On average of two years results, application of tea with BF1, BF2, BF3, and BF4 bio-fertilizer gave increases over control respectively of by %7.2, 7.9, 13.3, and 11.5% in Chlorophyll contents (SPAD), by 5.5, 7.8, 15.1, and 11.0% in anthocyanin (ACI) content and by 11.4, 12.7, 26.6, and 22.6% in second leaf area (Table 3). NPK applications, however, increased Chlorophyll contents

(SPAD) up to 2.2%, anthocyanin (ACI) content by 12.6 and second leaf area by 29.6. As an average of two years, mixed inoculations of tea with BF1, BF2, BF3, and BF4 increased N content of tea leaf by 22.8, 23.2, 33.8, and 27.6% as compared to the control. NPK applications, however, increased leaf N content up to 30.9%.

The study clearly indicates that development of stable formulation of PGPR is of great importance and is a promising approach to a sustainable agriculture. Bacterial formulations caused high growth, leaf weight and other parameters tested, but it was strongly dependent on the inoculant strain formulations and parameters evaluated. Among the bacterial formulations, the best treatment was the combination of all three strains, followed by dual inoculations. Mixed inoculations were much more effective than dual inoculations. Of the effective bacterial formulations tested consistently gave growth and yields of tea equal to chemical NPK fertilizers applied. The highest values of leaf weight was obtained from the application of NPK in the first harvest. On the other hand, efficiency of different bacteria gets closer to mineral fertilizer application in the second harvest. In this case, the mineral fertilizer efficiency decreases with time, whereas the bacterial activity becomes longer and more persistent. Inoculation of effective bacteria with multiple traits could significantly increase the nutrient use efficiency and improve tea growth and possibly reduce environmental pollution due to leaching during periods with excess rain in the area.

Inoculation with rhizobacteria-based bio-formulations stimulated overall plant growth, including leaf yield, chlorophyll and anthocyanin content, N, P and K content of Turkish tea clones Pazar-20. As reported previously, the effect of PGPR is a complex process, and depends on the bacterial strain and population, on the plant-bacterial strain combination, the plant genotype, the growth parameters evaluated, and environmental conditions (Şahin et al. 2004; Çakmakçı et al., 2006). Previous studies demonstrated the application of PGPR in soil have resulted in significant increase in growth of young tea bushes and help in the reduction of the use of chemicals in tea plantations (Chakraborty et al., 2006, 2012; Çakmakçı et al., 2013, 2014, 2015; Zhan et al., 2016; Çakmakçı, 2016).

The application of NPK fertilisers, three strains combinations of BF3 and BF4 formulation increased the chlorophyll and anthocyanin content in tea leaves, and enhanced the growth and yield parameters of tea. Chlorophyll, the main component of the colour in green tea, may influence the net photosynthesis rate and the tea quality. Anthocyanin are responsible for leaf color, antioxidant activity, physiological and biochemical processes. Chlorophyll is a highly important pigment as its amount determines the final colour of non-fermented green tea infusion. Fermentation process, transforms chlorophylls into pheophorbides and pheophytins, which give rise to the dark colour of black tea as reported by Ošťádalová et al. (2014), who suggested that chlorophylls may be an important stable indicator of the tea quality. Increasing contents of chlorophylls and anthocyanin would contribute to the production of quality teas, which was in agreement with result of Joshi et al. (2015), who reported that tea from anthocyanin-rich cultivars could be specialty teas with high antioxidant activity.

CONCLUSION

Field trials indicate that a higher yield potential can be expected from acidic soils with PGPR inoculation. In particular, newly IAA-producing, N₂-fixing, and P-solubilizing acid-tolerant bacteria based formulation tested BF3 (*Pantoea agglomerans* RC58 + *Bacillus megaterium* RC07 + *Pseudomonas fluorescens* RC77) and BF4 (*Bacillus megaterium* RC3D + *Bacillus subtilis* RC63 + *Pseudomonas fluorescens* RC512) stimulated overall plant growth, including shoot development and leaf yield, improving N, P and K nutrient uptake, and chlorophyll and anthocyanin content of tea plant. The use and application of such bioformulations can result in the reduction of application of harmful chemicals, protect the environment and biological resources and prevent the accumulation of nitrates and phosphates in agricultural soils. Screening of rhizobacteria that show multiple PGP traits suggests that they have better potential for field testing and applications in improving the growth of tea.

ACKNOWLEDGEMENTS

We are very grateful to Scientific and Technological Research Council of Turkey (TOVAG; 112 O 313) for their generous financial support for our study and would like to thank the Ataturk Tea Garden Cultures Research Institute.

REFERENCES

- Bhatt, B., Chandra, R., Ram, S., 2012. Long-term application of fertilizer and manure on rice productivity and soil biological properties. *International Journal of Agriculture Environment & Biotechnology*, 5, 429-433.
- Bremner, J. M. 1996. Nitrogen-total. In *Methods of soil analysis Part 3: Chemical methods*, eds. J. M. Bartels, and J. M. Bigham, 1085–121. Madison, Wisconsin: SSSA-AS Agronomy.
- Çakmakçı, R., 2016. Screening of multi-trait rhizobacteria for improving the growth, enzyme activities, and nutrient uptake of tea (*Camellia sinensis*). *Communications in Soil Science and Plant Analysis*, 47: 1680–1690.
- Çakmakçı, R., Dönmez, F., Aydın, A., Şahin, F., 2006. Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology and Biochemistry*, 38: 1482-1487.
- Çakmakçı, R., Dönmez, M. F., Ertürk, Y., Erat, M., Haznedar, A., Sekban, R., 2010. Diversity and metabolic potential of culturable bacteria from the rhizosphere of Turkish tea grown in acidic soils. *Plant and Soil*, 332, 299-318.
- Çakmakçı, R., Ertürk, Y., Atasever, A., Ercişli, S., Şentürk, M., Erat, M., Haznedar, A., Sekban, R., 2011. The use of plant growth promoting rhizobacteria for organic tea production in Turkey. *Proceedings of Tea-Organic-Low Carbon International Symposium*. 6-9 June, pp. 89-97, Guangyuan, China.
- Çakmakçı, R., Ertürk, Y., Sekban, R., Haznedar, A., Varmazyari, A., 2013. The effect of single and mixed cultures of plant growth promoting bacteria and mineral fertilizers on tea (*Camellia Sinensis*) growth, yield and nutrient uptake. *Soil Water Journal, Special Issue for AGRICASIA 2 (1)*: 653-662.
- Çakmakçı, R., Ertürk, Y., Atasever, A., Kotan, R., Erat, M., Varmazyari, A., Türkyılmaz, K., Haznedar, A., Sekban, R., 2014. Development of plant growth-promoting bacterial based bioformulations using solid and liquid carriers and evaluation of their influence on growth parameters of tea. In: *9th International Soil Science Congress on the Soul of the Soil and Civilization*, 14-16 October 2014, Side, 801-808.
- Çakmakçı, R., Ertürk, Y., Varmazyari, A., Atasever, A., Kotan, R., Erat, M., Türkyılmaz, K., Sekban, R., Haznedar, A., 2015. The effect of mixed cultures of plant growth promoting bacteria and mineral fertilizers on tea (*Camellia sinensis* L.) growth, yield, nutrient uptake, and enzyme activities. *International Soil Science Congress “Soil Science in International Year of Soils 2015*, 19-23 October, Sochi /Russia. Article Book, 67-71.
- Chakraborty, U., Chakraborty, B., Basnet, M., 2006. Plant growth promotion and induction of resistance in *Camellia sinensis* by *Bacillus megaterium*. *Journal of Basic Microbiology*, 46: 186-195.
- Chakraborty, U., B. N. Chakraborty, and A. P. Chakraborty. 2012. Induction of plant growth promotion in *Camellia sinensis* by *Bacillus megaterium* and its bioformulations. *World Journal of Agricultural Sciences*, 8:104–12.
- Han, W.-Y., L.-F. Ma, Y.-Z. Shi, J.-Y. Ruan, and S. J. Kemmitt. 2008. Nitrogen release dynamics and transformation of slow release fertiliser products and their effects on tea yield and quality. *Journal of the Science of Food and Agriculture*, 88:839–846.
- Hirono, Y., Watanabe, I. Nonaka, K., 2009. Trends in water quality around an intensive tea-growing area in Shizuoka, Japan. *Soil Science and Plant Nutrition*, 55:783–92.
- Joshi, R., Rana, A., Gulati, A., 2015. Studies on quality of orthodox teas made from anthocyanin-rich tea clones growing in Kangra valley, India. *Food Chemistry*, 176: 357-366.
- Kottur, G., S. Venkatesan, R. S. S. Kumar, and S. Murugesan. 2011. Impact of genotype, seasons and manufacturing process on the activities of peptidase and lipoxygenase in tea. *European Food Research and Technology*, 232:335–341.
- Liu, Z., Yang, J., Yang, Z., Zou, J., 2012. Effects of rainfall and fertilizer types on nitrogen and phosphorus concentrations in surface runoff from subtropical tea fields in Zhejiang, China. *Nutrient Cycling in Agroecosystems*, 93:297–307.
- Nath, R., Sharma, G.D., Barooah, M., 2013. Screening of endophytic bacterial isolates of tea (*Camellia sinensis* L.) roots for their multiple plant growth promoting activities. *International Journal of Agriculture Environment & Biotechnology*, 6: 211-215.
- Ošťálová, M., Tremlová, B., Pokorná, J., Král, M., 2014. Chlorophyll as an indicator of green tea quality. *Acta Veterinaria Brno*, 83: 103-109.
- Şahin, F., Çakmakçı, R., Kantar, F., 2004. Sugar beet and barley yields in relation to inoculation with N₂-fixing and phosphate solubilizing bacteria. *Plant and Soil*, 265:123-129.
- Saikia, D. N., Sarma, J., Dutta, P. K., Baruah, D. K., 2011. Sustainable productivity of tea through conservation of biomass, addition of bio-fertilizers and reduction of inorganic fertilizer. *Two and a Bud*, 58: 109-117.
- Salehi, S.Y., Hajiboland, R., 2008. A high internal phosphorus use efficiency in tea (*Camellia sinensis* L.) plants. *Asian Journal of Plant Sciences*, 7:30–36.
- Xue, D., Yao, H., Huang, C. Y., 2006. Microbial biomass, N mineralization and nitrification, enzyme activities, and microbial community diversity in tea orchard soils. *Plant and Soil*, 288: 319-331.

MORPHOLOGICAL AND YIELD EVALUATION OF FENNEL (*Foeniculum vulgare* L.) POPULATIONS OF DIFFERENT ORIGIN

Mahmut ÇAMLICA¹, Gülsüm YALDIZ¹

¹Department of Field Crops, Faculty of Agriculture and Natural Sciences, Bolu Abant İzzet Baysal University, Bolu, Turkey

Corresponding author email: mcamlica25@outlook.com

Abstract

Fennel (*Foeniculum vulgare* L.) is one of the most valuable plant among the medicinal and aromatic plants as coriander, cumin and dill cultured in the world and also in Turkey. In this study, five local fennel populations obtained from different provinces (Aydın, Erzurum, Burdur, Eskişehir, Denizli) for control and 43 fennel populations which were obtained from USDA (United States Department of Agriculture) were used as material. This experiment was conducted according to Augmented trial design to determine agricultural properties of these populations. 32 fennel populations adapted to Bolu ecological conditions among these 43 populations. As a result of the study, seedling emergence, plant height, number of branche per plant, number of umbel and umbellate number were found between 9.8-20.6 days, 39.22-104.64 cm, 3.76-8.50 number/plant, 0.78-14.43 umbel number/plant and 6.80-22.43 umbellate number/plant, respectively. Average values for other the characters were found; days to flowering time is 56.1-115.1 days, days to fruit-linking time is 102.55-140.35 days, number of seed per plant is 7.83-1248.44, weight of the thousand seed is 1.92-8.14 g, seed yield is 0.15-8.85 g/plant. The highest seed yield and number of seed were found in Ames 30290 (Tunisia population). Also, the lowest seed yield and the lowest number of seed per plant were seen from PI 288283 (India population).

Keywords: Fennel, *Foeniculum vulgare*, yield, Bolu.

INTRODUCTION

Fennel (*Foeniculum vulgare* L.) is an annual, biennial or perennial plant in Mediterranean basin as typical, well-known medicinal and aromatic plant belonging to the Apiaceae family. Origin of fennel is Mediterranean region and West Asia and it has spread to many parts of the world in ways (Ceylan, 1997). It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits (Rather et al., 2012). The seeds of fennel have an active substance, which is called essential oil and most important constituent is anethole (Miraldi, 1999; Sephidkon, 2001). Mature fruits and essential oil of fennel are used as flavoring agents in food products such as liqueurs, bread, pickle, pastries and cheeses (Zoubiri et al., 2014).

The fennel has been used as spices and vegetables especially in the Mediterranean countries since ancient times. Fennel grows in Northern Anatolia region, naturally. It can be used as tranquillizer, tonic and soporific drug in the Turkish folk medicine (Davis, 1978; Asimgal, 1997; Baytop, 1999).

Yield and quality values can change according to genotypes and environmental conditions. For this reason, it has become necessary to develop the most appropriate varieties in terms of efficiency and quality against the changing environmental conditions. The new variant for a breeding area is that it will not fall below average yield even in poor environmental conditions, it must have the stability that it will give high yield (Özgen, 1994).

This study was carried out to determine the performance of the fennel populations obtained from different countries of the world and Turkey in the ecological conditions of Bolu representing West Black Sea Region.

MATERIAL AND METHODS

The present experiment was carried out during growing season in 2017 at Bolu Abant İzzet Baysal University, Bolu. The field experimental site was located at research and application area of Agriculture and Natural Sciences Faculty, is between 40° 44' 45" N latitude, 31° 37' 46" E longitudes with altitude of 752 m. 43 fennel populations seeds were obtained from USDA and 5 fennel populations were obtained from different provinces (Erzurum, Aydın, Denizli, Kırşehir and Burdur) of Turkey as a control. Average climatic data were recorded 16.08 °C temperature; 41.37 kg/m² rainfall; 69.2% humidity during the vegetation period for 2017 (Anonymous, 2018). Experimental area soils are clay-loam with a pH value of 7.8, organic matter content of 4.7%, phosphorus ratio of 10.3 ppm and potassium ratio of 235 ppm (Anonymous, 2015).

Table 1. Information about fennel populations used in the study.

No	Plant Accession	Origin Country	No	Plant Accession	Origin Country
1	Ames 20029	Ukraine	25	PI 601795	U.S., California
2	Ames 23130	Italy	26	PI 649460	Italy, Lotium
3	Ames 27588	Italy	27	PI 649463	China, Shanxi
4	Ames 30289	Tunisia, Stax	28	PI 649464	China, Guangxi
5	Ames 30290	Tunisia, Stax	29	PI 649465	Uzbekistan
6	Ames 30693	U.S., Oregon	30	PI 649466	France, Loire-Atlantique
7	Ames 7551	U.S., Illinois	31	PI 649469	Syria
8	NSL 6409	United States, California	32	PI 649470	China, Yunnan
9	PI 172898	Turkey, Mardin	33	PI 649471	Morocco
10	PI 174212	Turkey, Şanlıurfa	34	Bucak pop.	Turkey, Burdur*
11	PI 174213	Turkey, Şanlıurfa	35	Denizli pop.	Turkey, Denizli*
12	PI 194892	Ethiopia	36	Erzurum pop.	Turkey, Erzurum*
13	PI 251085	Former Serbia and Montenegro	37	Eskişehir pop.	Turkey, Eskişehir*
14	PI 273659	Ethiopia	38	Nazilli pop.	Turkey, Aydın*
15	PI 273660	Ethiopia, Harer	39	PI 649467	France, Morbihan
16	PI 288283	India, Uttar Pradesh	40	Ames 7550	Italy
17	PI 288285	India, Rajasthan	41	Ames 24365	Former Serbia and Montenegro
18	PI 288477	India	42	Ames 21654	Unknown
19	PI 358460	Macedonia	43	PI 268383	Afghanistan, Kabul
20	PI 414189	Egypt, Cairo	44	PI 649472	U.S., Pennsylvania
21	PI 414190	U.S., Maryland	45	PI 325869	Spain, Madrid
22	PI 273660	Ethiopia, Harer	46	Ames 27589	Italy
23	PI 414191	U.S., Maryland	47	Ames 27419	Uzbekistan
24	PI 414192	U.S.	48	NSL 92013	U.S., New York

*Control populations

These population seeds were sown (17 April 2017) with Augmented block design having four blocks with sixteen entries in each block and having block size of 7.2 mx3.0 m with spacing of 45 cm. In the experiment, 6 kg/da diammonium phosphate (DAP) as base fertilizer and 4 kg/da ammonium nitrate (AN) as top fertilizer were applied. All recommended agronomic practices were followed timely for successful raising the crop. Ten competitive plants from each row were randomly selected and morphological and yield properties were taken for recording of data pertaining to fennel populations from these plants. The experiment harvest was started on 20 August and the final harvest made on 7 September. The first harvest was made in PI 288283 population, the last harvest was made Denizli and PI 194892 populations.

The data were analyzed using XLSTAT statistic software program and obtained results were subjected to analysis of variance and Least Significant Difference Test (LSD) to find differences among the fennel populations and controls at p=0.01.

RESULTS AND DISCUSSION

In the experimental year, 32 fennel populations which include 15 different countries, adapted to Bolu ecological conditions from these 48 populations. A number of morphological properties and yield values such as seedling emergence, plant height, number of branche per plant, number of umbel and umbellate number, days to flowering time, fruit linking days, seed yield, weight of the thousand seed and number of seed per plant were studied for characterization and yield component.

Table 2. Comparison of fennel populations in terms of morphological and yield values and LSD groups.

No	Plant Accession	GP	PH	NB	NU	NUT	DFT	FLD	NS	1000SW	SY (g)
1	Ames 23130	17.80a-e	55.18i-n	4.24ijk	12.58a-d	15.53e-k	59.10ijk	111.95fgh	760.78bcd	4.39e-i	3.16c-f
2	Ames 27588	17.40a-g	54.64j-o	8.26ab	5.12j-m	16.97c-f	101.90abc	121.15cd	420.68c-f	4.28ghj	1.85e-f
3	Ames 30289	17.60a-f	91.36ab	7.10a-f	7.27h-k	21.24a-b	86.90def	117.55de	1248.44a	6.99abc	8.85a
4	Ames 30290	16.60a-g	84.06bcd	7.90a-d	9.07b-j	16.74c-g	74.90fgh	102.55n	1131.19ab	5.79b-h	6.69ab
5	Ames 30693	20.60a	76.81b-g	6.80a-g	5.07j-m	12.43h-n	75.90fgh	102.55n	202.04fg	2.99ij	0.71f
6	Ames 7551	15.20c-i	73.54c-h	6.80a-g	7.23h-k	20.33a-d	91.10cde	122.35cd	492.30c-f	4.83d-i	2.45def
7	Ames 20029	18.60a-d	60.36h-n	6.40a-i	7.47h-k	11.64j-p	67.90h-k	105.55lmn	374.44d-g	4.29ghj	1.70ef
8	NSL 6409	17.40a-g	68.10e-j	6.67a-h	7.92e-k	18.13b-e	93.90bcd	118.15de	1030.48ab	5.00c-i	5.25bcd
9	PI 172898	13.80e-j	70.27d-i	5.74d-k	10.78a-h	22.43a	59.10ijk	107.95h-m	816.38bc	5.14b-h	4.07b-e
10	PI 174212	18.80a-d	52.17k-o	4.44h-k	11.18a-h	15.70e-j	69.10h-k	109.95h-k	323.38efg	6.69a-d	2.07def
11	PI 174213	16.60a-g	64.76f-i	6.50a-i	12.07a-f	15.29e-l	73.90fgh	105.55lmn	459.69c-f	6.34a-g	2.98c-f
12	PI 194892	16.40a-g	62.44g-m	4.26ijk	7.52h-k	6.80q	102.90abc	121.15cd	289.68efg	4.00hij	1.20ef
13	PI 251085	20.60a	61.96g-m	8.50a	3.74klm	11.94i-o	73.90fgh	106.55k-n	333.24efg	5.81b-h	2.00def
14	PI 273659	19.40abc	70.54d-i	3.76k	5.12j-m	7.47pq	81.90d-h	115.15ef	445.68c-f	4.40e-i	2.02def
15	PI 273660	19.40abc	86.84bc	6.66a-h	9.72b-i	10.30mq	81.90d-h	114.15efg	331.28efg	5.13b-h	1.75ef
16	PI 288283	10.80j	70.47d-i	3.94jk	0.78m	11.68j-p	56.10k	104.95mn	7.38g	6.99abc	0.15f
17	PI 288285	15.80b-h	69.07d-j	5.74d-k	10.73a-h	16.20d-h	57.10jk	106.95j-m	496.38c-f	5.01c-i	2.31def
18	PI 288477	18.60a-d	73.56c-h	8.10abc	5.07j-m	11.19l-p	69.90g-k	107.55i-m	468.24c-f	4.42e-i	2.17def
19	PI 358460	19.40abc	65.74e-k	7.26a-f	13.10ab	12.47h-n	77.90e-h	108.15h-m	390.28d-g	6.67a-d	2.67c-f
20	PI 414189	18.20a-e	45.57no	6.33a-i	5.18j-m	10.00m-q	73.10f-i	110.35g-k	240.44efg	4.59d-i	1.27ef
21	PI 414190	18.20a-e	86.84bc	6.60a-h	10.73a-h	10.93m-q	68.10h-k	105.35lmn	353.90efg	4.82d-i	1.82ef
22	PI 414191	20.20ab	56.49i-n	6.50a-i	14.43a	13.17f-m	74.10fgh	138.35a	467.10c-f	4.88c-i	2.35def
23	PI 414192	17.80a-e	39.22o	7.04a-f	1.18m	7.91opq	115.10a	139.95a	379.78d-g	1.92j	0.34f
24	PI 601795	13.20f-j	48.14mno	6.90a-g	4.93j-m	12.53	102.10abc	132.35b	468.63c-f	4.97c-i	2.40def
25	PI 649460	20.20ab	69.46d-j	7.44a-f	12.72abc	12.33h-n	72.10ghj	111.35f-i	272.10efg	5.31b-h	1.56ef
26	PI 649463	15.40c-h	81.24b-e	6.16b-j	2.32lm	20.93abc	81.90d-h	114.15efg	1135.48ab	5.89b-h	6.81ab
27	PI 649464	17.80a-e	92.37ab	5.54e-k	11.98a-g	16.42d-h	72.10ghj	111.95fgh	1214.78a	4.82d-i	5.77abc
28	PI 649465	18.20a-e	49.99l-o	5.90c-k	6.10n-q	8.88n-q	107.10abc	140.35a	567.23c-f	5.17b-h	2.96c-f
29	PI 649466	18.20a-e	55.24i-n	4.67g-k	7.08h-k	15.37e-l	71.10g-j	114.35efg	629.41cde	5.35b-h	3.36c-f
30	PI 649469	9.80j	69.17d-j	5.34f-k	7.68f-k	11.33k-p	71.10g-j	110.95ghj	310.38efg	5.42b-h	1.50ef
31	PI 649470	10.80j	103.07a	7.74a-e	7.38h-k	16.28d-h	72.10ghj	111.95fgh	357.58efg	8.14a	2.93c-f
32	PI 649471	15.40c-h	104.64a	5.36f-k	7.07h-k	18.21a-e	83.90d-g	114.15efg	502.68c-f	4.37e-i	2.26def
33	Denizli pop.	14.50d-i	78.33b-f	7.73a-e	14.33a	16.06e-i	70.00g-k	107.50i-m	622.05cde	6.50a-e	4.06b-e
34	Erzurum pop.	13.75e-j	80.03b-f	6.23a-j	8.15d-k	12.97f-n	74.75fgh	108.50h-m	427.55c-f	6.08a-h	2.63c-f
35	Bucak pop.	13.00g-j	68.20e-j	6.45a-i	7.62g-k	10.97m-q	74.50fgh	110.00h-k	296.85efg	6.43a-f	1.94ef
36	Eskişehir pop.	11.75hij	72.18c-h	6.33a-i	8.28c-j	11.50j-p	74.50fgh	109.00h-l	353.83efg	5.95b-h	2.17def
37	Nazilli pop.	18.00a-e	59.56h-n	6.88a-g	12.27a-e	16.10d-i	79.75d-h	110.75g-j	584.90c-f	7.17ab	4.36b-e
	Average	16.63	69.50	6.33	8.13	13.96	78.18	113.81	519.1	5.32	2.83
	F value	5.07**	5.34**	1.37**	8.64**	6.31**	1.06**	1.89**	2.44**	0.97**	2.16**
	CV %	14.73	9.98	15.62	20.11	14.43	8.99	1.69	39.82	15.03	49.70
	LSD (0.01)	4.57	15.61	2.29	4.45	4.26	14.66	4.03	397.26	2.11	3.29

*GP: Seedling emergence, PH: Plant Height, NB: Number of Branche, NU: Number of Umbel, NUT: Number of Umbellate, DFT: Days to Flowering Time, FLD: Fruit Linking Days, NS: Number of Seed, 1000SW: 1000 Seed Weight, SY: Seed Yield

** : Different letters at each column indicate significant differences at $p \leq 0.01$ probability level by LSD test

The results of the analysis of variance indicated that there were significant for all characters studied at $p < 0.01$ (Table 2).

The seedling emergence of fennel populations changed between 9.8-20.6 days as showing in table 2. The earliest seedling emergence was determined in PI 649469 (9.8 days) and followed by PI 649470 and PI 288283 with 10.8 days. The latest seedling emergences were found in PI 251085 and Ames 30693 populations with 20.6 days. It can be see that 14 fennel populations were found later and 3 populations were seen earlier than control populations.

Yıldırım and Kan, (2006) reported that fennel must be sown to annealed soil and given irrigation water or must be subsequent rainfall after sown. They also said seedling emergence of fennel changed between 17.3-24.3 days. The obtained results were found similarly with Yıldırım and Kan (2006).

The variation with respect to plant height at flowering ranged from 39.22-104.64 cm, showing broad variability among the populations. PI 649471 gave the highest plant height at flowering (104.64 cm) followed by PI 649470 (103.07cm), PI 649464 (92.37) and Ames 30289 (91.36 cm), while PI 414192 gave the lowest plant height at flowering (39.22 cm) followed by PI 414189 (45.57 cm) (Table 2). 8 populations were found higher and 9 populations were found lower than control populations in terms of plant height. Uzun et al., (2011) reported that the plant height range between 70.61-80.40 cm in fennel lines. Lopes et al., (2009) indicated that plant height of fennel changed between 30-51.7 cm. Similarly, Zahid et al., (2008) reported 69-129 cm for plant height in fifty local fennel accessions which agrees the current findings.

There were significant differences among the populations and controls in terms of number of branch. The number of branch was seen between 3.76-8.50 number/plant. While the highest number of branch was obtained from PI 251085 (8.50 number) and Ames 27588 (8.26 number), the least number of branch was obtained from PI 273659 (3.76 number) and PI 288283 (3.94 number) populations. Denizli population was found higher than 27 fennel populations and Nazilli populations were seen higher than 22 populations in terms of number of branch. It was reported that number of branch varied from 3.1 to 9.7 number/per plant in variation locations of Turkey (Arabacı and Bayram, 2005; Yıldırım and Kan, 2006; Uzun et al., 2011; Dirican, 2013).

The variation in umbel number per plant ranged from 0.78-14.43 showing variability among the populations for this character (Table 2). PI 414191 (14.43 number) gave the highest umbel number per plant, followed by Denizli population and PI 358460 (14.33 and 13.10, respectively). The lowest umbel number was found from PI 288283 (0.78 number) and followed by PI 414192 (1.18 number). Only one population (PI 414191) was higher umbell number than control populations. It is noted that number of umbel was found between 5.6-9.7 in Tokat conditions (Özyılmaz, (2007), 6.7-10.9 in Diyarbakır conditions (Kızıl et al., 2001). Similarly, Özkan (1999) reported that number of umbel was determined as 3.78, Kapoor et al., (2004) as 10-14 and Piccaglia and Marotti (2001) as 12-340 in fennel. Our results were also found lower than reported by Alipoor et al., (2016) between 17.07 and 19.1, Arabacı and Bayram (2005) as 22.7-27.9 and Oğuz (2000) between 29.3-64.90 number/plant in bottom land and barren land. These differences can be due to the genotype difference, environmental and growing conditions.

Variability among the fennel populations was observed for umbellate number per umbel between 6.80-22.43 (Table 2). PI 172898 gave the highest umbellate number per umbel (22.43) followed by Ames 30289 and PI 649463 (21.24 and 20.93, respectively). The lowest number of umbellate was found in PI 194892 (6.80 number) and followed by PI 273659 (7.47 number). Compared to controls, 11 fennel populations had higher umbellate number per plants. The genetic structure of the plant is an important element that determines the number of umbellate, but it can be showed variability depending on number of branch, size of umbel, morphological structure such as where it is located (Dirican, 2013). Uzun et al., (2011) reported 10.92-18.89 range for umbellate number per umbel in 9 fennel lines and 1 control in Samsun and Amasya provinces of Turkey. Umbellate number of fennel populations changed between 11.1-15.2 in different row spacing and seeding rate (Özyılmaz, 2007). Similarly, Dirican (2013) reported that the number of umbellate in main umbel varied between 14.5 and 34.0. It was noted that number of umbellate varied among the years as 55.2-501.2 in 2012 and 165.6-499.1 in 2013, 66.5-192.3 in 2014 in Tokat-Kazova conditions (Özyılmaz, 2015).

The obtained results were similar with Özyılmaz (2007) and Dirican (2013), but found different with Özyılmaz (2015). It was noted that number of umbellate could be affected by climate, sowing time, maturity time, seeding rate (Özkan and Gürbüz, 2000; Uzun et al., 2011).

Days to flowering time (50%) ranged from 56.1 (PI 288283) to 115.1 days (PI 414192). While the earliest days to flowering time was seen in PI 288283 (56.1 days) followed by PI 288285 (57.1 days), the latest days to flowering time was seen in PI 414192 (115.1 days) and followed by PI 649465 (107.1 days) and PI 194892 (102.9 days). Flowering times of 12 populations were found later than control populations and 8 populations were seen earlier than controls.

It was noted that 50% flowering time of the fennel lines varied between 72-75 days by Uzun et al., (2011). Lopes et al., (2010) reported that time to beginning of flowering of 1 fennel commercial cultivar and 9 wild bitter fennel accessions changed between 192-200 days collected from Northern Portugal. Özyılmaz (2015) reported that flowering times (between seedling emergence and flowering time) of 33 fennel populations varied from 58 to 71 days in Tokat-Kazova conditions. The first flowers were seen about 114 days after planting in Pakistan conditions (Ahmad et al., 2004). The obtained results were found partly similar with researchers except Lopes et al., (2010). It is thought that the different results could be occur from different ecological conditions (increase in the length of days and summer temperatures) (Kızıl et al., 2001), genetic varieties or growing conditions.

Fruit linking days (50%) varied from 102.55 to 140.35 days with average 113.81 days. The latest fruit linking days were observed from PI 649465 (140.35) and PI 414192 (139.95) and the earliest fruit linking days were seen in Ames 30290 and Ames 30693 with 102.55 days and followed by PI 288283 (104.95 days). 19 fennel population was found later than controls and 8 populations were found earlier than controls including PI 288285 population. It was reported that fruit linking changed between 54-102 days in Tokat-Kazova condition among the 2012-2014 years (Özyılmaz, 2015). Besides environmental factors, the seed yield and amount of essential oil from a unit area of fennel seeds also depends on the fruit-linking of the plants. It can be seen decreased number of fruits per plant due to insufficient pollination (Falzari et al., 2005). The obtained results were found higher than Özyılmaz (2015). This situation can be explained genetic differences, environmental or growing conditions.

Seed number per plant ranged from 7.38 (PI 288283) to 1248.44 (Ames 30289) with the mean of 519.10 (Table 2). The highest seed number was found in Ames 30289 population (1248.44) and followed by PI 649464 (1214.78), PI 649463 (1135.48), Ames 30290 (1131.19) and NSL 6409 populations (1030.48). The lowest seed number was found in PI 288283 with 7.38 number and followed by PI 414189 (240.44) and Ames 30693 (202.04) populations. While, 8 fennel populations had higher seed number and 5 fennel populations had lower seed number compared to control populations in terms of seed number. It is reported that seed number changed between 364-1666 in Tokat-Kazova conditions (Özyılmaz, 2015). It is important to selection of high efficient species to determination of genetic effects of seed number such as plant height, number of branche (Sengupta et al., 2014). The obtained results were found partly similar with Özyılmaz (2015). It was thought that environmental conditions, genetic differences and growth conditions may be the cause of differences.

The variation in 1000 seed weight ranged from 1.92 to 8.14 g showing variability among the populations for this character. PI 649470 gave the highest 1000 seed weight per plant (8.14 g), followed by Nazilli pop. with 7.17 g. The lowest 1000 seed weight was found in PI 414192 (1.92 g) and followed by Ames 30693 population (2.99 g). The average of 1000 seed was measured as 5.32 g fennel populations including controls. While PI 649470 population was only found higher than controls, 26 fennel populations was seen lower than all controls.

It is reported that the 1000 seed weight in the fennel can change depending on the use of water in spite of environmental differences (Kumar et al., 2002).

Yıldırım and Kan (2006) reported 1000 seed weight range from 8.0 to 9.2 g in different nitrogen and zinc doses. Uzun et al. (2009) reported that 1000 seed weight ranged from 5.08 to 8.46 g in different ecological condition. It was also noted that 1000 seed weight changed between 4.1 and 8.40 g (Karaca and Kevseroğlu, 1999; Özkan et al., 2000; Arabacı et al., 2005; Özyılmaz, 2007). The obtained values were found partly similar with researchers.

Seed yield is the most important part of the plant because of including essential oil, raw oil or using as spice or medicine. The range for seed yield per plant was 0.15-8.85 g with mean value of 2.83 g (Table 2). Ames 30289 population gave the highest seed yield per plant (8.85 g) followed by PI 649463 (6.81 g) and Ames 30290 (6.69 g) populations. The lowest seed yield per plant was registered for PI 288283 (0.15 g) and PI 414192 (0.34 g). Kızıl et al., (2001) reported seed yield per plant range between 0.78-3.68 g. Similarly, Uzun et al. (2011) reported 0.72-5.90 g range for seed yield per plant in 10 Turkish fennels which finds similar with the data. Obtained data about seed yield was found different reported from Arabacı et al., (2005) (15-22 g). The results were found similar with researchers, except Arabacı et al., (2005). It can be considered that the differences in results are based on genetic variety, environment and grown conditions.

CONCLUSION

Fennel populations were compared with local fennel populations according to morphological properties and yield values in the study in terms of seedling emergence, plant height, number of branche per plant, umbel number in per plant, umbellate number in per plant, days to flowering 50%,

fruit-linking day 50%, seed number, 1000 seed weight and seed yield in per plant. Among the fennel populations and controls investigated, Ames 30289 was significantly superior in terms of seed yield per plant (8.85 g) and number of seed (1248.44). It also took place among the first ten populations in terms of other properties except seedling emergence and umbel number.

In conclusion, populations of Ames 30289, Ames 30290, PI 649463 and PI 649464 came into prominence in terms of investigated values according to experimental year.

REFERENCES

- Ahmad, M., Hussain, S.A., Zubair, M., Rab, A., 2004. Effect of sowing and row spacing on seed production of fennel (*Foeniculum vulgare*). Pakistan Journal of Biological Sciences, 7 (7): 1144-1147.
- Alipoor, F., Alizadeh, K., Arshad, M., 2016. The impact of plant density on morphological characteristics of fennel (*Foeniculum vulgare* Mill). Journal of Chemical and Pharmaceutical Research, 8(5): 403-410.
- Anonymous, 2015. Bolu directorate of provincial food agriculture and livestock.
- Anonymous, 2018. Turkish state meteorological service, Bolu.
- Arabacı, O., Bayram, E., 2005. Rezenede (*Foeniculum vulgare* Mill.) farklı ekim zamanı ve tohumluk miktarının verim bazı önemli özellikler üzerine etkisi. Türkiye VI. Tarla Bitkileri Kongresi, 5-9 Eylül, Antalya.
- Asimgal, A., 1997. Sıfalı Bitkiler. İstanbul: Timas Yayınları. pp. 239-240.
- Baytop, T., 1999. Therapy with medicinal plants in Turkey (Past and Present) (1st ed.). Publication of Istanbul University, p. 320, İstanbul, Turkey,
- Ceylan, A., 1997. Tıbbi Bitkiler II. Ege Üniversitesi Ziraat Fakültesi Yayınları No:481. Bornova/İzmir.
- Davis, P.H., 1978. Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh, 4: 352-377.
- Dirican, A., 2013. Tokat florasında yayılış gösteren yabancı rezene (*Foeniculum vulgare* Mill) popülasyonlarının morfolojik ve kimyasal özelliklerinin belirlenmesi. Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Gaziosmanpaşa Üniversitesi, Tokat.
- Falzari, L.M., Memary, R.C., Dragar, V.A., 2005. Reducing fennel stand density increases pollen production, improving potential for pollination and subsequent oil yield. Hort Science, 40(3): 629-634.
- Kapoor, R., Giri, B., Mukerji, K., 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill on mycorrhizal inoculation supplemented with p-fertilizer. Bioresource Technology, 93: 307-311.
- Karaca, A., Kevseroğlu, K., 1999 Farklı orjinli kişniş (*Coriandrum sativum* L.) ve rezene (*Foeniculum vulgare* Mill.) bitkilerinin önemli tarımsal özellikleri üzerine bir araştırma. J. Agric., Fac. O.M.U., 14(2): 65-77.
- Kızıl, S., Arslan, N., İpek, A., 2001. Rezene (*Foeniculum vulgare* Mill. var. dulce) de farklı ekim zamanlarının verim ve verim unsurlarına etkisi. Türkiye 4. Tarla Bitkileri Kongresi, 17-21 Eylül 2001. Cilt II, 331-334, Tekirdağ.
- Kumar, A., Singh, R., Chhillar, R.K., 2002. Influence of Irrigation and Fertilizer Levels on Growth, Seed Yield and Water-Use Efficiency by Fennel (*Foeniculum vulgare*). Indian Journal of Agronomy 47(2): 289-293.
- Lopes, V.R., Barata, A.M., Farias, R., Mendes, M.D., Lima, A.S., Pedro, L.G., Barroso, J.G., Figueiredo, A.C., 2010. Morphological and essential oil variability from nine portuguese (*Foeniculum vulgare* Mill.) accessions. IV. International Symposium on Breeding Research on Medicinal and Aromatic Plants, 38(1): 33-49.
- Miraldi, E., 1999. Comparison of the essential oils from ten *Foeniculum vulgare* Miller samples of fruits of different origin. Flavour and Fragrance Journal, 14: 379-382.
- Oğuz, A., 2000. Rezenede (*Foeniculum vulgare* var. dulce) farklı üretim yöntemlerinin verim ve uçucu yağ üzerine etkileri. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, Adana.
- Özgen, M., 1994. Orta Anadolu koşullarında kışlık arpanın verim ve verim öğelerinde adaptasyon ve stabilite analizi. Turkish Journal of Agriculture and Forestry, 18: 169-177.
- Özkan, F., 1999. Tatlı rezenede bitkisi sıklığının verim ve verim öğeleri üzerine etkileri. Yüksek Lisans Tezi, A.Ü. Tarla Bitkileri Anabilim Dalı, Ankara.
- Özkan, F., Gürbüz, B., 2000. Tatlı rezene (*Foeniculum vulgare* Mill. var. dulce) de bitki sıklığının verim ve verim özellikleri üzerine etkileri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, (9): 1-2.
- Özyılmaz, B., 2007. Farklı sıra aralığı ve ekim normlarının rezene (*Foeniculum vulgare* Mill. var. dulce) de verim, verim unsurları ve bazı kalite özelliklerine etkileri. Yüksek Lisans Tezi, Gaziosmanpaşa Üniv., Fen Bilimleri Enstitüsü Tarla Bitkileri Anabilim Dalı, Tokat.
- Özyılmaz, B., 2015. Farklı yörelerden temin edilen tatlı rezene (*Foeniculum vulgare* var. dulce) popülasyonlarının karakterizasyonu. Doktora Tezi, Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, Tarla Bitkileri Anabilim Dalı, p. 42-45.
- Piccaglia, R., Marotti, M., 2001. Characterization of some Italian types of wild fennel (*Foeniculum vulgare* Mill.). Journal of Agricultural and Food Chemistry, 49: 239-244.
- Rather, M.A., Dar, B.A., Sofi, S.N., Bhat, B.A., Qurishi, M.A., 2012. *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology and safety. Arabian Journal of Chemistry, <http://dx.doi.org/10.1016/j.arabjc.2012.04.011>.
- Sengupta, S.K., Verma, B.K., Naidu, A.K., 2014. Genetic variability study in fennel (*Foeniculum vulgare* Mill.). International Science Journal, 1(1): 62-64.

- Sephidkon, F., 2001. Evalution of quantitative and qualitative of fennel (*Foeniculum vulgare*) essential oil in different stages. Iranian Journal of Medicinal and Aromatic Plants, 4: 85-101.
- Uzun, A., Kevseroğlu, K., Yılmaz, S., 2011. Orta Karadeniz Bölgesi için geliştirilen rezene (*Foeniculum vulgare* Mill. var. *dulce*) hatlarının bazı tarımsal özellikleri bakımından incelenmesi. GOÜ, Ziraat Fakültesi Dergisi, 28(2): 1-8.
- Yıldırım, N., Kan, Y., 2006. Farklı dozlarda uygulanan azot ve çinkonun rezene (*Foeniculum vulgare* Mill. var. *dulce*)’de verim ve verim öğeleri üzerine etkisi. Selçuk Üniversitesi, Ziraat Fakültesi Dergisi, 20(40): 94-101.
- Yıldırım, B., Tunçtürk, M., Okut, N., Türközü, D., 2008. Farklı sıra arası mesafeleri ve fosfor dozlarının rezene (*Foeniculum vulgare* Mill.) bitkisinde bitki gelişimi ve verime etkisi. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 13(2): 69-72.
- Zahid, N.Y., Abbasi, N.A., Hafiz, I.A., Ahmad, Z., 2008. Morphological characteristics and oil contents of fennel (*Foeniculum vulgare* L.) accessions from different regions of Pakistan. The Journal of the Chemical Society of Pakistan, 30(6): 889-895.
- Zoubiri, S., Baaliouamer, A., Seba, N., Chamouni, N., 2014. Chemical composition and larvicidal activity of Algerian *Foeniculum vulgare* Seed essential oil. Arabian Journal of Chemistry, 7(4): 480-485.

TIP SHAPE OF LEAF AND BASE SHAPE OF LEAF OF TURKISH TOBACCO POPULATIONS AND AKHISAR 97 CULTIVAR IN BOLU ECOLOGICAL CONDITIONS

Mahmut ÇAMLICA¹, Gülsüm YALDIZ¹

¹Department of Field Crops, Faculty of Agriculture and Natural Sciences, Bolu Abant İzzet Baysal University, Bolu, Turkey

Corresponding author email: mcamllica25@outlook.com

Abstract

Tobacco (*Nicotiana tabacum* L.) is an annual plant belongs to Solanaceae family. This plant is grown all over the world because it adapts to all ecological conditions. One of the most important parts of this plant is leaves, because generally, its leaves are used in the World as pleasure because of including special component as nicotine and reduced sugar. In this study, 30 tobacco samples collected from different regions of Turkey were observed in terms of leaf shapes. Tip shape of leaf and base shape of leaf were determined as sessile and petiolate, acute, straight and rounded, respectively among the regions. Black Sea populations and one population of Marmara Region (Bursa population) were found as a petiolate. Other region populations and Akhisar-97 cultivar were determined as sessile. In terms of tip shape of leaf, Black Sea tobaccos were found straight-pointed, Southeast Anatolian tobaccos (Hatay populations) and Akhisar 97 were seen as round-slightly pointed, generally. Other populations were seen as rounded-pointed, straight-pointed, straight-slightly pointed (Aegean Region and East Anatolia Region). In conclusion, while the Black Sea Regions have showed similarity in terms of the examined properties, the tobacco populations of the other regions were showed variability.

Keywords: Tobacco, population, Turkey, Bolu.

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is cultivated as an annual plant that is propagated by seed. This plant is widely known for its leaves, which are smoked, chewed, or sniffed for various impacts. It includes chemical nicotine which is known very well to be harmful to humans (Atrens, 2001).

It is known that South America was the origin and that the people in these areas first began to use it. In 1519 the seafarer Gazolo De Oviedo was brought to Europe by Y. Valdes and his team and spread throughout the world with the discovery of America. Tobacco, which began to spread from Europe to the east, was brought to Istanbul between 1601-1605 by British and Venetian sailors (Esendal, 2007; Öztürk et al., 2014).

Leaf is the most important and utilized part of tobacco plant. The size and form of the leaf are the most important factors used in the differentiation of tobacco varieties. They have a great role in blending (Anonymous, 2018). Tobacco leaves are used as the basic material of industries of cigarette and quality of them effects directly the cigarette quality and style (Song et al., 2007).

Although leaf shape and structure are kind characters, they are also affected by environmental conditions. The leaf of tobacco showed wide variations in terms of leaf form as anchusa, kidney and heart. It has also hereditary criterias. The most important properties among these criterias are being sessile and petiolate (Er and Yıldız, 2014).

In this work, tip shape of leaf and base shape of leaf of Turkish tobacco populations and Akhisar 97 cultivar were described in Bolu ecological conditions.

MATERIAL AND METHODS

This study was conducted in 2015 growing seasons at the experimental site of Bolu Abant İzzet Baysal University, Faculty of Agriculture and Natural Sciences, Department of Field Crops, Bolu-

Turkey (40°44'46" N, 31°37'46" E, altitude 752 m). Seeds of tobacco were obtained from different provinces and Aegean Agricultural Research Institute (AARI) and one cultivar (Akhisar-97) (Table 1). They were grown in 2015 growing seasons.

Table 1. List of tobacco populations and cultivar used in the current study.

No	Code	Populations/cultivar names	Obtained place or person	Regions	Geographic coordinates	
					Latitude	Longitude
1	AKHSR-1	Akhisar 97	Manisa/Cultivar	AR	38°55'3.5904" N	27°50'11.8320" E
2	MNS-1	Manisa (AARI, 64062)	Aegean Agricultural Research Institute	AR	38°36'50.5188" N	27°25'46.4232" E
3	AS-1	Akhisar-Sarılar	Manisa/Akhisar/Sarılar/Ufuk Özcan	AR	39°6'26.9100" N	28°0'8.7084" E
4	SLHLK-1	Salihli Kale Village	Manisa/Salihli/Kale Village/İbrahim Zeybek	AR	38°43'18.6780" N	28°8'17.9700" E
5	BLKSR-1	Balıkesir (AARI, 42986)	AARI	MR	-	-
6	BLKSR-2	Balıkesir (AARI, 64073)	AARI	MR	-	-
7	AYK-1	Agonya-Yarış Village	Çanakkale/Agonya/Yarış Village/Anıl Özyurt	MR	39°47'1.6332" N	27°15'51.7320" E
8	BRS-1	Bursa (AARI, 42884)	AARI	MR	-	-
9	BRS-2	Bursa (AARI, 78215)	AARI	MR	-	-
10	BCLKHN-1	B. Çelikhhan 97	Adıyaman/Çelikhhan	ESR	38°1'59.0520" N	38°14'13.9128" E
11	MŞ-1	Muş (AARI, 42094)	AARI	ESR	-	-
12	BTLS-1	Bitlis (AARI, 42076)	AARI	ESR	-	-
13	BTLS-2	Bitlis (AARI, 80111)	AARI	ESR	-	-
14	BMEK-1	Bitlis Mutki Erler Village-1	Bitlis/Mutki/Erler Village/Sait Sülün	ESR	38°28'46.7472" N	41°43'56.5536" E
15	BMEK-2	Bitlis Mutki Erler Village-2	Bitlis/Mutki/Erler Village/Yusuf Kesim	ESR	38°28'46.7472" N	41°43'56.5536" E
16	HTY-1	Hatay (AARI, 42126)	AARI	ESR	-	-
17	HTY-2	Hatay (AARI, 42128)	AARI	ESR	-	-
18	HTY-3	Hatay (AARI, 42132)	AARI	ESR	-	-
19	YYLDG-1	Yayladağı Sebenoba	Hatay/Yayladağı/Sebenoba/Ayşe Şahin	ESR	36°2'48.2424" N	36°1'15.0384" E
20	ESK-1	Eski Tütün-Hatay	Hatay	ESR	36°12'1" N	36°10'34" E
21	BFR-1	Bafra Gökçeada	Samsun/Bafra/Gökçeada/Şefik Kara	BSR	41°32'45.9204" N	35°45'41.4324" E
22	BFR-2	Bafra Paşayeh	Samsun/Bafra/Paşayeh/Cemil Yüksel	BSR	41°28'50.8260" N	35°44'18.0888" E
23	SMSN-1	Samsun (AARI, 49184)	AARI	BSR	-	-
24	SMSN-2	Samsun (AARI, 49188)	AARI	BSR	-	-
25	SMSN-3	Samsun (AARI, 49219)	AARI	BSR	-	-
26	SMSN-4	Samsun (AARI, 49224)	AARI	BSR	-	-
27	SMSN-5	Samsun Tekkeköy Hamzalı	Samsun/Tekkeköy/Hamzalı/Mustafa Anıl	BSR	41°12'19.8756" N	36°32'15.2844" E
28	SMSN-6	Samsun Terme Akçay	Samsun/Terme/Akçay/Mümin Bayram	BSR	41°8'0.7332" N	37°9'22.2264" E
29	SMSN-7	Samsun Tekkeköy Balcalı	Samsun/Tekkeköy/Balcalı/Alı Doğru	BSR	41°9'14.8932" N	36°34'5.4624" E
30	SMSN-8	Samsun Tekkeköy Kahyalı	Samsun/Tekkeköy/Kahyalı/Osman Kul	BSR	41°10'21.5508" N	36°32'21.6564" E

AARI: Aegean Agricultural Research Institute, AR: Aegean Region, MR: Marmara Region, ESR: Eastern and Southeastern Anatolia Region, BSR: Black Sea Region.

The experiment was carried out on the experimental field of the research and application area of Department of Field Crops, Natural and Agricultural Sciences, Bolu Abant İzzet Baysal University (Turkey) (40° 44' 44" N, 31° 37' 45" E, 881 m altitude). The soil was clay-loam with a pH value of 7.8, 4.7% organic matter content, 10.3 ppm phosphorus ratio and 235 ppm potassium ratio. Average climatic data were recorded 19.10 °C temperature; 259,1 mm rainfall; 71.8% humidity during the vegetation period for 2015 (Anonymous, 2015).

Experimental design was randomized complete block design with three replicates. Each plot consisted of four rows which were 2.10 m long. Row spacing was 40 cm and plant spacing within rows was 15 cm, and a total of 56 seedlings were used in each plot.

4 kg/da Diammonium phosphate (DAP) and 2 kg/da Ammonium nitrate (AN) was applied to the plots as a base and top fertilizer. Tobacco populations and cultivar were regularly irrigated in vegetation periods and controlled against weeds. Examined properties were determined according to reported by Peksüslü et al. (2002) and Er and Yıldız (2014).

RESULTS AND DISCUSSION

The obtained observations, tip shape of leaf and base shape of leaf of Turkish tobacco populations and Akhisar 97 cultivar were given in table 2 and 3.

Table 2. Tip shape of leaf in tobacco cultivar and populations.

No	Populations/Cultivar	Tip Shape of Leaf	No	Populations/Cultivar	Tip Shape of Leaf
1	AKHSR-1	Round-Mild Acute	16	HTY-1	Round-Mild Acute
2	MNS-1	Round-Acute	17	HTY-2	Round-Mild Acute
3	AS-1	Round-Acute	18	HTY-3	Round-Mild Acute
4	SLHLK-1	Straight-Medium Acute	19	YYLDG-1	Round- Mild Acute
5	BLKSR-1	Round-Acute	20	ESK-1	Straight-Medium Acute
6	BLKSR-2	Round-Acute	21	BFR-1	Straight-Acute
7	AYK-1	Straight-Acute	22	BFR-2	Straight- Acute
8	BRS-1	Round-Acute	23	SMSN-1	Straight-Acute
9	BRS-2	Round-Acute	24	SMSN-2	Straight-Acute
10	BCLKHN-1	Round-Acute	25	SMSN-3	Straight-Acute
11	MŞ-1	Straight-Medium Acute	26	SMSN-4	Straight-Acute
12	BTLS-1	Round-Acute	27	SMSN-5	Straight-Acute
13	BTLS-2	Round-Acute	28	SMSN-6	Straight-Acute
14	BMEK-1	Round Acute	29	SMSN-7	Straight-Acute
15	BMEK-2	Round Acute	30	SMSN-8	Straight-Acute

Samsun and Bafra populations were found as straight-acute, Bitlis (BTLS) populations were round-acute, origin of Hatay tobacco populations (HTY-1,2,3, YYLDĞ-1) and Akhisar-97 were round-mild acute. Tip shape of tobacco was divided to 5 groups. AYK-1 and Bafra and Samsun (Black Sea Region) populations of leaf tip shapes straight-acute, Balıkesir (BLKSR), Bitlis (BTLS), Bursa (BRS), Akhisar-Sarılar (AS), B. Çelikhhan (BCLKHN-1) and Manisa (MNS) populations were seen as round-acute, Akhisar-91 (AKHSR-1) cultivar and Hatay (HTY) and Yayladag (YYLDG-1) populations were seen as round-mild acute, Muş (MŞ) and ESK-1 populations were found as straight-medium acute and lastly, SLHLK-1 population was found as straight-medium acute in terms of tip shape of tobaccos (Table 2, Fig. 1).

Table 3. Base shape of leaf shape in tobacco cultivar and populations.

No	Populations/Cultivar	Base Shape	No	Populations/Cultivar	Base shape
1	AKHSR-1	Sessile	16	HTY-1	Sessile
2	MNS-1	Sessile	17	HTY-2	Sessile
3	AS-1	Sessile	18	HTY-3	Sessile
4	SLHLK-1	Sessile	19	YYLDG-1	Sessile
5	BLKSR-1	Sessile	20	ESK-1	Sessile
6	BLKSR-2	Sessile	21	BFR-1	Petiolate
7	AYK-1	Sessile	22	BFR-2	Petiolate
8	BRS-1	Petiolate	23	SMSN-1	Petiolate
9	BRS-2	Petiolate	24	SMSN-2	Petiolate
10	BCLKHN-1	Sessile	25	SMSN-3	Petiolate
11	MŞ-1	Sessile	26	SMSN-4	Petiolate
12	BTLS-1	Sessile	27	SMSN-5	Petiolate
13	BTLS-2	Sessile	28	SMSN-6	Petiolate
14	BMEK-1	Sessile	29	SMSN-7	Petiolate
15	BMEK-2	Sessile	30	SMSN-8	Petiolate

Tobacco populations grown in Samsun, Bafra and Bursa (BRS-1, 2) provinces were observed as petiolate, other populations and cultivar were observed as sessile (Table 3, Fig. 2). It was reported that tip shape and base shape of tobacco changed between the regions in Turkey. These shapes were determined as petiolate in Black Sea, as petiolate or sessile Marmara Region and as sessile in Eastern

and Southeastern Anatolia Region (Peksüslü et al., 2002; Er and Yıldız, 2014). The findings were found similar with researchers.



Fig 1. Tip shape of leaves of tobacco.

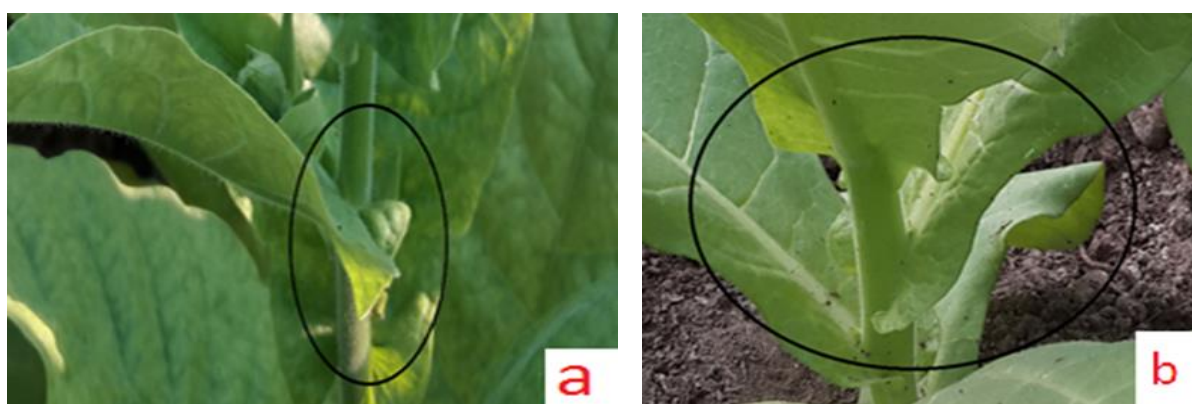


Fig 2. Base shape of leaves of tobacco.

CONCLUSION

In our study, tip and base shapes of tobacco cultivar and populations were determined in Bolu ecological conditions as sessile, petiolate, round, straight and acute generally. Tip shape was divided 5 groups among the regions and base shape of 12 populations were found as petiolate and others were seen sessile. Tip and base shape were found differently among the regions and it was seen that these properties can change between the tobacco populations in Turkey.

ACKNOWLEDGEMENTS

This study is part of a MSc Thesis and we would like to thank to the Aegean Agricultural Research Institute, İzmir/Turkey for supplying seeds of some tobacco populations in this study.

REFERENCES

- Anonymous, 2015. Bolu directorate of provincial food agriculture and livestock.
Anonymous, 2018. Harmancılık açısından tütün yaprağının özellikleri. Tütün Eksperleri Derneği, <http://www.tutunekspert.org.tr>, (19 September).
Atrens, D.M., 2001. Nicotine is an addictive substance: a critical examination of the basic concepts and the empirical evidence. *Journal of Drug Issues*, 32: 325-394.
Esendal, E., 2007. Tütün tarımı. NKÜ Tekirdağ Ziraat Fakültesi Yayınları (unpublished), Tekirdağ.
Er, C., Yıldız, M., 2014. Keyf Bitkileri. Ankara Üniversitesi Ziraat Fakültesi Yayınları, No:1616, Ankara.

- Öztürk, M., Güvensen, A., Altay, V., Altundağ, E., 2014. Tütünün Botaniksel Özellikleri Hakkında Genel Değerlendirme, Mucizeden Belaya Yolculuk Tütün. Tarihçi Kitapevi. Ocak-2014, İstanbul.
- Peksüslü, A., Yılmaz, İ., İnal, A., Kartal, H., 2002. Tütün genotipleri. Anadolu, 22(2): 82-90.
- Song, Q., Zheng-yin, W., Jun-xiong, S., 2007. Quality characteristics of tobacco leaves with different aromatic styles from Guizhou province, China. Agricultural Sciences in China, 6(2): 220-226.

DETERMINATION OF DIURNAL VARIABILITY IN SUMMER SAVORY (*Satureja hortensis* L.) SPECIES IN ITS NATURAL GROWTH AREA

Memet İNAN¹, Muzaffer KIRPIK¹, Durmuş Alpaslan KAYA²

¹Adıyaman University Kahta Vocational School, Department of Medicinal and Aromatic Plants, Adıyaman, Turkey

²Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Hatay, Turkey

*Corresponding Author email: memetinan@gmail.com

Abstract

Summer savory (*Satureja hortensis* L.) is a species of the Lamiaceae family which grows annually. This study was carried out for determining the diurnal variability of summer savory (*Satureja hortensis* L.) plant picked from its natural growing areas. For that purpose, the plant was harvested in different times of the day (6 a.m., 9 a.m., 12 p.m., 3 p.m., 6 p.m., and 9 p.m.). This study was carried out in order to determine the diurnal variability of the summer savory picked from its natural growth areas for different purposes. The plant was harvested for six times in the full flowering period in the plants found in the natural spreading area (37051'45.48"N and 38038'21.48"E) as Kahta district of Adıyaman province. Some plant characteristics (plant height, number of branches, fresh herbs), essential oil content in leaves and essential oil components were determined. It was determined that there were significant differences between the contents of essential oils and their components during the day according to the data obtained from six harvests. The highest essential oil content (2.07%) was determined at 3 p.m., and the lowest essential oil content (1.61%) was at 9 a.m. Carvacrol (34.22-47.52%), γ -terpinene (23.17-26.27%) and o-cymene (16.96-21.26%) were the most remarkable components in the essential oil. It was also determined that carvacrol as the main component reached to the highest values in the harvests between 6 p.m. and 9 p.m. It was concluded that the most suitable harvests should be conducted between 3 p.m. and 6 p.m. considering both the volatile oil and the carvacrol ratios.

Keywords: Summer savory, diurnal variability, cutting time, carvacrol

INTRODUCTION

The genus of *Satureja* as a member of Lamiaceae family is represented with 200 species spread in the continents of Asia and America including Mediterranean as the leading (Ghannadi, 2002). In Turkey, 28% out of 14 species are endemic (Baydar, 2013). *Satureja hortensis* L. as one of these species (çibriska, çipriska, çibrika, yer kekiği, çam kekiği, ebem kekiği, çay kekiği) naturally grows more on the east of Turkey. Agriculture of this species that has been used as spice or for the treatment of some diseases by the local people is engaged generally in the west of Turkey (Başer et al., 2004).

This species that grows up to 30-35cm in its natural growing area and that has lilac, violaceous and white flowers is generally encountered on rocky sloping lands subjected to erosion (Davis, 1982). Fresh and dried leaves of the plant are used for flatulation and as diaphoretic, appetizer, gastric and stimulant drug (Baytop, 1999). Besides these effects, it has been reported by many researchers that the plants has antibacterial, antifungal, antimicrobial and antioxidant effects (Bandoniene et al., 2002; Güllüce et al., 2003; Adıgüzel et al., 2007; Mihajilov-Krstev et al., 2009; Djenane et al., 2011; Shojaee-Aliabadi et al., 2013).

In our country, especially the species of *Satureja cuneifolia*, *S. wiedemanniana*, *S. thymbra*, *S. hortensi* and *S. cilicica* have remarkable places in trade, and these are generally picked from the Aegean and Mediterranean regions. These aforementioned species are estimated to be picked annually about 600-700 ton (Satıl et al., 2002). It has also been reported that the species of *S. hortensis* picked from different regions of Turkey has essential oil ratio varying between 1,28 and 4,75%, the main component in the plants picked from the west of our country is carvacrol (42-63%), however, the main component in the plants picked from the east is thymol (29-43%) (Başer et al., 2004). It has been determined for cultivated *S. hortensis* species that, the essential oil ration varies between 1,73 and 3,77% in different harvest periods, and the main components of γ -terpinen (17,38- 64,17%) and

carvacrol (15,38- 51,07%) are at a high ratio in this essential oil (Aşçı et al., 2010). In a study that was carried out under Iran conditions, it was reported that different nitrogen and CaCO₃ implementations had positive effects upon essential oil rate and components (Mumivand et al., 2011). In the studies carried out on essential oils in leaves of *S. hortensis* plant, the main components were determined to be the components such as γ -terpinene, carvacrol, α -terpinene, p-cymene, and myrcene (Ghannadi, 2002; Başer et al., 2004; Aşçı et al., 2010; Mumivand et al., 2011; Mohammadhosseini and Beiranvand, 2013; Kargar et al., 2014).

Several factors such as geographical location, altitude, climatic conditions, soil structure, growing conditions and time of harvest are efficient upon essential oil ratio and components of the plants (Telci et al., 2009; Mihajilov-Krstev et al., 2010). In their study upon the species of *Thymbra spicata*, Kaya et al., (2013) determined that the oil ration in fresh and dry herbs was high during the cool morning and evening hours, and the essential oil ratio decreased at midday hours when the temperature increased; the highest carvacrol, γ -terpinen and cymol rates were obtained from the harvests at morning and evening hours. Similarly, it was also reported that the essential oil ratio and components determined at early hours of the morning were higher in the species of *Coridothymus capitatus* L. cultivated naturally at different hours during the day (Arabacı et al., 2015).

This study was carried out for determining the diurnal variability of the essential oil ratio and components in the species of *Satureja hortensis* L. that has a natural narrow spreading area in Kahta district of Adıyaman province and some characteristics of the plant.

MATERIAL AND METHOD

Material

Kahta district of Adıyaman province is located on 37°51'45.48"N and 38°38'21.48"E coordinates; and the plants belonging to the species of *Satureja hortensis* L. picked from its natural spreading areas at 678 m altitude created the material of the study.

Method

The species of *Satureja hortensis* L. that grows in summer in our region starts to blossom in the later August. For that reason, the plant was harvested on 20.09.2017 as the full flowering period following the progress of blossoms in plants. Before harvests, 20 plants representing the population were determined, and some measurements were performed in these plants in order to determine their herbal characteristics. Average and standard deviations of the obtained data were calculated in Excel. For determining diurnal variability in essential oil ratio and components, the plants were harvested at three-hour intervals as of 6 a.m.; and the last harvest was completed at 9 p.m. Totally 6 more harvests were also engaged in different plants of the same population during the day. The essential oil rates determined at temperature, humidity and harvest times when the plants were harvested were presented in Table 1. The weighing was processed labeling the plants in the study area in order to determine the fresh herb and leaf weights in the harvested plants. The thousand seed weight were also harvested in the plants in the same population in order to determine thousand seed weight of the species on 10.10.2017.

Table 1. Temperature, humidity and essential oil rates at hours of harvest for the species of *Satureja hortensis*

Time for Harvest	Temperature (°C)	Humidity (%)	Essential Oil Ratio (%)
6 a.m.	18.3	35	1.92
9 a.m.	24.0	24	1.61
12 p.m.	24.8	21	1.71
3 p.m.	26.9	17	2.07
6 p.m.	25.4	20	1.97
9 p.m.	23.0	22	1.66

Essential Oil Ratio

After the harvests, leaf and stalks were separated. Essential oil rate was obtained by hydrodistillation method using Clevenger apparatus. 300 ml distilled water was added on 20 g leaf sample, distillation occurred in 3 hours and the essential oil rate was calculated.

Determining the Essential Oil Components

The components of the essential oils obtained from the plants were determined in Gas Chromatograph-Mass Spectrometer (GC-MS) device in Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, Medicinal and Aromatic Plants Analysis Laboratory. The essential oil components were determined in Thermo Scientific ISQ Single Quadrupole branded gas chromatography device - TG-Wax MS branded- (5% Phenyl Polysilphenylene-siloxane, 0,25mm inner diameter*30m length, 0,25µm film thickness) using colons. The ionization energy was calibrated as 70 eV, and the mass interval was m/z 1.2- 1200amu. The scan mode was used as the screening more in data collection. MS transfer line temperature was 250°C, MS ionization temperature was 220°C, and whereas colon temperature was 50°C at the beginning, then it was increased up to 220°C with 3°C/min rate of temperature increase. The structure of each component was defined using mass spectrums (Wiley 9) with Xcalibur software.

RESULTS AND DISCUSSION

The results related to some plant characteristics obtained from the harvests engaged in natural growing area of the species of *Satureja hortensis* were presented in Table 2. It was determined in measurements that the length of the plant in the species varied between 32,00 and 47,00 cm; and average plant length of 20 plants was determined to be 38,50 cm. Whereas these determined average plant length values were higher rather than the values reported by some researchers (Baytop, 1999; Adıgüzel et al., 2007; Jadcak, 2007; Aşçı et al., 2010), these were similar to the values reported by some others (Mumivand et al., 2011; El-Gohary et al., 2015). These similarities and differences were arisen from the plant's growing in natural conditions or culture. It was observed that the length of the plant decreased to 15-20 cm in dry stream and stony areas.

The branches that appeared on the plant lined apparently, facing as one another and regularly. The number of branches per plant varied between 10.00 and 19.00, and average number of branches was determined to be 15.00 number/plant. Average number of branches was higher rather than the values of El-Gohary et al. (2015) who reported the number of branches in plants cultivated at different dates as 7.71 and 11.46 number/plant.

Significant differences were also determined between fresh herb productivities per plant. Fresh herb productivity varied between 4.62 and 14.00 g/plant. In a study investigating different fertilization implementations, it was reported that fresh herb productivity per plant varied between 169.00 and 220.00 g (Mumivand et al., 2011), and the productivity per plant was 21.28- 35.64g in different plant periods (El-Gohary et al., 2015). It was determined that fresh herb productivity in cultivated plants varied between 790.5- 1085.1 kg/da (Aşçı et al., 2010) and 2735- 4298 kg/da (Jadcak, 2007). Both per plant productivity and per area productivity differences were arisen from the factors such as growing environments, planting dates and caring conditions of the plant.

As in fresh herb productivity, significant variations appeared in dry herb (2.60- 7.31 g/plant), fresh leaf (1.65- 4.22 g/plant) and dry leaf (0.81- 2.11 g/plant) productivities. These findings were coherent with the values reported by Alizadeh et al. (2010); in their study, Mumivand et al. (2011) determined that the dose of nitrogen created a positive effect upon plants, and dry herb productivity per plant varied between 37.95 and 52.75 g. These reported values were fairly higher rather than the ones we determined. And this revealed the species to have a better performance under culture conditions.

It was specified while analyzing the seeds of the species that thousand seed weight varied between 0.22 and 0.26 g, and average thousand seed weight was 0.24 g (Table 2). Thousand seed weight of *S. hortensis* species was reported to be at 0.5- 0.8 g interval (Anonymous, 2018). These reported values were higher rather than our findings. This difference was probably arisen from plants' growing in different geographical regions.

Table 2. Some herbal properties related to the species of *Satureja hortensis*.

	Min	Max	Avg.	Standard Deviation
Plant Length (cm)	32.00	47.00	38.50	5.30
Number of Branches (number/plant)	10,00	19.00	15.00	3.13
Fresh Herb (g/plant)	4.62	14.00	7.31	2.75
Dry Herb (g/plant)	2.60	7.31	3.74	1.45
Fresh Leaf (g/plant)	1.65	4.22	2.52	0.84
Dry Leaf (g/plant)	0.81	2.11	1.29	0.41
Thousand Seed Weight (g)	0.22	0.26	0.24	0.01

Essential Oil Rate and Components

It was determined that essential oil rates varied between 1.61- 2.07 % during the day (Table 3). The lowest essential oil ratio in flowery leafs was obtained during the harvests at 9 a.m., and the highest essential oil ratio was obtained from the harvest at 3 p.m. in harvests at 6 a.m. and 6 p.m., essential oil rate was determined to be 1.92 and 1.97 %, respectively. This proved that there was no remarkable difference between essential oil rates at sunrise and sunset. In other words, it was necessary for plants of the species to be picked either at early hours of the morning or at 3-6 p.m. Similar to our findings, it was also reported in the studies carried out on the species of *Thymbra spicata* (Kaya et al., 2013) and *Coridothymus capitatus* (Arabacı et al., 2015) that high essential oil rates were determined during the harvests engaged at cool hours of the day.

Table 3. The results related to the diurnal variations of essential oil and components in leaf of *Satureja hortensis*

		Time for Harvest					
		6 a.m.	9 a.m.	12 p.m.	3 p.m.	6 p.m.	9 p.m.
Essential Oil Rate (%)		1.92	1.61	1.71	2.07	1.97	1.66
RT	Compound Name						
3,43	α -Pinene	0.98	1.08	1.06	1.18	0.65	0.63
3,49	α -Phellandrene	0.65	0.95	1.01	0.98	0.99	0.90
4,79	β -Pinene	0.51	0.60	0.65	0.69	0.27	0.25
6,05	β -Myrcene	1.11	1.16	1.17	1.17	1.06	1.05
6,4	α -Terpinene	1.60	1.89	1.98	1.96	1.93	1.76
6,88	l-Limonene	0.24	0.25	0.25	0.25	0.22	0.21
8,23	γ-Terpinene	25.93	23.17	26.27	24.38	24.51	24.10
9,01	o-Cymene	21.26	16.96	17.69	18.60	20.28	20.35
20,89	Terpinen-4-ol	0.19	0.20	0.21	0.21	0.26	0.27
40,91	Thymol	7.72	15.42	7.21	12.64	0.12	0.12
41,69	Carvacrol	36.48	34.54	34.22	34.40	47.01	47.52
Total (%)		96.67	96.22	91.72	96.46	97.30	97.16

Totally 11 components in the essential oil obtained from the species of *S. hortensis* harvested from the nature created at least 91 % of the essential oil. Among these components, carvacrol, γ -terpinene, o-cymene and thymol were remarkable, respectively. Diurnal carvacrol rate varied between 34.22 and 47.52%. The highest carvacrol rate was determined to be 47.01 % and 47.52 % in the last two harvests, and the lowest rate was determined to be 34.22 %-34.54% in harvests at 9 a.m. and 3

p.m. Whereas γ -terpinene as the highest second component was measured to be 26.27 % at midday, the lowest rate as 23.17 % was determined at harvests engaged at 9 a.m. During the cool hours of the day (6 a.m., 6 p.m. and 9 p.m.) o- cymene rate was between 20.28 and 21.26%, and at hot hours (9 a.m.-3 p.m.) the rate of o-cymene decreased down to 16.96- 18.60 %, and the rate for thymol was determined to be 15.42 % and 12.64 %, respectively at these hours. At the last two harvests, the rate for thymol decreased down to 0.12%. In their study upon *T. spicata*, Kaya et al. (2013) reported that the lowest carvacrol rate was obtained during the midday (1 p.m.-2 p.m.), and the highest carvacrol value was obtained from the harvests at 3 a.m. and 6 a.m.; moreover, in their study upon the species of *C. capitatus*, Arabacı et al. (2015) determined that the highest carvacrol rate was obtained from the harvests at 2 p.m., and they also obtained high carvacrol rate from the harvest at 6 a.m. Depending upon these reported findings and the results we obtained from the study we carried out, it was possible to mention that the carvacrol rate in essential oils of this plant was higher in hours with cool weather.

CONCLUSION

It has been known that there are several environmental factors affecting the essential oil rate and components in plants. Furthermore, there have been various studies related to plants' including active agents at different amounts during the day. In this study carried out on determining the diurnal variability in the species of *Satureja hortensis*, it was determined that the highest essential oil ratio was obtained from the harvests at 3 p.m. and 6 p.m., and the rate of carvacrol as a remarkable component increased at these hours. For that reason, it was specified that it would be better for the species of *Satureja hortensis* to be harvested after the noon time in our local area.

REFERENCES

- Adıgüzel, A., Özer, H., Kılıç, H., Cetin, B., 2007. Screening of antimicrobial activity of essential oil and methanol extract of *Satureja hortensis* on foodborne bacteria and fungi. Czech Journal of Food Sciences, 25 (2): 81- 89.
- Alizadeh, A., Khoshkhui, M., Javidnia, K., Firuzi, O., Tafazoli, E., Khalighi, A., 2010. Effect of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in *Satureja hortensis* L. (Lamiaceae) cultivated in Iran, Journal of Medicinal Plants Research, 4(1): 33- 40.
- Anonim, 2018. *Satureja hortensis* L., Plant Resources of South- East Asia, http://uses.plantnet-project.org/en/Satureja_hortensis (PROSEA)
- Arabacı, O., Tokul, H.E., Öğretmen, N.G., Bayram, E., 2015. Doğal yayılış gösteren *Coridothymus capitatus* L. genotiplerinde diurnal varyabilitenin verim ve kaliteye etkisi, Ege Üniversitesi Ziraat Fakültesi Dergisi, 52 (2): 141- 150.
- Aşçı, M., Kırıcı, S., Turk, M., İnan, M., 2010. Determination of variability of essential oil and agricultural characterization of *Satureja hortensis* L. on date of harvest during flowering period in the Çukurova condition, , 6th Conference on Aromatic and Medicinal Plants of Southeast European Countries (6th CMAPSEEC), p:1025- 1036, April 18- 22, 2010, Antalya/TURKEY.
- Başer, H. K. C., Özek, T., Kırimer, N., 2004. A Comparative Study of the Essential Oils of Wild and Cultivated *Satureja hortensis* L., J. Essent. Oil Res., 16, 422-424.
- Bandoniene D., Venskutonis P. R., Gruzdiene D., Murkovic M., 2002. Antioxidative activity of sage (*Salvia officinalis* L.), savory (*Satureja hortensis* L.) and borage (*Borago officinalis* L.) extracts in rapeseed oil. European Journal of Lipid Science and Technology, 104(5): 286- 292.
- Baydar, H., 2013. Tıbbi ve Aromatik Bitkiler Bilimi ve Teknolojisi (4. Baskı), Süleyman Demirel Üniversitesi, Yayın No: 51, 224.
- Baytop, T., 1999. Therapy with Medicinal Plants in Turkey (Past and Present), Publication of Istanbul University, No. 3255, p.332. ISBN:975-420-021-1
- Davis P.H., 1982. Flora of Turkey and East Aegean Islands. 7,314-322, Edinburg University Pres.
- Djenane, D., Yanguela, J., Amrouche, T., Boubrit, S., Boussad, N., Roncales, P., 2011. Chemical composition and antimicrobial effects of essential oils of *Eucalyptus globulus*, *Myrtus communis* and *Satureja hortensis* against *Escherichia coli* O₁₅₇ :H₇ and *Staphylococcus aureus* in minced beef. Food Science and Technology International, 17(6): 505- 515.
- El-Gohary, A.E., El Gendy, A.G., Hendawy, S.F., El-Sherbeny, S.E., Hussein, M.S., Geneva, M., 2015. Herbage yield, essential oil content and composition of summer savory (*Satureja hortensis* L.) as affected by sowing date and foliar nutrition, Genetic And Plant Physiology, 5(2): 170- 178.
- Ghannadi, A., 2002. Composition of the essential oil of *Satureja hortensis* L. seeds from Iran, J. Essent. Oil Res., 14: 35- 36.

- Güllüce, M., Sökmen, M., Daferera, D., Ağar, G., Özkan, H., Kartal, N., Polissiou, M., Sökmen, A., Sahin, F., 2003. *In vitro* antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of *Satureja hortensis* L. Journal of Agricultural and Food Chemistry, 51 (14): 3958- 3965.
- Jadcza, D., 2007. Effect of Sowing Date on the Quantity and Quality of the Yield of Summer Savory (*Satureja hortensis*) Grown for a Bunch Harvest. Herba Polonica, 53 (3): 22- 26.
- Kargar, V., Alizadeh, A., Namayandeh, A., 2014. Essential oil constituents of *Satureja sahendica* Bornm. and *Satureja hortensis* L. cultivated in Iran, International Journal of Farming and Allied Sciences, 3(1): 91- 94.
- Kaya, D., A., Arslan, M., İnan, M., Baskaya, S., 2013. Diurnal Changes on Content and Composition of *Thymbra Spicata* L. Essential Oil, Research Journal of Biological Sciences 8 (1): 6- 10.
- Mihajilov-Krstev, T., Radnovic, D., Kitic, D., Stojanovic-Radic, Z., Zlatkovic, B., 2009. Antimicrobial activity of *Satureja hortensis* L. essential oil against pathogenic microbial strains. Biotechnology & Biotechnological Equipment, 23(4):1492- 1496.
- Mohammadhosseini, M., ve Beiranvand, M., 2013. Chemical composition of the essential oil from aerial parts of *Satureja hortensis* as a potent medical plant using traditional hydrodistillation, Journal of Chemical Health Risks, 3(4): 43- 54.
- Mumivand, H., Babalar, M., Hadian, J., Fakhr-Tabatabaei, M., 2011. Plant growth and essential oil content and composition of *Satureja hortensis* L. cv. Saturn in response to calcium carbonate and nitrogen application rates, Journal of Medicinal Plants Research, 5(10), 1859- 1866.
- Satıl, F., Dirmenci, T., Tümen, G., 2002. Türkiye’deki *Satureja* L. Türlerinin Ticareti ve Doğadaki Durumu-I, 14. Bitkisel İlaç Hammadeleri Toplantısı, 29- 31 Mayıs 2002, Eskişehir, 94- 100.
- Shojaee-Aliabadi, S., Hosseini, H., Mohammadifar, M. A., Mohammadi, A., Ghasemlou, M., Ojagh, S. M., Hosseini, S. M., Khaksar R., 2013. Characterization of antioxidant-antimicrobial kappa-carrageenan films containing *Satureja hortensis* essential oil, International Journal of Biological Macromolecules, 52: 116- 124.
- Telci, I., Demirtas, I., and Sahin, A., 2009. Variation in plant properties and essential oil composition of sweet fennel (*Foeniculum vulgare* Mill.) fruits during stages of maturity, Industrial Crops and Products, 30:1; 126–130.

EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON ROOTING OF *Laurus nobilis* L. STEM CUTTINGS

Aysun ÇAVUŞOĞLU¹, M. Ufuk KASIM¹, Rezzan KASIM¹

¹Kocaeli University Arslanbey Agricultural Vocational School, Kocaeli, Turkey

Corresponding author email: cavusoglu@kocaeli.edu.tr

Abstract

In this study, effect of Arbuscular Mycorrhizal mix application on rooting of softwood stem cuttings prepared as terminal or base cutting of laurel (*Laurus nobilis* L.) at greenhouse condition, were studied. Cuttings were planted after inoculation with mycorrhizal powder mix level as; 0 g/kg (control) 1 g/kg, 2 g/kg or 3 g/kg in peat as rooting media at the time of bedding. The planted cuttings were kept 120 days in the rooting medium and after the time; cuttings viability rate (%), rooting rate (%), average root number per rooted cutting and average root length per rooted cutting were calculated from the removed cuttings. Data show that inoculation of the mycorrhizal mix at rate of 1 g/kg peat media, root length in terminal cuttings (3 cm/cutting) significantly affected. In base cutting when the mycorrhizal mix used as 2 g/kg peat; root number (3.42/cutting) and root length (3.29 cm/cutting) reached maximum data. When two cutting types are compared; rooting rate, root number and root length in base cuttings showed statistically higher results than terminal cuttings.

Keywords: *Laurus nobilis*, Mycorrhiza, rooting, cuttings

INTRODUCTION

Laurel (*Laurus nobilis* L.) is an utilized plant in different branches of industry, medicine, agriculture and forestry (Anonymous, 2018). Laurel plant has some important phytochemicals (Sayyah et al., 2002; Patrakar et al., 2012; Goudjil et al., 2015) that can vary according to initial mother plants, geographical regions and plant parts. Since the culture of the laurel plant is not yet widespread, it is obtained in the form of collecting from the its natural habitat. In this case it is inevitable that causes destruction in the nature. In this instance new laurel plantations and orchards should be set up under the congruous areas. For this purpose, healthy seedling or sampling must be obtained in the first step.

Vegetative propagation through cutting is the one of the most studied propagation methods for sampling in laurel besides seed germination (Herrera et al., 2004; Parlak, 2008; Vlad and Vlad, 2008; Şirin and Tekintaş, 2010; Nissim-Levi et al., 2014). Most of the studies had reached possitive results on rooting but data showed that rooting percentage are not enough because of certain reasons.

Mycorrhizal inoculation is mostly known as rooting progressive agent and a kind of helper for phosphorus uptake in plants. There are some scientific studies and reviews on mycorrhizal benefits in plant propagation and in agriculture (Powell and Bagyaraj, 1984; Bolan, 1991; Palenzuela et al., 2002; Koide and Mosse, 2004; Porras-Piedra et al., 2005; Bidarnamani and Mohkami, 2014; Javanmardi et al., 2014). Although the mentioned studies, at present there are very few works available in terms of laurel-mycorrhizal relationships and laurel propagation by mycorrhizae (Vallini et al., 1993; Hassiotis and Dina, 2011; Pirlak, 2012).

We aimed in this study, effect of Arbuscular Mycorrhizal mix application on rooting of softwood stem cuttings prepared as terminal or base cutting of laurel (*Laurus nobilis* L.).

MATERIAL AND METHODS

The pot experiment was conducted in the greenhouse, Arslanbey Vocational School, Kocaeli University, under Kocaeli Province ecological condition, Turkey in between March 31-July 29, 2015. The treated cuttings were obtained from seedlings (Fig. 1A) which germinated from seeds that obtained from only one determined fruitful mother plant. The cuttings were prepared as terminal and

base cuttings in 10 cm and all the leaves were removed from cuttings, after media preparation, just before planting in the same day (Fig. 1B). Commercial peat was used as growing medium that originated from Lithuania as sphagnum peat, consisted from nitrogen, phosphorus pentaoxide, potassium oxide, boron, copper, iron, manganese, molybdenum, zinc, chlorine at different levels according to company statement. The treated commercial arbuscular mycorrhizal mix was imported from U.S.A. as endo roots soluble mycorrhizas consisted from eight different species of *Glomus* genus and one species of *Gigaspora* sp. according to company statement.

The peat was prepared by hand and mycorrhizal mix added as powder in the dose of 1 g/kg, 2 g/kg and 3 g/kg peat, besides control (0 g/kg). After the addition, peat mix was well stirred for obtain uniform rooting mix. The prepared peat, mixed with mycorrhizal powder and control, shared the pots as 500 g after than prepared cuttings were placed as 1/3 portion in peat for rooting. All pots were irrigated as needed at same rate during the experiment. After the 120 days, cuttings viability rate, rooting rate (in viable cuttings), root number (in rooted cuttings), root lenght (in rooted cuttings) were calculated. The study designed as Completely Radomized Design replicated thrice (Fig. 1C) and statistical analyzes were done by using SPSS 16.0 programme. Each pot consisted 6 terminal and 6 base cuttings. Totally each treatment consisted 18 sample. The data were transformed by the arcsine square for percentage means.

RESULTS AND DISCUSSION

According to the results (Table 1) viability rates of cuttings are between 94.4-100 % in terminal cuttings and 73.3-100 % in base cuttings. According to a previous study (Cavusoglu and Sulusoglu, 2014) laurel cutting viability rate changed between 17-100% in perlite media. But cutting time, initial mother plants, media and treatment differences should not be ignored.

Table 1. The Effect of Mycorrhizal Inoculation on Rooting-Related Parameters of *Laurus nobilis* L.* ,** ,***

TREATMENTS	Viability Rate (%)		Rooting Rate (%)*		Root Number (number/cutting)		Root Lenght (cm/cutting)	
	Terminal Cutting	Base Cutting	Terminal Cutting	Base Cutting	Terminal Cutting	Base Cutting	Terminal Cutting	Base Cutting
Control	95.8 a	100 a	14.3 a	73.3 a	2.75 a	2.57 a	2.28 b	2.66 a
1 g/kg Mycorrhizae	94.4 a	73.3 a	9.5 a	76.7 a	2 ab	2.83 a	3 a	2.88 a
2 g/kg Mycorrhizae	100 a	93.3 a	5.6 a	56.7 a	1 bc	3.42 a	0.5 c	3.29 a
3 g/kg Mycorrhizae	95.2 a	100 a	0 a	43.3 a	0 c	2.72 a	0 d	2.12 a
Total	96.35 A	91.65 A	7.35 B	62.5 A	1.44 B	2.89 A	1.45 B	2.74 A

* Data were transformed by the arcsine square for percentage means.

**In the table, lower cases (a, b or c) in the columns are presented the effect of mycorrhizal mix doses on different cutting types (significantly differences at P<0.05)

***In the table, capital letters (A or B) are presented different cutting types effect on rooting parameters (significantly differences at P<0.05)

Rooting rate in base or terminal cutting are not show significantly importance according to mycorrhizal inoculation but in totally base cuttings are statistically give more rooting rate (62.5%) than terminal cutting (7.35%) (Fig. 1D, E). In a study (Parlak, 2008), different cutting time, plant growth regulator doses and rooting media were studied, and the highest rooting rate in laurel was found at the rate of 63.3% in July when treated with 5000 ppm IBA.

Our data show that inoculation of the mycorrhizal mix at rate of 1 g/kg peat media, root lenght in terminal cuttings (3 cm/cutting) statistically possitively affected. In the base cutting when the mycorrhizal mix used as 2 g/kg peat; root number (3.42/cutting) and root lenght (3.29 cm/cutting) reached maximum data. Similarly Porras-Piedra et al., (2005) worked on olive cuttings with three

different *Glomus* spp. treatments at nursery and older phases. They stated that early inoculation of the roots of cuttings with VAM fungi stimulates their growth during the nursery and early development stage.

Zai et al. (2007) studied on *Prunus maritima* cuttings with tree different *Glomus* species and they have found that *Glomus* fungi has beneficial effects on root induction and growth of cuttings, in addition hardwood cuttings was found better than softwood cuttings. In our study, when mycorrhizal inoculation ignored, and two cutting types are compared; rooting rate (62.5%), root number (2.89) and root length (2.74 cm) in base cuttings showed statistically higher results than rooting rate (7.35%), root number (1.44) and root length (1.45 cm) in terminal cuttings.



Fig 1. The step of the study of Laurel (*Laurus nobilis* L.) rooting with the aid of mycorrhizal inoculation; A) Mother plants derived from seed, B) Obtained base and terminal cuttings from a mother plant, C) Pot experiment, D) Removed base cuttings from peat inoculated with 2 g/kg mycorrhizal mix. E) Removed terminal cuttings from peat inoculated with 3 g/kg mycorrhizal mix.

CONCLUSION

According to the results of the experiment, under the experimental condition when rooting rate is zero in terminal cuttings at 3 g/kg mycorrhizal inoculation, at the same dose also gave the lowest data in rooting rate, root number and root length in base cuttings. In the base cuttings, the highest rooting rate was obtained from 1 g/kg mycorrhizal inoculation and the highest root number and root length was obtained from 2 g/kg mycorrhizal inoculation in peat media numerically. When two cutting types compared, base cuttings also gave highest rooting rate, root number and root length statistically. Despite the fact that this work is hopeful, further studies needs to be tested by additional experiment because cutting time, media, pot size, dose, initial plants etc. can be cause different results.

REFERENCES

- Anonymous, 2018. Defne Eylem Planı. www.ogm.gov.tr/ekutuphane/Yayinlar/Defne_Eylem_Planı.pdf (date of access: 22.06.2018).
- Bidarnamani, F., Mohkami, Z., 2014. Enhanced rooting of leaf bud cuttings of *Schefflera arboricola* using mycorrhizal fungi. Annual Research & Review in Biology, 4(18): 2892-2900.
- Bolan, N.S., 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil, 134: 189-207.
- Cavusoglu, A., Sulusoglu, M., 2014. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) on rooting of female and male *Laurus nobilis* L. cuttings. International Journal of Biosciences, 4(11): 206-216.
- Goudjil, M.B., Ladjel, S., Bencheikh, S.E., Zighmi, S., Hamada, D., 2015. Study of the chemical composition, antibacterial and antioxidant activities of the essential oil extracted from leaves of Algerian *Laurus nobilis* Lauraceae. Journal of Chemical and Pharmaceutical Research, 7(1): 379-385.
- Hassiotis, C.N., Dina, E.I., 2011. The effects of laurel (*Laurus nobilis* L.) on development of two mycorrhizal fungi. International Biodeterioration & Biodegradation, 65: 628-634.
- Herrera, T.I., Ono, E.O., Leal, F.P., 2004. Efeitos de auxina e boro no enraizamento adventicio de estacas caulinares de louro (*Laurus nobilis* L.). Biotemas, 17(1): 65-77.
- Javanmardi, J., Zarei, M., Saei, M., 2014. Influence of arbuscular mycorrhizal fungi on physiology and fruit quality of pepino (*Solanum muricatum* Ait.) in vermicompost amended medium. Adv. Hort. Sci., 28(1): 35-42.
- Koide, R.T., Mosse, B., 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza, 14: 145-163.
- Nissim-Levi, A., Ovadia, R., Kagan, S., Oren-Shamir, M., 2014. Shading stock plants with photoselective nets affects the yields and rooting quality of their cuttings. Journal of Horticultural Science & Biotechnology, 89(6): 693-699.
- Palenzuela, J., Azcon-Aguilar, C., Figueroa, D., Caravaca, F., Roldan, A., Barea, J.M., 2002. Effects of mycorrhizal inoculation of shrubs from Mediterranean ecosystems and composted residue application on transplant performance and mycorrhizal developments in a desertified soil. Biol Fertil Soils, 36: 170-175.
- Parlak, S., 2008. Propagation of bay laurel (*Laurus nobilis* L.) by cuttings. Ege Forestry Research Institute, İzmir-Turkey, Technical Bulletin No: 42: 71 p.
- Patrakar, R., Mansuriya, M., Patil, P., 2012. Phytochemical and pharmacological review on *Laurus nobilis*. International Journal of Pharmaceutical and Chemical Sciences, 1(2):595-602.
- Pırlak, İ.T., 2012. Defne (*Laurus nobilis* L.) ve keçiboynuzu (*Ceratonia siliqua* L.) türlerinin büyüme ve gelişmesi üzerine ortam ve mikoriza aşılmasının etkisi. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, 77p.
- Porrás-Piedra, A., Soriano-Martin, M.L., Porrás-Soriano, A., Fernández-Izquierdo, G., 2005. Influence of arbuscular mycorrhizas on the growth rate of mist-propagated olive plantlets. Spanish Journal of Agricultural Research, 3(1): 98-105.
- Powell, C.L., Bagyaraj, D.J., 1984. Effect of mycorrhizal inoculation on the nursery production of blueberry cuttings. New Zealand Journal of Agricultural Research, 27: 467-471.
- Sayyah, M., Valizadeh, J., Kamalinejad, M., 2002. Anticonvulsant activity of the leaf essential oil of *Laurus nobilis* against pentyleneetetrazole-and maximal electroshock-induced seizures. Phytomedicine, 9: 212-216.
- Şirin, U., Tekintaş, F.E., 2010. Anatomical and histological investigations of adventive root formation on laurel (*Laurus nobilis* L.) cuttings. ADÜ Ziraat Fakültesi Dergisi, 7(1): 35-41.
- Vallini, G., Pera, A., Avio, L., Valdrighi, M., Giovannetti, M., 1993. Influence of humic acids on laurel growth, associated rhizospheric microorganisms, and mycorrhizal fungi. Biol Fertil Soil, 16: 1-4.
- Vlad, M., Vlad, I., 2008. Stimulation of striking roots at the cuttings of laurel tree (*Laurus nobilis*) with the aid of bioactive substances of the radistim type. Not. Bot. Hort. Agrobot. Cluj, 36(2): 32-34.
- Zai, X., Qin, P., Wan, S., Zhao, F., Wang, G., Yan, D., Zhou, J., 2007. Effects of arbuscular mycorrhizal fungi on the rooting and growth of beach plum (*Prunus maritima*) cuttings. Journal of Horticultural Sciences & Biotechnology, 82(6): 863-866.

DETERMINATION OF AGRONOMIC AND QUALITY CHARACTERISTICS OF BLACK CUMIN SPECIES (*Nigella sativa* L. and *N. damascena* L.) SOWED AT DIFFERENT TIMES

Mehmet Necat İZGİ¹, İsa TELCİ²

¹Kızıltepe Technical Vocational School of Higher Education, Mardin Artuklu University, Mardin, Turkey

²Department of Field Crops, Faculty of Agriculture, Suleyman Demirel University, Isparta, Turkey

Abstract

This study aims to determine the proper sowing time for the growers of black cumin, a species of the *Nigella sativa* and *N. damascena* species of *Nigella* genus. The study was carried out under the climatic conditions of Mardin plain during 2011-2012 and 2012-2013 vegetation periods. The plants were sowed in four different times: two times in autumn (November 10 and November 25) and two times in spring (March 03 and March 17). – Agronomical and quality characteristics of the species sown four different times were investigated in the study. It was found a significant difference between sowing times for all the characteristics examined in the study. In the 2-year average, the seed yield of *sativa* (3725.8 kg/ha) was found to be higher than that of *damascena* (2829.0 kg/ha). Higher yields were obtained in both species in autumn sowing. The study determined that *damascena*, which is more susceptible to cold climate, is not affected by winter sowing in the region and that autumn sowing should be preferred more for both species to obtain higher yields.

Keywords: *Nigella sativa*, *N. damascena*, black cumin, sowing time, seed yield

INTRODUCTION

Black cumin (*Nigella sativa* L.), belonging to the Ranunculaceae family, is an annual herbaceous plant with high economic value. There are a total of 20 species belonging to *Nigella* genus in the world and 14 species in Turkey flora (Seçmen et al., 2000, Davis, 1965). Two species have commercial value. *Nigella sativa* L. is grown for use as medicine and spice whereas *Nigella damascena* L. is grown as ornamental plant besides its general use (Telci et al., 2014, Ertaş 2016).

Commercial black cumin species are annual, herbaceous, 20-60 cm long and its branches can grow to a certain extent. Its flowers comprise 5 parts, are bluish and show protandry during fertilization (Ceylan, 1996). The nectar-rich flowers are at the edge of the main stem and side branches; after fertilization, capsules are formed in the flowers which contain seeds in excess (Baytop, 1999, Ceylan, 1996). The seeds of the plants are three-sided, black colored, 1.5-2 mm long and have a bitter taste and aromatic scent (Baytop, 1999). Black cumin seeds are used in a variety of bakery products (bread, muffins, biscuits, etc.) and some cheese varieties (Akgül, 1993; Er and Yıldız, 2007; Filippo et al., 2002).

Black cumin is grown in Turkey in the cities such as Afyon, Uşak and Kütahya in the central western Anatolia region, especially in the Göller Region. In recent years, the sowing area of this species has increased in the region. Production areas of black cumin are increasing in recent years especially in Southeastern Anatolia. Agro-technical studies are important for the new growers of the regions. For this reason, the study was set up to determine the appropriate sowing times of two commercial black cumin species.

MATERIALS AND METHOD

Two black cumin species (*Nigella sativa* and *N. damascena*) were used in the study. *N. sativa* was obtained from Tokat and *N. damascena* from Diyarbakır and uniformed as a results of selection studies for 4 years at Gaziosmanpaşa University. The study was carried out during two vegetation periods, 2011- 2012 and 2012- 2013 under the plain conditions of of Mardin according to the

randomized blocks split plot design with three replications. The main plots consisted of species while the sub-plots consisted of sowing times.

The plants were sowed in 4 different times: two of them in the autumn (November 10 and November 25) and the other two in the spring (March 03 and March 17). There were five rows of plots with a height of 3 m and a distance of 20 cm in-between. During the study, fertilizer with 6 kg/da N and 4 kg/da P₂O₅ (TSP) was given to the plants. Half of the nitrogenous fertilizer was given in the field as the bottom fertilizer and the other half was spread before the bolting. The irrigation was made when the plant needed water and the weed control was made by hand. Samples were taken from the plants at the stage of maturation from the parts of the plant remained after the edge effects were taken (1 row from the edge, 25 cm from the beginning of the plot) to investigate characteristics such as harvest, plant height (cm), number of branches (number/plant), number of capsules (piece/plant), thousand seed weight (g), seed yield (kg/da), fixed oil ratio (%) and fixed oil yield (L/da). Seed oil content were determined with hexane extraction in Soxhalet apparatus in field crops laboratory of Süleyman Demirel University. F values were calculated by performing variance analysis on the obtained values and important features were grouped by Duncan test.

RESULTS AND DISCUSSION

In this study, which was conducted with the aim of determining the impact of different sowing times on the agronomic and quality characteristics of black cumin species (*Nigella sativa* L. and *N. damascena* L.), there was a significant difference between sowing times for the all examined characteristics according to the results of the variance analysis. There was no significant difference for all the characteristics between years; but there was a significant difference among the species only in 1000 seed weight. The year x sowing time was found to be significant for plant height and the year x species interactions was found to be significant for the number of branches (Table 1). The characteristics examined in the study are explained under the subheadings below.

Plant Height

Plant height changed between 43.13 and 63.25 cm in *N. sativa* and between 43.38 and 61.60 cm in *N. damascena* (Table 2). The average values were 52.39 cm in *sativa* and 52.65 cm in *damascena* and the difference between the species was found to be insignificant (Table 2). For both species, higher plant heights were obtained in the autumn sowing when compared to the spring sowing. Higher average values were obtained in autumn sowing compared to spring sowing. Due to long vegetation period, the fact that plants are not harmed during winter and the spring rainfall is well evaluated by plants, the plants sowed in the autumn in the region grew higher than those sowed in the spring. It was found out that although *damascena* is more sensitive to winter than *sativa*, and although it damaged by winter colds in northern high altitude areas (Ertaş 2016), it was not damaged by winter colds during both years in the region.

Table 1.F-values and significance calculated as a result of variance analysis of the characteristics of *N. sativa* and *N. damascena* species sowed at different times under the conditions of Mardin.

Variation sources	Plant height (cm)	Number of branches	Number of capsules	Thousand seed weight (g)	Seed yield (kg/ha)	Seed oil rate (%)	Seed oil yield (L/ha)
Year (Y)	0.07	0.07	0.54	1.28	0.13	0.12	0.09
Sowing time (ST)	37.73**	12.32**	11.94**	24.70**	15.87**	1.5	11.11**
Species (S)	0.03	1.21	0.07	14.82**	3.22	0.38	2.25
Y×ST	4.45*	0.21	0.36	0.77	0.52	0.55	0.36
Y×S	0.42	6.02*	0.84	0.05	0.01	0.30	0.01
ST×S	1.75	1.70	0.51	0.70	2.01	1.07	1.41
Y×ST×S	0.20	0.35	0.65	0.43	0.12	0.40	0.08

* p<0.05, ** p<0.01

Number of Branches

The number of branches varied from 3.33 to 5.72 in *N. sativa* and 3.48 to 4.73 in *N. damascena*. The average values for species were determined as 4.39 in *sativa* and 4.14 in *damascena* and the difference between species was found to be insignificant (Table 2). In both species, more branches were obtained in autumn sowing than in spring sowing. Similarly, the average values of were higher in autumn than in spring. Because the plant height in autumn sowing is longer due to the reasons explained above, the number of branches was also greater. The study revealed that the number of branches of black cumin varies according to populations and agricultural operations. Baytöre (2011) stated that the number of branches varied between 3.45 and 4.42 per plant in different populations; Özel et al (2009) stated that it varied between 2.30 and 4.43 per plant and Telci (1995) stated that it varied between 4.19 and 5.22 per plant.

Table 2. Values regarding the characteristics of *N. sativa* and *N. damascena* species sowed at different times under the conditions of Mardin.

	Plant height (cm)	Number of branches	Number of capsules	Thousand seed weight (g)	Seed yield (kg/ha)	Seed oil rate (%)	Seed oil yield (L/ha)
<i>Nigella sativa</i>							
1st Sowing	63.25	5.72	9.78	1.97	310.40	35.1	109.0
2nd Sowing	55.72	4.58	9.30	2.02	372.58	35.9	133.8
3rd Sowing	47.47	3.33	6.63	1.72	166.60	36.5	60.8
4th Sowing	43.13	3.93	6.90	1.70	198.97	35.6	70.8
Mean Avg.	52.39	4.39	8.15	1.85	262.14	35.8	93.8
<i>Nigella damascena</i>							
1st Sowing	60.80	4.73	9.02	1.92	263.22	34.7	91.3
2nd Sowing	61.60	4.65	9.70	1.85	282.90	36.4	103.0
3rd Sowing	44.85	3.70	7.13	1.53	202.03	36.3	73.3
4th Sowing	43.38	3.48	7.22	1.53	168.78	36.6	61.8
Mean	52.66	4.14	8.27	1.71	229.23	36.0	82.5
Mean							
1st Sowing	62.03a*	5.23a	9.40a	1.94a	286.81a	34.89	100.1a
2nd Sowing	58.66a	4.62a	9.50a	1.93a	327.74a	36.18	118.4a
3rd Sowing	46.16b	3.52b	6.88b	1.63b	184.32b	36.35	67.1b
4th Sowing	43.26b	3.71b	7.06b	1.62b	183.88b	36.11	66.3b

*The differences between the averages indicated by the same letter are not significant.

Number of Capsules Per Plant (number/plant)

The number of capsules per plant was found to be 6.63-9.78 in *N. sativa* and 7.13-9.70 in *N. damascena* (Table 2). The average values of species were determined as 8.15 in *sativa* and 8.26 in *damascena* and the difference between species was found to be insignificant. The average number of capsules for both species was greater in autumn than in spring. The average number of capsules in autumn sowings was found to be 9.40 and 9.50, respectively, and 6.88 and 7.06 for spring.

The change intervals for black cumin were found to be greater. According to Özel et al (2009), it ranged from 2.57 to 15.97. Number of capsules per plant can even be up to 25.10 capsule/ plant, according to Tektaş (2015). This change depends on factors such as ecology, sowing time and frequency.

Thousand Seed Weight (g)

Thousand seed weights were found to be 1.70-2.02 g in *N. sativa* and 1.53-1.92 g in *N. damascena*. Average values by the species were determined as 1.85 g in *sativa* and 1.70 g in *damascena*; and sowing time had a significant impact on all the characteristics examined; a significant difference was found among species only in terms of thousand seed weights. For both species, thousand seed weights were found to be higher in autumn than in spring. There was a significant difference in thousand seed weight in autumn and in spring (Table 2).

The values for *N. sativa* were found to be higher than those of *N. damascena* in terms of thousand seed weight. Thousand seed weight of *N. sativa* was recorded to vary from 2 to 3 g. This change varies according to genotype, aquaculture and ecology, as it is in other characteristics (Tektaş 2015, Özel et al 2009, Telci 1995, Kılıç and Arabacı 2016).

Seed Yield (kg/da)

The seed yield varied between 1666.0 and 3725.8 kg/ha in *N. sativa* and between 1688.8 and 2829.0 kg/ha in *N. damascena*. The average values of the species were found to be 2621.4 kg/ha in *sativa* and 2292.3 kg/ha in *damascena* and the difference between the species was found to be insignificant.

The highest yield was obtained from the second sowing in the autumn with 3277.4 kg/ha, followed by the first sowing with 2868.0 kg/ha. However, the difference between these two values was found to be insignificant. In spring sowings, there was a significant decrease in yields compared to autumn sowings (3rd sowing 1843 kg/ha and 4th sowing 1839 kg/ha); both sowing times in the spring were categorized in the same group (Table 2).

The reasons for higher yields in the plants sowed in the region in autumn than those sowed in spring are the fact that the plants sowed in autumn have a longer vegetation period, they have a more developed root system, they make good use of spring rainfalls and that they are not damaged by winter cold (Kaya et al. 2000).

The range of change in seed yield of black cumin was found to be quite broad, varying depending on the climatic conditions of the area, agriculture and irrigation conditions and agro-technical applications such as sowing time and frequency. The values obtained in autumn were found to be in accordance with the high values obtained in the study conducted under the conditions of similar climate in Urfa (Özel et al., 2009) whereas these values were found to be higher than the values obtained in the studies conducted with summer sowings in Central Anatolia and Central Black Sea region (Arslan 1993; Telci, 1995; Kulan et al., 2012).

Seed Oil Content (%)

Fixed oil rates varied between 35.1 and 36.5% in *N. sativa* and between 34.7 and 36.6% in *N. damascena*. The average values of the species were found to be 35.8% and 36.0% in *sativa* and *damascena* respectively, indicating an insignificant difference between the species (Table 2). In addition, the difference between the sowing times was found to be insignificant. It was determined that the difference between species and sowing time is insignificant in terms of fixed oil ratio and that this characteristic is genetically controlled more strongly in the genotypes of the species examined.

Seed Oil Yield (L/ha)

It was determined that fixed oil yield is related to the seed yield and oil; a significant difference was found between the sowing times in terms of fixed oil yield and the difference between the species and years were found to be insignificant. The oil yield varied between 608 and 1338 kg/ha in *N. sativa* and 913 and 1033 kg/ha in *N. damascena*, respectively. In both species, the highest yield was obtained

in autumn sowings and especially in the second autumn sowings, and the fall sowings were categorized in the same statistical group (Table 2).

CONCLUSION

This study investigated the effects of different sowing times on agricultural quality for two different black cumin species (*Nigella sativa* L., *N. damascena* L.). Autumn sowings can be proposed for the growers who want to obtain the highest seed and oil yield in both species under the ecological conditions of Mardin plain. It was also determined that both species can get over winter colds in the region without being damaged.

REFERENCES

- Akgül A., 1993. Baharat Bilimi ve Teknolojisi, Gıda Teknolojisi Derneği Yayınları No:15, Damla Matbaacılık, Ankara, s:72–75.
- Arslan N., 1993. Ekim Zamanı ve Bitki Sıklığının Çörek Otunun (*Nigella sativa* L.) Verimine Etkisi. Uluslararası Katılımlı Bitkisel İlaç Hammaddeleri Bildiri Toplantısı, 73-80.
- Baytop T., 1999. Türkiye’de Bitkiler İle Tedavi (Geçmişte ve Bugün) 2. Baskı. ISBN:9754200211, Nobel Tıp Kitab Evleri.
- Baytöre F., 2011. Bazı Çörek Otu (*Nigella sativa* L.) Popülasyonlarının Verim ve Verim Kriterlerinin Belirlenmesi. Yüksek Lisans Tezi, Namık Kemal Üniversitesi, Tekirdağ.
- Ceylan A., 1996. Tıbbi Bitkiler–2. (Uçucu Yağ Bitkileri) Ege Üniversitesi Ziraat Fakültesi, Yayın No: 481
- Davis, P.H., 1965. *Nigella* L. In: Davis, P.H. (ed.). The Flora of Turkey and East Aegean Islands, Vol. 1 pp: 98–105. Edinburgh University Press, Edinburgh.
- Er C. ve Yıldız M., 2007. Keyf Bitkileri (Üçüncü Baskı), Ankara Üniversitesi, Ziraat Fakültesi Yayın No: 1555, Ders Kitabı No: 508.
- Ertas M.E., 2016. Tokat Kazova Ekolojik Koşullarında Kışlık ve Yazlık Ekilen Çörek Otu (*Nigella* sp.) Genotiplerinin Agronomik ve Kalite Özelliklerinin Belirlenmesi. Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi, Tokat.
- Filippo D’Antuono L., Moretti A., Lovato A.F.S., 2002. Seed Yield, Yield Components, Oil Content and Essential Oil Content and Composition of *Nigella sativa* L. and *Nigella damascena* L. Industrial Crops and Products 15, 15(1):59–69.
- Kılıç C. ve Arabacı O., 2016. Çörek Otu (*Nigella sativa* L.)’nda Farklı Ekim Zamanı ve Tohumluk Miktarının Verim ve Kaliteye Etkisi. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi; 13(2): 49 – 56.
- Kulan E.G., Turan Y.S., Gülmezoğlu N., Kara İ., Aytaç Z., 2012. Kuru Koşullarda Yetiştirilen Çörek Otunun (*Nigella sativa* L.) Bazı Agronomik ve Kalite Özellikleri. Tıbbi ve Aromatik Bitkiler Sempozyumu 13-15 Eylül 2012, Tokat, 177-181.
- Özel A., Demirel U., Güler İ., Erden K., 2009. Farklı Sıra Aralığı ve Tohumluk Miktarlarının Çörek Otunda (*Nigella sativa* L.) Verim ve Bazı Tarımsal Karakterlere Etkisi. Harran Üniversitesi Ziraat Fakültesi Dergisi; 13(1), 17-25.
- Seçmen Ö., Gemici Y., Gök G., Bekat L., Leblebici E., 2000. Tohumlu Bitkiler Sistematigi. Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No: 116. İzmir.
- Tektaş E., 2015. Harran Ovası Koşullarında Birim Alandaki Tohum Sayısının Çörek Otu (*Nigella sativa* L.)’nın Verim ve Bazı Bitkisel Özelliklerine Etkisi. Yüksek Lisans Tezi, Harran Üniversitesi, Şanlıurfa.
- Telci İ., 1995. Tokat Şartlarında Farklı Ekim Sıklığının Çörek otu (*Nigella sativa* L.)’unda Verim, Verim Unsurları ve Bazı Bitkisel Özelliklerine Etkisi. Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi, Tokat.
- Telci İ., Şahin-Yaglioglu A., Eser F., Aksit H., Demirtas I., Tekin S., 2014. Comparison of Seed Oil Composition of *Nigella sativa* L. and *N. damascena* L. During Seed Maturation Stages. Journal of the American Oil Chemists' Society. ISSN 0003-021X Volume 91, Number 10.

EVALUATION OF THE CURRENT STATUS OF IMPORTANT MEDICINAL AND AROMATIC PLANTS IN THE CONTEXT OF INCREASING THE EXPORT POTENTIAL

Önder Volkan BAYRAKTAR¹, Görkem ÖZTÜRK¹, Doğan ARSLAN², Hüseyin ARSLAN²
Sait ENGİNDENİZ³

¹Department of Agricultural Economics, Siirt University, Turkey

²Department of Field Crops, Siirt University, Turkey

³Department of Agricultural Economics, Ege University, Turkey

Abstract

Turkey in the trade of medicinal and aromatic plants are among the most important countries. Turkey has a rich genetic diversity and endemism in terms of geographical structure. In addition, given the fact that many plants are the gene centers, it is expected that the export of medicinal and aromatic plants will be at a much better level. On the other hand, the demand for medicinal and aromatic plants in the world is increasing day by day and the investments to meet this demand are also increasing. As a result, a rapidly growing market has been formed in the world for medicinal and aromatic plants. Thus, making efforts to increase the export potential of medicinal and aromatic plants in Turkey is of great importance. The present production and export status of coriander, carob, peppermint, lavender and black cumin are examined from important medicinal plants exported in this study. However, it is aimed to introduce proposals that contribute to the solution of the problems encountered in the export of these plants.

Keywords: Medicinal and aromatic plants, Turkey, Export

INTRODUCTION

Turkey is a significant export market of the medicinal and aromatic plants in the world. Turkey is besides being a rich country with regard to genetic diversity and endemism because of its geographical structure difference, is also gene center of many plants. Due to the high potential of Turkey for the export of medicinal plants, Turkey's exports in medicinal and aromatic plants are expected to be in a much better level. On the other hand, people's demand for medicinal plants have been increased and the investments to meet this demand have been also increased. As a result, a rapidly growing market has been formed in the world for medicinal and aromatic plants. Therefore, it is important that scientific researches should be carried out in terms of increase the export potential. Turkey is ranked 18th among 110 exporting countries for medicinal plants (Aydın et al., 2014).

In this study, present production and exports status of coriander, carob, mint, lavender and black cumin were examined and some suggestions are given to solution of the problems encountered in the exports of these medicinal and aromatic plants. Statistical data used in the study have been obtained from TurkStat.

RESULTS

Some of the medicinal and aromatic plants are cultivated while some are collected from nature. In Turkey, some plants such as thyme, cumin, anise, fennel, sage, black cumin, coriander, fenugreek, mint, poppy, hops are cultivated. Some plants such as bay leaf, mahleb, linden, sage and rosemary are collected from nature. There are 347 species in Turkey that are collected from nature and traded internally and externally, 139 which of are exported. (Özgüven et al., 2005; Faydaloğlu et al., 2011). The number of cultivated species of medicinal and aromatic plants is up to 6-7% of 400 cultivated plants. For this reason, the cultivation of medicinal and aromatic plants is very important (Arslan et al., 2015).

Coriander, carob, lavender and black cumin production increased while mint production decreased from 2013 to 2017 in Turkey. The most significant increase was in the black cumin due to

increase in cultivated area. The production of black cumin, which had been 352 tons in 2013, increased by 778.97% and reached to 3094 tons in 2017 (Table 1).

Table 1. Production and cultivated area of some important medicinal and aromatic plants according to years in Turkey

Year	Coriander		Carob		Mint		Lavender		Black Cumin	
	Area (da)	Amount (ton)	Area (da)	Amount (ton)	Area (da)	Amount (ton)	Area (da)	Amount (ton)	Area (da)	Amount (ton)
2013	11	1	5119	14261	10646	14143	709	105	3261	352
2014	11	1	6307	13985	10652	14700	2189	297	1717	140
2015	150	11	5244	12851	10577	14945	3218	400	4681	425
2016	503	42	5693	13405	10921	15550	5700	747	23160	2527
2017	410	29	6735	15016	10520	14213	6606	845	32560	3094

Source: TurkStat, 2017

As of 2013, the export amount of coriander is 228,56 tons and the export value 566,08 thousand dollars, these amounts decreased to 137,16 tons and 183,87 thousand dollars in 2017. In 2013, while the export amount of carob was 1416 tons, it decreased to 1367,48 tons in 2017. Although, while the export value was 1 million 85 thousand dollars in 2013, it is seen 1 million 98 thousand. As of 2013, the amount of mint export is 189,56 tons and the export value is \$ 1 million 10 thousand. These figures increased to 635,87 tons and 1 million 864 thousand dollars in 2017. While lavender exports of almost 10 thousand dollars with 0,38 tons were carried out in 2013, it is 16 thousand dollars with 0,44 tons were carried out in 2017.

As of 2013, the export amount of black cumin is 65,13 tons and the export value 219 thousand dollars. These amounts rose to 461,72 tons and 1 million 360 thousand dollars in 2017 (Table 2).

Table 2. Export quantities and export value of some important medicinal and aromatic plants according to years in Turkey.

Year	Coriander		Carob		Mint		Lavender		Black Cumin	
	Export Quantity (kg)	Export Value (\$)	Export Quantity (kg)	Export Value (\$)	Export Quantity (kg)	Export Value (\$)	Export Quantity (kg)	Export Value (\$)	Export Quantity (kg)	Export Value (\$)
2013	228596	566088	1416006	1085242	189560	1010017	378	9756	65131	219136
2014	71394	144727	948047	847173	205356	1045155	733	18562	57400	224544
2015	166743	307265	542793	600412	360585	1314201	450	25231	52445	244489
2016	152346	267851	1257614	987274	374293	1360420	194	19551	116116	461838
2017	137156	183873	1367481	1098836	635867	1864665	443	16198	461724	1360906

CONCLUSION AND RECOMMENDATIONS

Amounts and value of exports is not at the desired level although Turkey is an important country for the production and export of medicinal and aromatic plants. Despite the export potential of about 200 medicinal and aromatic plants among the plant species used for medicinal purposes in Turkey, only 70-100 species are exported (Başer, 2000). However, statistics are not included the vast majority of these species. Medicinal and aromatic plants are appeared in “others” chapter in the statistics expect for the most exported medicinal and aromatic plants. Therefore, the complete list of exported medicinal and aromatic plants from Turkey is not available (Öztürk et al., 2012). It is necessary to prepare a complete list and create a database of medicinal and aromatic plants in Turkey, which are collected from nature and cultivated, by working together with the collectors, intermediaries, export companies and relevant government institutions (Faydaloğlu and Sürücüoğlu, 2011). It is important that the statistics of the medicinal and aromatic plants which are collected from nature and cultivated should be given separately on the database and the database need to be updated continuously.

On the other hand, a low added value created by medicinal and aromatic plants in terms of Turkish economy because of most of Turkey’s medicinal and aromatic plants exports are made from unprocessed products. Cleaning, selecting, sorting, packaging operations are applied in a limited number of spice and tea production (Binici, 2002). Turkey’s exports of medicinal plants will be

increased by providing standardization for medicinal and aromatic plants and processing of medicinal and aromatic plants.

Cultivation and breeding of plants should be provided in order to increase the market share of Turkey's exports of medicinal and aromatic plants (Yücer and Altıntaş, 2012). The number of registered varieties in medicinal and aromatic plant species cultivated in Turkey is very small. In recent years, some varieties have been registered or registration studies have been initiated in medicinal and aromatic plants such as poppy, anise, coriander, fenugreek, thyme, sage. But, it is required to increase cultivation and breeding of medicinal and aromatic plants studies.

In addition, the lack of legislation on the collection from nature and production of medicinal and aromatic plants should be eliminated and trainings should be given to collectors in order to prevent the destruction of nature.

REFERENCES

- Arslan, N., Baydar, H., Süleyman, K., Karık, Ü., Şekeroğlu, N., Gümüşçü, A., 2005, Changes in the Production of Medicinal Plants and New Seeking, The Chamber of Agricultural Engineers VIII. Technical Congress, 12-16 January 2005, Ankara, pp: 483-507. (in Turkish)
- Aydın, E., Yurum, Ç., Kevseroğlu, K., Seyis, F., 2014, Risk Status of Important Medicinal and Aromatic Plants Collected Intensive from the Nature and Displaying a High Market Share, The Second Symposium of Medicinal and Aromatic Plants, 23-25 September 2014, Yalova, pp: 281-286. (in Turkish)
- Başer K.H.C., 2000. Sustainable Wild Harvesting of Medicinal and Aromatic Plants: An Educational Approach, Harvesting On Non-Wood Forest Products, Seminar Proceedings, Menemenİzmir, Turkey
- Bayramoğlu, M.M., Toksyo, D., Şen, G., 2009, Medicinal Plant's Trade in Turkey, The Second Socio-Economic Problems in Forestry, 19-21 February, Isparta, pp: 89-98. (in Turkish)
- Faydaoğlu, E., Sürücüoğlu, M. S., 2011, History of the Use of Medicinal and Aromatic Plants and their Economic Importance, Kastamonu Univ., Journal of Forestry Faculty, 11(1):52-67. (in Turkish)
- Metin, İ., Güngör, H., Çolak, Ö.F., 2012, Exports and Imports of Some Medicinal Plants, Symposium of Medicinal and Aromatic Plants, 13-15 September 2012, Tokat, pp: 326-333. (in Turkish)
- Öztürk, M., Temel, M., Tınmaz, A.B., Kil, L., 2012, Medicinal and Aromatic Plants on Turkey Foreign Trade, Symposium of Medicinal and Aromatic Plants, 13-15 September 2012, Tokat, pp:33-44. (in Turkish)
- Turkstat, 2016, Turkish Statistical Institute, www.tuik.gov.tr. [Accessed: March 2017].
- Unctad, United Nations Conference on Trade and Development, <http://unctad.org> [Accessed: March 2017].
- Yücer, A., Altıntaş, G., 2012, Foreign Trade of Medicinal and Aromatic Plants in Turkey, Symposium of Medicinal and Aromatic Plants, 13-15 September 2012, Tokat, pp:55-63. (in Turkish)

FORMULATION OF HAZELNUTS FUNCTIONAL PRODUCTS WITH HERBAL EXTRACTS

Zuleyhan BARAN¹, Halil Ibrahim UGRAS², Mehmet Akif SEN³, Dicle BARAN⁴, Bora KARAGUL⁴

¹University of Duzce, Akcakoca VHS, Duzce, Turkey

²University of Duzce, Faculty of Arts and Sciences, Duzce, Turkey

³University of Istanbul Medeniyet, Faculty of Tourism, Istanbul, Turkey

⁴University of Duzce, Institute for Graduate Studies in Sciences, Duzce, Turkey

*Corresponding author email: zuleyhanbaran@duzce.edu.tr

Abstract

Turkey is the biggest hazelnut grower and meets the requirement of hazelnut of 70% of the world. Black Sea region has appropriate climate conditions for growing hazelnut and a lot of city in the region grow hazelnut and it has important income for export. Hazelnut uses as dried nuts and also it is widely used in desert and chocolate industry. Functional products really gain value with born of nutritional (healthy) food concept in recent years. In this topic, consumer in demands nutritional value of a food with give healthiness or immune-enhancing properties. Because of that reason hazelnut has very important value for national economy and improvement with functional products is important to increase market share. This research is planned on research and development of hazelnut pastes with different herbal extracts. Products improve economical value of hazelnut, which grow in Black Sea region and functional foods and enlarge hazelnut-growing area too. As a result believed that Black Sea hazelnut will be more demand in domestic and foreign market with increasing economical potential

Keywords: Hazelnut products, functional products

INTRODUCTION

Turkey is the biggest hazelnut grower and meets the requirement of hazelnut of 70% of the world in our country. Our countries Black Sea region has appropriate ecological conditions to grow hazelnut. Hazelnut has 20% in our Agricultural export and it provides foreign exchange flow as totally national resources. In this scene, it is an idea which designs of new hazelnut products for locals which earn their money from hazelnut contributes publicity of Duzce and economy of locals. The study contains not only export but also research and development studies for improving of using area in the country of important income source, which is hazelnut. Firstly, research and development researches aim applying of experimental procedures of designed products and then expand of successful products to hazelnut growers. According that, it is thought that research makes a significant contribution to country economy and creates employment.

This research aims evaluation and improving economical efficiency of hazelnut, which is the most common agricultural product of Black Sea region. Some food products consume increase last years in our country with changing of food habits. Hazelnut paste cream and relevant is a most important product, which have high consume scale in breakfast. Their hazelnuts remain in Turkey. Additionally, some foreign companies said that and they increased their sales ranges in our country and international market.

One of The most important purposes of the study is providing added value top country economy with production of valuable agricultural product in own opportunities and then it will be a valuable exportation product. The purpose of the study is increase nutritional value of product as spreadable hazelnut with own natural growth production as local honey (chestnut, linden and bitter honey), local grape juice as known as molasses (grape, kaki persimmon, peat, mulberry, etc) chestnut flour, mulberry powder, 60 different types herbal extracts and powders. Other aims of study are increase

scale of hazelnut paste types and their nutritional values that are consumed from children and young and increase opportunity and types of balanced diet with safe food that is produced in our country.

Literature

Homeland of hazelnut is Anatolia and first growing and trade of hazelnut has been begun in Turkey (Gurses, 1997; Ozdemir, 1998). Turkey has 70% hazelnut grow range in the world (Sariyar, 1998). Other main growers are Italy and America (Table 1).

Table 1. World hazelnut production values

Countries	2009/10	2010/11	2011/12	2012/13	2013/14	2014/15	2015/16	2016/17
Turkey	500.000	600.000	430.000	660.000	549.000	412.000	646.167	420.000
Italy	120.000	107.000	140.000	84.000	132.000	100.000	125.000	130.000
USD	42.600	24.500	35.000	32.000	35.000	36.290	43.500	32.000
Azerbaijan	35.000	39.000	55.000	40.000	30.000	25.000	50.000	35.000
Georgia	32.000	40.000	30.000	28.000	35.000	35.000	40.000	40.000
Spain	18.000	20.000	22.000	16.000	19.500	19.500	20.000	21.000
Other	27.000	27.000	27.000	25.000	25.000	25.000	45.000	42.000
Total	774.600	857.500	739.000	885.000	825.500	660.773	969.500	720.000

Source: World Nut and Dried Fruit Congress, 2017

Important combination properties of hazelnut type researched important combinations in hazelnut (water, ash, oil/fat, protein, carbohydrate), minerals (phosphor, calcium, magnesium, manganese, potassium, zinc, iron, sodium), vitamins (B1, B2, E) and said that hazelnut combination changes type to type (Table 2) (Fallico vd., 2003).

Table 2. Nutritional values of hazelnuts

	In 100 g
Fat (g)	61
CHO (g)	17
Protein (g)	15
Leaf (g)	10
K (mg)	680
Ca (mg)	114
Vit A (mg)	20
Vit C (mg)	6
Fe (mg)	5
Na (mg)	0
Cholesterol (mg)	0

Source: TUİK, 2016

As it is known, in terms of ecological conditions for hazelnuts, the most suitable places are the sea level areas in the Black Sea Region in Turkey. Hazelnut cultivation at places above this height is not suitable for both quality and yield (Özbek, 1978). It is mainly classified as Levant quality or Giresun quality that symbolizes the Turkish hazelnut region commercially (Isleroglu et al., 2005). Functional foods are food or food components that provide additional benefits over human physiology and metabolic functions beyond the body's essential nutrient requirements, thereby contributing to the prevention of disease and a healthier life. There are numerous in vivo, in vitro and clinical trials that nutrition based on herbal products can reduce chronic disease, especially cancer risk (Berner and OinDonnell, 1998).

MATERIAL AND METHODS

At this research started in a food research and development department with to paste of raw and roasted hazelnuts and then 60 different kind of herbal extracts added in different percentages to remained pastes. Each addition to pastes recorded and tested with sensory analysis. Product which had highest point evaluated as successful product and optimizations started for production step of the product. As a result, it was decided that 15 of 500 remained products had score to release. Each of product procedures converted to production scheme one by one after point evaluate of products.

RESULTS AND DISCUSSION

Functional foods have positive proved effects on human health. Spread of consuming products, which are enriched with functional foods, is important because of nutritional value and positive effect on human health. In recent years, consumers have begun to look at food not only for basic nutrition, but also for health benefits. In this regard, functional food is an important plant source containing beneficial compounds and has potential as a natural source of phytochemicals.

One of more important other conclusion is enrichment of hazelnut, which has high nutritional value, and we have leadership on its growth with functional foods. Enrichment of hazelnut products is a new entrepreneurship in the way of both taste and health, which improve country economy. In the study, products were tested in sensory analysis and order of preference and then the best choice of the consumer list was improved. According to the research, the most preferred functional hazelnut product is hazelnut paste which contain propolis and then ginger, clove, cinnamon, turmeric, locust bean, grape core, flaxseed, opium poppy, chestnut, linden, daisy, coconut, mulberry and local molasses (plum, mulberry, smell grape, pear, etc.) respectively. According to the conclusion of the research, these products are going to offer to consumers and started to works. In this way, aim that the study is production of hazelnut paste types more than one which contribute national economy and study on providing employment is going on.

REFERENCES

- Berner, L. A. and O'Donnell, J. A., 1998. Functional Foods And Health Claims Legislation: Applications To Dairy Foods. *International Dairy Journal*, 8, 355-362.
- Demir, C. 1996. Investigation of aflatoxin formation in hazelnut stored at different humidity and temperatures. *Trakya University Graduate School of Natural and Applied Sciences, M.Sc. Thesis, Tekirdag.*
- Fallico, B., Arena, E., Zappalà, M. 2003. Rosating of hazelnuts. Role of oil in colour development and hydroxymethylfurfural formation. *Food Chemistry*.
- Gurses, M. 1997. Effects of Different Storage Conditions on Aflatoxin Formation in Inland Hazelnut. *Ankara University Graduate School of Natural and Applied Sciences Department of Food Engineering, MSc Thesis, Ankara.*
- INC XXXVI World Nut and Dried Fruit Congress (2017) - May 21, 2017 INC Annual Congress.
- Isleroglu, H., Yildirim, Z., Yildirim, M., 2005. Flaxseed As A Functional Food. *GOÜ. Journal of Agriculture Faculty*, 2005, 22 (2), 23-30.
- Ozbek, S. 1978. *Special Fruits*, Çukurova Agriculture Faculty, Publication: 128, Adana.
- Sariyar, L. 1998. Investigation of Lipolytic Activity of Some Molds in Hazelnut. *Istanbul Technical University, Graduate School of Natural and Applied Sciences, M.Sc. Thesis, İstanbul.*
- Sipahioğlu, H.N. 1998. Investigation of Lipase Effect Created by Some Molds During the Storage of Hazelnuts, *Istanbul Technical University, Graduate School of Natural and Applied Sciences, M.Sc. Thesis, İstanbul.*
- TUİK, (2016). http://www.tuik.gov.tr/PreTablo.do?alt_id=1001.

USING PROPOLIS EXTRACT AS A FUNCTIONAL FOOD IN HAZELNUT PASTE

Zuleyhan BARAN¹, Halil Ibrahim UGRAS², Mehmet Akif SEN³, Dicle BARAN⁴, Bora KARAGUL⁴

¹University of Duzce, Akcakoca VHS, Duzce, Turkey

²University of Duzce, Faculty of Arts and Sciences, Duzce, Turkey

³University of Istanbul Medeniyet, Faculty of Tourism, Istanbul, Turkey

⁴University of Duzce, Institute for Graduate Studies in Sciences, Duzce, Turkey

Corresponding author email: zuleyhanbaran@duzce.edu.tr

Abstract

Turkey is a country that holds the world leadership in the production of hazelnuts due to the appropriate climate and soil conditions. For this reason, hazelnut is an important agricultural product within the scope of our export products. On the other hand, the fact that the hazelnut products don't have a real purchase price and the fact that the producer does not satisfy the price market brings the quality and marketing problems of hazelnut production to the agenda when evaluated from a commercial point of view. Therefore hazelnut products to be introduced to diversify the domestic and foreign markets as a functional product, although the price of nuts is not based on agricultural products that will contribute to the development of hazelnut market by providing price increase indirectly due to adding flavor and value to the product which is located in hazelnut. In this issue, the fact that food is not only a nutritional ingredient but also a functional product that includes health-positive ingredients is an important development which has gained importance in recent years. Black Sea at the same time considering the richness of plants which is the production area of hazelnut is also considered as a center of important beekeeping products. This suggests apitherapy products where bee products are used for therapeutic purposes. The combination of a high energy level product, such as hazelnut, with a major health-care substance with antimicrobial properties, such as propolis, shows that a much more valuable substance will emerge in terms of health. For this reason, this research is based on the mixing of hazelnut paste additives at different ratios with propolis. In the subject research, flowers and linden blossom honey has been used as a sweetener which produced in the Black Sea region. As a result of the study, hazelnut paste which is 0.5% propolis and 17.5% flower honey product was tested on microbial, antimicrobial and nutritive value and it was decided to be used on product basis.

Keywords: Hazelnut, Functional Products, Propolis

INTRODUCTION

Turkey's hazelnut production in the World leadership because of it has suitable climate and soil conditions. Therefore, it is an important agricultural product which is evaluated within the scope of our hazelnut export products. On the other hand, when evaluated from a commercial point of view, elements such as the fact that hazelnut product does not have a real trading price and the lack of a price market that will satisfy the manufacturer raise the problems of quality and marketing in hazelnut production. For this reason, hazelnut products are diversified as functional products and introducing to the domestic and foreign market, hazelnut price is not on the basis of agricultural product, but because it adds flavor and value to the product, indirectly increasing the price will contribute to the development of hazelnut market.

It is an important development that has gained importance in recent years, and that food is considered not only as a nutritional element but also as functional products that include positive health effects. Black Sea, which is the production area of hazelnut, is also considered as an important beekeeping products Center when it is considered in terms of plant richness. This situation brings to mind the apiterapi products where bee products are used for therapeutic purposes. The combination of a substance with antimicrobial properties such as propolis, especially with a high energy level product such as hazelnuts, shows that a more valuable substance will emerge in terms of Health. For this reason, this research is based on the mixing of different proportions of propolis additives with

hazelnut paste. Flower and linden honey produced in the Black Sea region were used as sweeteners during the research. As a result of the research, it was decided that hazelnut paste, which is 0.5% propolis and 17.5% flower honey product, can be used on the basis of product by testing it through its microbial, antimicrobial and nutritive value.

Literature

Hazelnut is the homeland of Anatolia, the first in the world hazelnut production and trade has begun in Turkey. Turkey in the last five years has 70% share of world hazelnut production. The nutritional value of nuts with valuable components, another valuable functional food product propolis with the enrichment of the new hazelnut paste products to be produced is an important issue. Bee products such as honey, pollen, royal jelly, propolis and bee venom have been an important product that has been used to improve both nutrients and some diseases. In particular propolis; antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antitumoral and cytostatic activity comes to the fore with many biological features (Albayrak and Albayrak, 2008). Propolis is a resinous product in which bees collect and convert from plant secretions to cover hive holes (Marcucci et al., 2001). Propolis, Mg, Ca, I, K, Na, Cu, Zn, Mn and Fe with elements such as B1, B2, B6, C and E vitamins and a large number of fatty acids and all these besides glucose, fructose, sucrose, amino acids and various enzymes (Dogan and Hayoglu, 2012).

Propolis extract obtained from the hive by various methods can be used as a propolis extract after extraction (Kumova et al., 2002). Propolis, a highly valuable product, has a very high antioxidant effect (Graikou et al., 2016; Taffine et al., 2016; Buratti et al., 2007). The antimicrobial properties of propolis, depending on the type of solvent used, plant species, geographical origin and the compounds it contains, are related to the flavonoids pinosembrin, galangin, and pinobanksin and cinnamylidene acetic acid and caffeic acid esters (Dezmirean et al., 2017).

Considering the studies on the use of propolis in foods;

1. It can be used as a chemical preservative in meat products due to its antimicrobial effect (Han and Park, 1995)
2. Increased shelf life of frozen fish by 2-3 times (Donadieu, 1979),
3. Application on the cheese surface that *Aspergillus versicolor* type mold stops the development of the low concentration (250 ppm) (Aly and Elewa, 2007).
4. Using 0.6 % propolis extract that has been shown increase shelf life 1-2 weeks in Egypt type sausage (Ali et al., 2010).
5. It prevents the lipid oxidation on butter (Cottica et al., 2015).
6. In studies on yogurt, the addition of low concentration propolis has a slowing effect on the development of *S. aureus* and *E. coli*, while it has a low effect on *Bifidobacterium* and *Lactobacillus* (Jian-xin et al. 2011),
7. Studies on Italian type salami prevent oxidation during storage (Bernardi et al., 2013) .
8. Fruit juice (apple, orange, grape and tangerine) studies high antifungal effects on all kinds of mildew according to Na benzoate and especially extra antimicrobial effect in the samples of apple/orange juice (Koç et al., 2007, Gurs and et al., 2007; Yang et al., 2017).
9. In the studies on fruity yogurt, the effect of propolis on antioxidant and antimicrobial activity, chemical and sensory properties was observed. As a result of the analyzes, antioxidant activity increased in yogurt samples, microbial growth slowed down, propolis contribution between chemical and sensory properties did not cause a significant difference (Guney, 2016) expressed

MATERIAL AND METHODS

At this research started in a food research and development department with to paste of raw and roasted hazelnuts and then propolis extracts added in different percentages to remained pastes. Each addition to pastes recorded and tested with sensory analysis. Product which had highest point evaluated as successful product and optimizations started for production step of the product. As a result, it was decided that 3 of 27 remained products had score to release. Each of product procedures converted to production scheme one by one after point evaluate of products.

RESULTS AND DISCUSSION

Propolis is a valuable bee product that has been scientifically proven to have functional properties and positive health effects. Studies on the antimicrobial protective effect of propolis have revealed that this product has such a potential. However, studies on propolis have proved that the ingredients in propolis can be used as additives in food. In recent years, propolis-doped foods have been the subject of a lot of research on the additives. Especially antimicrobial and antioxidant properties, propolis contributed to the emergence of different food products. Today, the tendency of people towards natural additives shows that these foods with functional properties can be preferred because of their natural nature. However, propolis has a very strong use potential as a food additive because it is both nutritional and natural preservative. This study was conducted to determine the practical use of propolis in food products and to determine what it is preferable when presented to consumer tastes in a different format. This shows that the consumer's interest in healthy products is quite high, but it has also revealed that such enriched food products should be included in the market. Thus, the presentation of a delicious and nutritious food product, such as hazelnut paste, enriched with functional foods, will make it easier for the consumers to turn to healthy products in terms of availability and will contribute greatly to the country's economy.

REFERENCES

- Ali,F.H., Kassem, G, M., Atta-Alla, O, A. 2010. Propolis as a natural decontaminant and antioxidant in fresh oriental sausage. *Veterinari Italiana* Apr-Jun;46(2):167-72.
- Aly, S., and Elewa, N., 2007. The effect of Egyptian honeybee propolis on the growth of *Aspergillus versicolor* and sterigmatocystin biosynthesis in Ras cheese. *Journal of Dairy Research*, 74(1), 74-78.
- Bernardi, S., Favaro-Trindade, C, S., Trindade, M, A., Balieiro, J, C, C., Cavenaghi, A, D., Contrera-Castillo, C,J., 2013. Italian-type salami with propolis as antioxidant. *Ital. J. Food Sci.*, vol. 25 – 2013.
- Buratti, S., Benedetti, S. Cosio, M. S. 2007. Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis. *Talanta* 71 1387–1392 .
- Cottica, S. M., Sabik, H., Antoine, C., Fortin, J., Graveline, N., Visentainer, J. V., Britten, M., 2015. Characterization of Canadian propolis fractions obtained from two-step sequential extraction. *LWT - Food Science and Technology*, 60(1):609– 614.
- Dezmirean, D, S., Marghitaş, L, A., Chirila, F., Copaciu, F., Simonca, V., Bobiş, O., Erler, S., 2017. Influence of geographic origin, plant source and polyphenolic substances on antimicrobial properties of propolis against human and honey bee pathogens. *Journal of Apicultural Research*, Vol. 56, No. 5, 588–597.
- Doğan, N. ve Hayoğlu, İ.2012. Propolis ve Kullanım Alanları. *HR.Ü.Z.F. Dergisi*, 2012, 16(3): 39-48.
- Donadieu, Y. 1979. *La propolis*. Editions Maloine, Paris.
- Graikou, K., Popova, M., Gortzi, O., Bankova, V., Chinou, I., 2016. Characterization and biological evaluation of selected Mediterranean propolis samples. Is it a new type?. *LWT - Food Science and Technology* 65 261-267.
- Güney, F., 2016.Bazı propolis özülerinin meyveli yoğurtların biyokimyasal, fizikokimyasal ve raf ömrü üzerine etkilerinin araştırılması .Yüksek Lisans Tezi. Ordu Üniversitesi,Fen Bilimleri Enstitüsü.
- Han, S. K. and Park, H. K. 1995. A study on the preservation of meat products by natural propolis: effect of EEP on protein change of meat products. *Korean Journal Of Animal Science*, 37(5):551–557.
- Jian-xin, G., Hai-ying, C., Zhao-yun, L., 2011. The Influence of Propolis on *Bifidobacteria* And *Lactobacillus* in Yogurt. *Chinese Journal of Disinfection* 2011-02.
- Koç, A, N., Silici, S., Sarigüzel, F, M., Sağdıç, O. 2007. Antifungal Activity of Propolis in Four Different Fruit Juices. *Food Technol. Biotechnol.* 45 (1) 57–61.

- Kumova, U., Korkmaz, A., Avci, B. C., Ceyran, G. 2002. Önemli Bir Arı Ürünü: Propolis. Uludag Bee Journal May 2002.
- Marcucci M. C., Ferreres, F., Garca-Viguera, C., Bankova, V. S., De Castro S. L., Dantas, A. P., Valente, P. H. M., Paulino, N., 2001. Phenolic compounds from Brazilian propolis with pharmacological activities. J Ethnopharmacol 74:105–12.
- Sağdıç, O., Silici, S., Yetim, H., 2007. Fate of Escherichia coli and E. coli O157:H7 in apple juice treated with propolis extract. Annals of Microbiology, 57 (3) 345-348.
- Tafinine, Z. M., Ouchemoukh, S., Tamendjari, A., 2016. Antioxydant activity of some algerian honey and propolis. Industrial Crops and Products 88 85–90.
- Yang, W., Wu, Z., Huang, Z, Y., Miao, X., 2017. Preservation of orange juice using propolis. Journal of Food Science and Technology -Mysore- 54(11):1-9

THE HEAVY METAL, VOLATILE OIL PHYSICO-CHEMICAL and ANTIMICROBIAL PROPERTIES of SAGE PLANT COLLECTED FROM MUĞLA-BODRUM LOCATION

Erman DUMAN¹

¹Afyon Kocatepe University, Engineering Faculty, Food Engineer Department, Turkey

Corresponding author e-mail: eduman@aku.edu.tr

Abstract

In this research, physico-chemical and antimicrobial properties of volatile oil, heavy metal of sage plant (Salvia fruticosa Mill.) collected from Muğla-Bodrum location have been examined in detail. The results obtained were as follows: heavy metal contents (12 number, 0-401 mg / kg), essential oils (1.0%), essential oil compositions (40 number, maximum 1.8 cineole 49%) refractive index (1, 463), color (0,1-1,6-0-0,1), viscosity (4,73 mPa), oxidative stability on sunflower oil, total amount of phenolic substance were determined. In addition, these essential oils were obtained from three different molds (Aspergillus parasiticus NRRL 3357, Aspergillus parasiticus DSM 5771 and Zygosaccharomyces rouxii ATCC 28253) and 5 bacteria (Escherichia coli ATCC 25922, Escherichia coli O157: H7, Listeria monocytogenes ATCC 7644, Salmonella enteritis ATCC 13076, Staphylococcus aureus ATCC 2592). As a result, the results were found in accordance with European pharmacopoeias and sage essential oil was found effective at different rates on the indicated microorganisms.

Keywords: *Salvia fruticosa* Mill, heavy metal, volatile oil, physico-chemical, antimicrobial.

INTRODUCTION

Since the existence of human beings, plants have been the most important food and food source for both humans and animals. There are many definitions of medicinal and aromatic plants. Bayram et al. (2009) reported that medicinal and aromatic plants are "herbal medicine, unprocessed or processed by the composition of one or more plants containing the therapeutic properties or other people's health benefits derived from plants or products" (Bayram et al., 2009).

Essential oil is the compounds obtained by various distillation methods from medicinal and aromatic plants and are widely used as natural flavoring agents. Plants used for this purpose are juniper seeds, anise seeds, cinnamon bark, coriander seeds, clove buds, fennel seeds, turmeric roots, lemon balm leaves, mint, thyme leaves and vanilla seeds (Buesa et al., 2005).

Medicinal and aromatic plants and essential oils; when added to ready-to-eat food products, it increases the storage time of foods with the antimicrobial effect (Farg et al., 1989). Essential oils showing antimicrobial effect against bacteria and bacteria are marjoram, oregano, sage, rosemary, carnation, nigella, garlic and onion (Nychas, 1995). Fats that inhibit yeast and fungi should be particularly rich in phenol, aldehyde and alcohols (Bruni et al., 2003).

LITERATURE

Anatolian sage (*Salvia fruticosa* Mill.) is a species of commercial importance spreading from the northwest to the southwest in Turkey. This species, which has been collected from nature for many years, has a significant place in medical and aromatic plants both in domestic and foreign markets. Some of these components were highest components 1,8-cineole (37.3%) and camphor (8.1%) (Karık and Sağlam., 2017).

In Turkey, the sage in many species there are around 900 species in the world. In Turkey known as sage and used in 2 species. All species belonging to this genus are not used by the people (Anonymous, 2016). Sage has been reported to have antioxidant, anti-inflammatory, anti-carcinogenic and antimicrobial activity in the aqueous and oily extracts (Gali-Muhtasib, 2006).

On the other hand, *Salvia libanotica* an endemic plant species in the Mediterranean region, is used by people as a method of treatment for complaints of colds and abdominal pain. The amount of *Salvia libanotica* essential oil in Lebanon varies between 0.03-0.65%. The most important components of oil are 1,8-cineol and camphor (Gali-Muhtasib, 2006). Farhat et al., (2001) in the study published in different times of the year *Salvia libanotica* plant examined the composition of volatile oil. A total of 11 components have been reported in volatile oils containing the highest rates of cineol at different rates.

MATERIAL AND METHODS

Material

In this study, the species of sage (*Salvia fruticosa* Mill.) was obtained from Muğla-Bodrum location by Afyonkarahisar Medical and Aromatic Plants Center in Turkey and dried in a cool environment.

Methods

The supplied sage plant were investigated amount of essential oil (%), volatile oil components (%), refractive index, oxidative stability on sunflower oil (h), mineral substance (ppb) and antimicrobial effects of *Escherichia coli* ATCC 25922, *Escherichia coli* O157: H7, *Listeria monocytogenes* ATCC 7644, *Salmonella enterica* subsp. *enteritis* ATCC 13076, *Staphylococcus aureus* ATCC 2592 bacteria, *Candida zeyland* and *zygosaccharomyces rouxii* respectively.

Volatile Oil Content of Sage Plant (%)

Essential oils used in the study, Telci et al. (2006). Fifty grams of dry plant material was transferred to 1000 ml flasks and placed on the Clevenger device by adding 500 ml of water (1:10). Distillation was continued for two hours and the obtained oils were stored in glass bottles and stored at 4 °C in the refrigerator until they were used in the study.

Composition of Sage Volatile Oil (%)

The essential oil components of the sage plant were determined by Gas Chromatography (Agilent GC). In order to determine the essential oil components, Gas Chromatography - Flame Ionization Detection (GC-FID) system was used. Volatile oils obtained as a result of distillation were diluted with acetone at a ratio of 1:10 (v / v) and 0.1 µL volume was injected into the column with glass syringe (30m x 0.25mm x 0.25µm film). In the system, helium was used as carrier gas at 5 Psi pressure and injection block temperature was determined as 230 °C, FID detector temperature was 250 °C. The FID column temperature was started from 50 °C and was increased to 3 °C per minute and the final temperature was set to 230 °C. The samples were incubated at the starting temperature for 3 minutes and at the end temperature for 15 minutes. The proportional values of the components were determined as % according to FID field without using correction factor. Volatile oil components were determined by Gas Chromatography-Mass Spectrometry (GC-MS) (Agilent). The obtained spectra were defined by comparing the spectra of the components in WILLEY and NIST libraries (Çam et al., 2012).

Refractive Index of Sage Volatile Oil (n_D)

For this purpose, the samples of plant volatile oil taken with the help of spatula were poured on the prism of the refractometer and a reading of 20 °C (Anonymous, 1970).

Oxidative stability of sage volatile oil (h)

In this study, 3 grams of sunflower oil was used for the oxidation stability and the induction time of the Metrohm 743 Rancimat device was determined by the Rancimat device. The air flow rate set at 110 °C was measured at 10 L / h. The conductivity of the study was 0.055 µs of ultra pure water (Anonymous, 1992).

Color analysis of sage volatile oil

This analysis was carried out according to the principles of Mc Guire (1992).

Viscosity analysis of sage volatile oil

This analysis was carried out according to the principles of Lazaridou (2004).

Determination of heavy metal content of sage plant

This analysis was performed according to the principles of ICP-AES, Skujins (1998) and was calculated at mg / kg.

Determination of antimicrobial activity of sage volatile oil

This analysis was carried out used disc diffusion and microdilution method according to the principles of Güllüce et. al. (2004).

Statistical analysis

The standard deviations of the data obtained from the study were calculated and each sample was evaluated in three parallel (Subramanian et.al., 2012).

FINDINGS AND DISCUSSION

Sage Plant Volatile Oil Content (%)

Table 1. Sage plant volatile oil content (%).

Plant Name	Variety Name	% Volatile oil	(±)Std mean
Sage	<i>Salvia fruticosa</i> Mill.	1,0	0.055

As can be seen in Table 1, the volatile oil ratio was found to be 1.0%. In the study on the amount of essential oils of the Lebanese sage, the researchers reported a volatile fat content of 0.03-0.65% (Gali-Muhtasib et.al., 2006). The difference between the volatile oil ratios determined shows that the plants can vary according to their growing conditions, climate, variety and years.

Heavy Metal and Mineral Contents of Sage Plant (mg/kg)

Table 2. Heavy Metal and Mineral Contents of Sage Plant (mg/kg).

Plant Name	Variety Name	Cr	Mn	Fe	Co	Ni	Cu
Sage	<i>Salvia fruticosa</i> Mill.	0,805	4,337	401,800	0,174	0,995	4,206

Table 2 (continued). Heavy Metal and Mineral Contents of Sage Plant (mg/kg).

Plant Name	Ag	Cd	Hg	Pb	As	Se	Zn
Sage	0,006	0,007	0,008	0,517	0,086	0	36,109

As seen in Table 2, chrome, manganese, iron, cobalt, nickel, copper, zinc, cadmium, lead and arsenic have been determined as common in the plant we investigated. The highest iron content of these minerals was determined. In addition to these determinations, the highest percentage of iron in sage was determined. In terms of mercury content was also determined in sage.

When we compare the literature with Abu-Darwish (2014) sage heavy metal content of his research on the different concentrations of Co, Cu, Fe, Mn and Zn, Pb, Cd heavy metals were detected. The difference between the heavy metals we detect and the heavy metals made in other studies shows that the plants may vary according to their growing conditions, climate, variety and years. In addition, the heavy metals we encountered in sage plants were determined under the maximum values of WHO (2007) and European Pharmacopoeia (2010) and were found to be suitable for heavy metal content.

Refractive Indices of Volatile Oils of Sage Plant (n_D)

Table 3. Refractive indices of volatile oils of sage plant (n_D).

Plant Name	Variety Name	Refractive Indices	($\pm$) Std mean
Sage	<i>Salvia fruticosa</i> Mill.	1,463	0.12

As seen in Table 3, the lowest volatile oil refractive index ratio; 1,463 n_D and sage samples were determined. Compared to literature information, refractive index value is an important quality criterion in determining the quality of fats and oils (Nakamura et.al., 1999). The higher the refractive index, the higher the density of the substance dissolved in the liquid.

Viscosity Values of Volatile Oils of Sage Plant (mPass-24°C)

Table 4. Viscosity values of volatile oils of sage plant (mPass-24°C).

Plant Name	Variety Name	Viscosity	($\pm$) Std mean
Sage	<i>Salvia fruticosa</i> Mill.	4,73	0.11

As shown in Table 4, the viscosity values of the volatile oil were determined as 4.73 mPa.

Color Values of Volatile Oils of Sage Plant

Table 5. Color values of volatile oils of sage plant (R:Red, Y:Yellow, B: Blue, D: Dark).

Plant Name	Variety Name	R-Y-B-D	($\pm$)Std mean
Sage	<i>Salvia fruticosa</i> Mill.	0,1-1,6-0-0,1	0.029

As can be seen in Table 5, the volatile oil color values of the sage plant, which is the lightest color of the volatile oils; 0.1 red and at least dark sage volatile oil was detected. No information was found in the literature regarding this feature. The results determined in this aspect are unique.

The Oxidative Stability Values of Volatile Oil of Sage Plant on Sunflower Oil

Table 6. The oxidative stability values of volatile oil of sage plant on sunflower oil (h-110°C).

Volatile Oil Name	Variety Name	Rancimat (h)	($\pm$)Std mean
Sage	<i>Salvia fruticosa</i> Mill.	2,32	0.076

In Table 6, the oxidative stability values of volatile oil of sage plant on sunflower oil were determined as 2.32 (hour-110°C). Compared to the literature, oxidative stability; depending on many factors such as the applied environment, temperature, composition. Temperature is an important parameter. Because the high temperature of 110°C in the Ransimat test can prevent the active substances in the volatile oils or show a reverse mechanism, it is thought that the active volatile components are lost by flying away from the environment (Turek and Stintzing, 2012).

Sage Plant Volatile Oil Composition (%)

Table 4.7. Sage plant volatile oil composition (%).

Peak	R.T.	Component name	Pct Total (%)
1	6,301	1s- α -pinene	6,764
2	7,173	camphene	2,267
3	8,093	2-.beta.-pinene	3,033
4	9,448	beta.-myrcene	2,951
5	9,939	alpha.-terpinene	0,262
6	10,481	dl-limonene	2,189
7	10,775	1,8-cineole	49,027
8	11,863	gamma.-terpinen	0,15
9	12,68	p-cymene	2,434
10	17,486	beta.-thujone	3,84
11	18,089	alpha.-thujone	1,593
12	18,257	1-octen-3-ol	0,167
13	19,527	copaene	0,151
14	20,485	camphor	8,113
15	21,274	linalool	0,245
16	22,324	(-)-bornyl acetate	0,375
17	22,795	caryophyllene	3,599
18	23,012	terpinene-4-ol	0,736
19	23,059	(+)-aromadendrene	0,297
20	24,924	alpha.-humulene	0,771
21	25,059	delta.-terpineol	1,072
22	25,765	alpha. terpineol	2,318
23	25,944	borneol	1,403
24	32,762	(-)-caryophyllene oxide	0,797
25	33,147	methyleugenol	0,08
26	33,217	santolina triene	0,096
27	33,536	humulene oxide II	0,311
28	34,045	veridiflorol	1,703
29	34,472	(+) spathulenol	0,156
30	34,905	thymol	0,181
31	34,973	eugenol	0,172
32	35,077	3,4-diethylphenol	0,092
33	35,177	decane, 1-bromo-2-methyl-	0,145
34	35,247	phenol, 2-ethyl-4,5-dimethyl-	0,097
35	35,355	carvacrol	0,808
36	35,997	cis-z.-alpha.-bisabolene epoxide	0,235
37	36,103	caryophylla-4(12),8(13)-dien-5.beta.-ol	0,352
38	36,396	2-butyryl 3-phenylpropanoate	0,621
39	36,448	patchoulane	0,079
40	36,719	caryophylla-3,8(13)-dien-5.beta.-ol	0,317
Total			99,99

As seen in Table 4.7, a total of 40 components were found in the composition of sage essential oil. The highest amounts of these components are 1,8-cineole (49,02%), camphor (8,11%), 1s- α -pinene (6,76%) and beta-thujone (3,84%), respectively. In a study by Karık and Sağlam, (2017), *Salvia fruticosa mill.* obtained from Turkey was characterized by the volatile oil composition gc and gc-ms. As a result of this study, a total of 19 components were reported. Some of these components were highest compenents 1,8-cineole (37.3%) and camphor (8.1%). Also low amounts of thujones (α -thujone 1.2%- 3.3% and β -thujone 0.9%- 9.9%) were stated (Abu-darwish et. Al., 2013). Compared with the literature, sage essential oil components were found to be similar

Antibacterial and Antifungal Effects of Volatile Oils of Sage Plant

The results of antimicrobial activity of volatile oils used in the study are given in Table 8.1 and 8.2. In our study, we investigated the antimicrobial effect of these herbs which are used as medicinal plants. According to the results of the experiment, antibacterial activity could be observed on all bacteria.

Table 8.1. Antibacterial effects of volatile oils of sage plant.

Volatile oils of Sage		Test Bacteria				
		<i>S. Enteritis</i> ATCC 13076	<i>E. coli</i> ATCC 25922	<i>L.monocytogenes</i> ATCC 7644	<i>S. aureus</i> ATCC 2592	<i>E.coli</i> O157:H7
<i>Salvia fruticosa</i>	DD	50,70	24,82	52,24	51,51	26,32
	MIC	15,62	62,50	15,62	15,62	15,62
	MIC	15,62	15,62	31,25	31,25	31,25

3.DD: Disk Diffusion Method (millimeter)

4.MIC: Minimum Inhibition Concentration (Micro gram / milliliter)

Table 8.2. Antifungal effects of volatile oils of sage plant (inhibition zone diameter mm).

Volatile oils of Sage	<i>Aspergillus parasiticus</i> NRRL 3357	<i>Aspergillus parasiticus</i> DSM 5771	<i>Zygosaccharomyces rouxii</i> ATCC 28253
<i>Salvia fruticosa</i>	NA	NA	15,83

NA: No active

As shown in Table 8.1, antimicrobial activity studies using disk diffusion method revealed that sage oil used in the study was effective on bacteria and formed very high inhibition zones on bacteria. *S. Enteritis* essential oil sage (50.70 mm) acting on *ATCC 13076* strain. It was observed that sage (52.24 mm) was active on *L. monocytogenes ATCC 7644* strain. Again sage oil was effective on *S. aureus ATCC 2592* and 51.51 inhibition zones were determined.

The antifungal effects of essential oil on 2 different mold and 1 yeast species were also investigated (Table 8.2). It was determined that all of the sage oils did not show activity against the fungi tested. Sage samples were not only effective against mold types but also had low effect on yeast species. Inhibition zones of sage oils were not formed. The only yeast species used in the study was sage oil on *Zygosaccharomyces rouxii ATCC 28253*.

The results obtained from our study were found to be higher than the results obtained from the study of the antimicrobial activities of basil, rosemary, sage and thyme oils of Teixeira et. al. (2013). In our study, antimicrobial activity of sage was determined higher than the results of Ebrahimabadi et.al. (2010). The results of the study to determine the minimum inhibition concentration were in parallel with the disc diffusion method and sage oils were effective against the pathogenic bacteria tested at the lowest concentrations. The most effective sage on *L. monocytogenes ATCC 7644* was volatile and a concentration of 15.62 µg/ml was determined. The values obtained in our study were higher than the values of Al. Maqtari et.al. (2011).

CONCLUSION

Afyonkarahisar Medicinal and Aromatic Plants research center provided by the contribution of this project and various physicochemical and antimicrobial properties revealed sage plant and essential oil in the use of essential oils in the pharmaceutical, cosmetic and food areas, increasing the use of these plants as a source of healing and traditional medicine in terms of the transfer to our country people is recommended and need development also quality control parametres of sage plant and volatile oil.

ACKNOWLEDGMENTS

The author thanks for their supports to Turkish Republic Ministry of Forestry, Afyonkarahisar Medical and Aromatic Plant Center.

REFERENCES

- Abu-Darwish, M. S., Cabral C., Ferreira I.V., Gonçalves M.J., Cavaleiro C., Cruz M. T., Al-bdour T.H and Salgueiro L. 2013. Essential Oil of Common Sage (*Salvia officinalis* L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory Potential, *Biomed Res Int.* 2013; 2013: 538940.
- Abu-Darwish, M. S., 2014. Essential Oil Variation and Trace Metals Content in Garden Sage (*Salvia officinalis* L.) Grown at Different Environmental Conditions, *Journal of Agricultural Science*; Vol. 6, No. 3; 2014 ISSN 1916-9752, Published by Canadian Center of Science and Education.
- Al.Maqtari M.A.A., Alghalibi S.M., Alhamzy E.H. 2011. "Chemical composition and antimicrobial activity of essential oil of *Thymus vulgaris* from Yemen". *Turkish Journal of Biochemistry.* 36 (4), 342–349.
- Anonim, 1970. Türk Standartları Enstitüsü, Refraktif İndeks Analizi Standardı-894.
- Anonymous, 1992. American Oil Chemists' Society Sampling and analysis of commercial fats and oils: Oil Stability Index. Cd 12b-92 Volume-1.
- Anonymous, 2016. . <http://web.ogm.gov.tr/birimler/merkez/odundisiurun/dkmanlar/envanter/planlama/sube/mudurlugu/Sage.pdf>. Erişim tarihi: 15.06.2016
- Bayram, E., Kırıcı, S., Tansı, S., Yılmaz, G., Arabacı, O., Kızıl, S., Telci, İ. 2009. Tıbbi ve Aromatik Bitkilerin Üretiminin Artırılması Olanakları. www.zmo.org.tr/resimler/ekler/09e9d4bcc8157c0_ek.pdf. Erişim Tarihi: 12.07.2016.
- Bruni, R., Medici, A., Andreotti, E., Fantin, C., Muzzoli, M. ve Dehesa, M. 2003. Chemical composition and biological activities of Ishpingo essential oil, A traditional Ecuadorian spice of *Ocotea quixos* (Lam) Kosterm. (Lauraceae) şower calices. *Food Chemistry.* 85(3): 415-421.
- Buesa, C.; Echarri, J. M.; Torrecilla, C. 2005. Alimentos funcionales. Los Fitobióticos o fitoquímicos. Desarrollo de la alimentación funcional en Cataluña. Barcelona. European Spice Association.
- Çam, H. Karakoç, Ö.Ç. Gökçe, A. Telci, İ. Demirtaş, İ. 2012. Farklı nane türlerine ait klonların Volatile oillerinin buğday bitine fumigant etkisi, *Türk. entomol. derg.*, 2012, 36 (2): 255-263.
- Ebrahimabadi A.H., Mazoochi A., Kashi F.J., Bidgoli Z.D., Batooli H. 2010. "Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran. *Food and Chemical Toxicology.* 48, 1371-1376.
- European Pharmacopoeia 7.0, 2010. Council of Europe, Strasbourg.
- Farag, R. S., Daw, Z. Y., Hewedi, F. M. ve El-Baroty, G.S.A. 1989. Antimicrobial activity of some egyptian spice essential oils. *Journal of Food Protection.* 52: 665-667.
- Farhat, G. N. Affara, N. I. and Gali-Muhtasib, H. U. 2001. "Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice.," *Toxicon*, vol. 39, no. 10, s.1601–1605.
- Gali-Muhtasib, H. 2006. "Anticancer and medicinal properties of essential oil and extracts of East Mediterranean sage (*salvia triloba*).," *Adv. Phytomedicine*, vol. 2, s.169–180.
- Güllüce, M., Sokmen, M., Sahin, F., Sokmen, A., Adiguzel, A., and Ozer, H. 2004. "Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L.) Druce ssp. *Serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey". *Journal of the Science of Food and Agriculture*, 84, 735–741.
- Lazaridio, A., 2004, Composition thermal and rheological behaviour of selected Greek honeys, *Journal of Food Engineering*, 64, 9-21.
- Karık, Ü. and Sağlam, C., 2017, Tekirdağ Ekolojik Koşullarında Anadolu Adaçayı (*Salvia fruticosa* Mill.) Popülasyonlarının Verim ve Kalite Özelliklerinin Belirlenmesi. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi* 2017, 26 (2): 203–215. (in Turkish).
- Mc Guire, R. G., 1992, Reporting of objective color measurements, *Hortscience*, 27 (12), 1254-1255.
- Nakamura, C.V.; Ueda- Nakamura, T.; Bando, E.; Melo, A.F.N.; Cortez, D.A.G. and Filho, B.P.D. 1999. Antibacterial activity of *Ocimum gratissimum* L., Volatile oils, *Mem Inst Oswaldo Cruz*, Rio de Janeiro, Vol. 94(5): 675-678.
- Nychas, G. J. E. 1995. Natural antimicrobials from plants. In: Gould, G. W. (Ed.), *New Methods of Food Preservation.* (58pp) London, Blackie: Academic Profesional.
- Skujins S. 1998. Handbook for ICP-AES (Varian-Vista). A hort Guide To Vista Series ICP AES Operation. Varian Int. AG Zug. Version 1.0. pp 29. Switzerland.
- Subramanian R., Gayathri S. , Rathnavel C., Raj V. 2012. Analysis of mineral and heavy metals in some medicinal plants collected from local market. *Asian Pacific Journal of Tropical Biomedicine* (2012)S74-S78.
- Teixeira, B., Marques, A., Ramos, C., Neng N.R., Nogueira J.M.F., Saraiva J.A, Nunes M.L. 2013. "Chemical composition and antibacterial and antioxidant properties of commercial essential oils. *Industrial Crops and Products.* 43, 587-595.
- Telci, İ., Bayram, E., Yılmaz, G. and Avcı, B. 2006. Variability in essential oil composition of Turkish basil (*Ocimum basilicum* L.). *Biochemical Systematics and Ecology*, 34: 489-497.

- Turek and Stintzing, 2012. Impact of different storage conditions on the quality of selected essential oils. *Food. Res. Int.*, 46 (2012), pp. 341–353
- World Health Organization, 2007. *Guidelines for Assessing Quality of Herbal Medicines with Reference to Contaminants and Residues*, Geneva.

INDIGO PLANT (*Isatis glauca* L.) SEED OIL AND SOME PHYSICO-CHEMICAL PROPERTIES

Sabire DUMAN¹, Erman DUMAN²

¹Afyon Kocatepe University, Afyon Medicinal Highschool, Nutrition and Dietetic Department, Afyonkarahisar, Turkey

²Afyon Kocatepe University, Engineering Faculty, Food Engineering Department, Afyonkarahisar, Turkey

Corresponding author email: eduman@aku.edu.tr

Abstract

In this research, for centuries primarily textile dyeing, also used for medical purposes; The seed of *Isatis glauca* L. was analyzed. The fatty acid compositions (%) of seed oil (%) of indigo seeds obtained from Afyonkarahisar Medical and Aromatic Plant Centers of Turkish Republic Ministry of Forestry were investigated using Soxhlet and gas chromatography. In our study, the oil content of indigo seeds was found to be 12.53% and a total of 19 fatty acids were detected. The three most common fatty acids in the seed of *Isatis glauca* L. are as follows; oleic acid (23.77 %), linoleic acid (16.53 %), erusic acid (38.45 %). It was found to be rich in unsaturated fatty acids. There are also positive effects on health not only in textile dyeing but shall be attention cause erusic acid content.

Keywords: *Indigo plant, Isatis glauca* L., oil, refractive index, fatty acids composition.

INTRODUCTION

Indigo was the most universally important dyestuff (Gilbert and Cook, 2001) and natural indigo as a textile dye have been used since Bronze Age (-7000) (Pawlak et al., 2006). Natural indigo can be derived from many plants belonging to different species, generation and families but the most commonly used species was *Isatis tinctoria* in temperate climates (Gilbert and Cook, 2001).

Turkey has a large number of plants with paint containing dyestuff and dyeing feature. Some parts of the plants are used for staining while others are used only in flowers, leaves, bark or roots (Öztürk, 1999). As in the past, many of the dyeing plants are used in textile, as well as in different areas such as medicine, cosmetics and coloring of foods (Piccaglia and Venturi, 1998). Most of the herbs used in this context have the same or separate active substances as herbal teas. They contain antioxidant, antibacterial and antimicrobial agents. When these dye plants are not collected within a year, they dry out and return to the soil (Karadağ, 2007).

One of the rare colors of the dyes used in natural dyeing is blue. The blue color is obtained from two main sources: Indigo (*Indigofera tinctoria*) and Indigo plant (*Indigo tinctoria*) (Kızıl and Arslan, 2001). The dyestuff obtained from indigo was used in textile fields as well as in painting areas such as wall paintings. Nowadays, it is very difficult to determine the origin of the image used in painting and fabric dyeing by chemical analysis because the places where indigo comes from are different. It is known that 300 different indigofera plants are obtained from Africa, Asia or America (Delammare and Guineau, 2007).

Indigo is a plant belonging to the genus *Isatis* of the family Brassicaceae. When analyzed morphological aspects it is one of the most difficult to breed in Turkey. It is very difficult to separate species levels. It is very difficult to differentiate in terms of flowering time of *Isatis* species. During the period between May and October, the plant goes into flowering time. When the leaf is examined formally, it is divided into two as bottom and trunk leaves. Bottom leaves are usually long and unsealed. The leaves that are at the bottom are poured when the plant starts to bloom. The plumage of the leaves is not characteristic, can be seen as hairy or hairless. Flower condition is usually yellow flowers and flowers are located at the ends of the branches. The body of the plant is generally green in color and varies between 15-40 cm and 125-175 cm according to the species. When we examine

the structure of the plant weed, we present as a herbaceous plant on rocky slopes, shrubs and empty terrain. (Anonymous, 1991).

The homeland of indwelling is the Caucasus slopes and spread from the far east to the Himalayas. Today, culture is being made in North Africa and Europe. Nearly 30 types of woad is grown naturally in Turkey. However, it is unknown when it comes to Turkey (Karadağ, 2007). Indigo in Turkey of 'distribution area is referred to as the Southern and Eastern Anatolia. In terms of province, the distribution area of the weed is Osmaniye, Hakkari, Bitlis, Antalya, Bingöl, Manisa, Niğde (Kaya, 2016).

It grows wildy on the edges of the fields in Central and Western Anatolia. All types of *Isatis* species are known for their yellow flowers. Cube dye indigo is obtained by a complex fermentation and oxidation process (Enez, 1987).

A variety of shades of blue are obtained as a result of the fermentation process. The variety of color tones obtained is related to the number of times the dye was sprayed in the dye bath. First, the dark blue navy blue color of the second dye in the dyeing process was obtained. After 1577, indigon has lost its importance by supplying indigo from outside. The Romans used the indigo to make the ink called ebi indicum Rom. Indigo is one of the water-insoluble dyestuffs and is reduced by fermentation. It dissolves in the base environment. The fabrics immersed in this mixture become oxidized to blue color when they are removed into the yellow color and then as they come into contact with the air (Eyüboğlu et al., 1983).

In addition to being a dye plant, *I. tinctoria* is also known as a medicinal plant. Leaves, roots and seeds of *I. Tinctoria* contain indole-derived compounds (principally glucosinolate) with anti-inflammatory and anti-tumoral properties (Frechard et al., 2001; Hamburger, 2002; Oberthür et al., 2005). The roots are used for pharyngitis, laryngitis, erysipelas, and carbuncle, and to prevent hepatitis A, epidemic meningitis, cancer and inflammation and also antibiotic, antiseptic and anti-viral activities were also reported (Han et al., 2011).

For this purpose, in our study, the researches on the oil and some physicochemical properties of *Isatis glauca* L. from *Brassicaceae* family, also known as indigo, have been carried out in our country.

MATERIAL AND METHODS

Material

In this study used indigo seeds (*Isatis glauca* L.) which we obtained from the center of Afyon Medicinal and Aromatic Plants, oil amount determination and fatty acid composition (GC) analysis were performed.

Methods

% Oil content

In our study, fixed oil analysis of indigo seed was determined by using Soxhlet method. 10 grams of ground sham (*Isatis glauca* L.) seeds were weighed into the Soxhlet cartridge, extracted at 80 °C and 6 hours with n-hexane. The n-hexane in the extract was removed from the rotary evaporator under vacuum at 60 °C. The remaining solvent was evaporated at 103 ± 2 °C for 20 minutes. After cooling the desiccator, the amount of fat was determined by using the weighing differences (AOAC., 2000). During the analysis, oil samples were stored in 25 mL brown bottles at -18 ° C and nitrogen gas was applied to prevent air in the bottle.

Fatty acid composition

In our study, fatty acid composition of indigo seed oil was determined by modifying AOCS Ce c-89 (Anonymous, 1989).

RESULTS AND DISCUSSION

The results of the oil analysis in the indigo seed (*Isatis glauca* L.) are given in Table 1.

Table 1. İndigo seed oil content (%).

Seed oil content	%	Std
<i>Isatis glauca</i> L. seed	12.53	0.85

As can be seen in Table 1, the oil content of the seed weed was 12.53 % and this rate was found to be lower than the values found by Müller et.al. (1965) 33% and Pavlov (1947) 29 % while Scrimgeour (1976) % 4 found higher than the value. This may be caused by the difference between variety and the cultivation environment. Also it was determined that the oil content of indigo seed (%12.53) was higher in fenugreek seed (7.24 %) (Brummer et al., 2003) and basil seed (11.55 %) (Razavi et al., 2009).

The results fatty acid composition in the indigo seed (*Isatis glauca* L.) oil are given in Table 2.

Table 2. İndigo seed oil fatty acid composition (%).

Fatty acids	Sample	<i>Isatis glauca</i> Boiss. Scrimgeour (1976)	<i>Isatis lusitanica</i> L. Miller et.al., (1965)	<i>Isatis auseri</i> Boiss. Miller et.al., (1965)
	<i>Isatis glauca</i> L.			
1 Caproic acid (C6:0)	0,04	-	-	-
2 Undecanoic acid (C11:0)	0,02	-	-	-
3 Myristic acid (C14:0)	0,08	-	-	-
4 Palmitic acid (C16:0)	7,45	4.6	9.0	3.0
5 Palmitoleic acid (C16:1)	0,08	-	-	-
6 Heptadecanoic acid (C17:0)	0,08	-	-	-
7 Stearic acid (C18:0)	2,12	0.6	8.0	1.0
8 Oleic acid (C18:1)	23,77	11.1	23.0	15.0
9 Linoleic acid (C18:2)	16,53	13.4	6.0	12.0
10 Linolenic acid (C18:3)	0,09	29.4	20.0	31.0
11 Arachidic acid (C20:0)	1,82	-	2.0	1.0
12 Gadoleic acid (C20:1)	1.16	10.9	10.0	9.0
13 11-cis,14-cis Eicosadienoic acid (C20:2)	-	1.3	-	0.5
14 Cis11,14,17-eicosatrienoic acid (C20:3)	0,26	-	-	-
15 Cis-13,16-docosadienoic acid (C 22:2)	0,52	-	-	-
16 Behenic acid (C22:0)	-	1.0	0.1	-
17 Erusic acid (C22:1)	38,45	23.3	23.0	24.0
18 Docosadienoic acid (C22:2)	-	0.5	-	-
19 Lignoceric acid (C24:0)	0,793	0.3	-	-
20 Nervonic acid (C24:1)	5,622	3.5	-	2.0
21 Docosahexaenoic acid (C 22:6 n-3)	0,248	-	-	-

Table 2. shows the results of the analysis of fatty acids composition in the indigo seed (*Isatis glauca* L.). As a result of this study have been identified 19 fatty acids. *Isatis glauca* L. seed fixed oil in terms of saturated fatty acids, especially as palmitic acid (7.54 %) and oleic acid (23.77 %), linoleic acid (16.53 %) and erusic acid (38.45 %) were found to be rich in unsaturated fatty acids. When

compared to the literature (Scrimgeour, 1976 and Miller et.al., 1965), it was determined that the fatty acid composition. In addition, determined was changed according to other varieties. But it is seen high in all varieties percentage of erusic acid.

CONCLUSION

The three most common fatty acids in the seed of *Isatis glauca* L. are as follows; oleic acid (23.77 %), linoleic acid (16.53 %), erusic acid (38.45 %). It was found to be rich in unsaturated fatty acids. There are also positive effects on health not only in textile dyeing but shall be attention cause erusic acid content.

ACKNOWLEDGEMENTS

The author thanks for their supports to Turkish Republic Ministry of Forestry, Afyonkarahisar Medical and Aromatic Plant Center and Afyon Kocatepe University Science Research Coordinator (BAP).

REFERENCES

- Anonim., 1991. Bitkilerden Elde Edilen Boyalarla Yün Liflerin Boyanması. TC. Sanayi ve Ticaret Bakanlığı Küçük Sanatlar ve Sanayi Bölgeleri ve Siteleri Genel Müdürlüğü, Ankara.
- Anonymous, 1989. American Oil Chemists' Society Fatty Acid Composition by GC, cis and trans isomers, Official Method Ce c-89 (93) Volume-1.
- Anonymous., 2000. Official Methods of Analysis of AOAC International (17th ed.). AOAC International Suite Suit 500, 481 North Frederick Avenue Gaithersburg. Maryland, 2417-2877, USA.
- Brummer, Y., Cui, W. ve Wang, Q., 2003. "Extraction, purification and physicochemical characterization of fenugreek gum", Food Hydrocolloids, 17:229–236.
- Delamare, F., Guineau, B., 2007, Renkler ve Malzemeleri. Çev. Türkay, Orçun. Yapı Kredi Kültür Sanat Yayıncılık, 2007.
- Enez N., 1987. Doğal Boyamacılık Anadolu'da Yün Boyamacılığında Kullanılmış Olan Bitkiler ve Doğal Boyalarla Yün Boyamacılığı. Marmara Üniversitesi Yayın No 449. Fatih Yayınevi.
- Eyüboğlu., Okaygün I., Yaraş F., 1983. Doğal Boyalarla Yün Boyama Uygulamalı ve Geleneksel Yöntemler. (1. Baskı). İstanbul: Ofset yapımevi, 19.
- Frechard A., Fabre N., Pean C., Montaut S., Favvel M., Rollin P. and Fauraste L., 2001. Novel indoletype glucosinolates from woad (*Isatis tinctoria* L). Tetrahedron Letters, 42(51): 9015-9017.
- Gilbert G.K. and Cooke D.T., 2001. Dyes from plants: Past usage, present understanding and potential. Plant Growth Regulation, 34: 57-69.
- Hamburger M., 2002. *Isatis tinctoria* – from the rediscovery of an ancient medicinal plant towards a novel anti-inflammatory phytopharmaceutical. Phytochemistry Reviews, 1: 333-344.
- Han J., Jiang X. and Zhang L., 2011. Optimisation of extraction conditions for polysaccharides from the roots of *Isatis tinctoria* L. by response surface methodology and their in vitro free radicals scavenging activities and effects on IL-4 and IFN- γ mRNA expression in chicken lymphocytes. Carbohydrate Polymers, 86 (3): 1320-1326.
- Karadağ, R., 2007. Doğal Boyamacılık. Geleneksel El Sanatları ve Mağazalar İşletme Müdürlüğü, 40-42, 1. Baskı, Ankara.
- Kaya, Ü., 2016. Pamuk, Yün ve İpek Kumaşların Çivit Otu ile Boyanması ve Bazı Haslık Değerlerinin İncelenmesi, Gazi Üniversitesi, Güzel Sanatlar Enstitüsü, Tekstil Tasarımı Anabilim Dalı, Yüksek Lisans Tezi, s.18.
- Kızıl, S., Arslan, N., 2001, Bazı Çivit Otu (*Isatis Tinctoria* L., *Isatis constricta* Davis) Türleri ile Yün Halı İpliklerinin Boyanması ve Elde Edilen Renklerin Bazı Haslık Değerlerinin Belirlenmesi Üzerine Bir Araştırma, Tarım Bilimleri Dergisi, 2001, 7 (3), 42-47.
- Miller, R.W., Earle, F.R., Wolff, I.A., Jones, Q., 1965, J. Amer. Oil Chem. Soc. 42(10), 817.
- Oberthür C., Jäggi R. and Hamburger M., 2005. HPLC based activity profiling for 5-lipoxygenase inhibitory activity in *Isatis tinctoria* leaf extracts. Fitoterapia, 76 (3): 324-332.
- Öztürk, İ., 1999. Doğal Bitkisel Boyalarla Yün Boyama, İzmir: Dokuz Eylül yayınları 1. Baskı.
- Pavlov, N.V., 1947. Rastitel'noe syr'e Kazakhstana (Moskwa-Leningrad, 1947), 52 s [in Russian]
- Pawlak K., Puchalska M., Miszczak A., Rosloniec E. and Jarosz M., 2006. Blue natural organic dyestuffs-from textile dyeing to mural painting. Separation and characterization of coloring matters present in elderberry, lowood and indigo. Journal of Mass Spectrometry, 41: 613-622.
- Piccaglia R., Venturi G., 1998. Dye plants: A Renewable Source Of Natural Colours, Agro- Food-industry Hi-tech, 27-30, July-August.

- Razavi, S.M.A., Mortazavi, S.A., Matia-merino, L., Hosseini-Parvar, S.H., Motamedzadegan, A. ve Khanipour, E., 2009. 'Optimisation study of gum extraction from basil seeds', International Journal of Food Science and Technology, 44 :1755–1762.
- Scrimgeour, C.M., 1976. Lipids 11(12), 877.

ELATERIDAE (INSECTA: COLEOPTERA) BIODIVERSITY OF TURKISH MEDICINAL PLANT FLORA

Nilay GÜLPERÇİN¹

¹Natural History Application and Research Center, Ege University, Bornova, İzmir, Turkey

Corresponding author email: nilay.gulpercin@gmail.com.tr

Abstract

Medicinal plants diversity of Turkey is quite rich as a result of its location at the intersection of three phyto-geographical regions, its being a bridge between the floras of South Europe and South-west Asia as well as being the center of origination and differentiation of many kinds and sections. As in whole world, use of plants existing in the natural flora for different purposes in public health have been a part of our traditional culture for many years. Medicinal plants also have great significance in terms of the sustainability of biodiversity of insects. Studies on the species belonging to the family of Elateridae (Insecta: Coleoptera) in the insect groups of these plants have been increasing in recent years in Turkey as well as in the world. In this study, the species belonging to Elateridae family in medicinal plants in Turkey were evaluated in light of previous studies, collected material from field and the data from the literature. As a result of these studies, totally 32 species belonging to 14 genera (*Adrastus*, *Aeoloderma*, *Agriotes*, *Ampedus*, *Athous*, *Cardiophorus*, *Dicronychus*, *Drasterius*, *Hemicrepidius*, *Melanotus*, *Peripontius*, *Prosternon*, *Selatosomus* and *Synaptus*) of Elateridae have been determined. The most important medicinal plants which were visited by these species are *Petroselinum crispum*, *Spartium junceum*, *Glycyrrhiza glabra*, *Papaver somniferum*, *Rumex acetosella*, and *Urtica* spp. Among them, *Urtica* spp. are the most preferred plants with 11 species by click beetles. In this study, it noticed that the Elateridae fauna in Turkish medicinal plant flora is very rich and has the potential to be used in biodiversity studies. The present investigation can be used as a reference case study in similar biodiversity studies in the future.

Keywords: Elateridae, fauna, medicinal plants flora, biodiversity, Turkey

INTRODUCTION

Medicinal plants are important part of our natural wealth. They serve as important therapeutic agents as well as valuable raw materials for manufacturing numerous traditional and modern medicines. The history of medicinal plant use for treating diseases and ailments is probably dates back to the beginning of human civilization. Our natural substance that they could find to ease their sufferings caused by acute and chronic illnesses, physical discomforts, wounds and injuries, an even terminal illnesses. Since ancient times, plants with therapeutic properties have secured an important place in the healing practices and treatment of diseases. Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The fact is that, use of herbal treatments is independent of any age groups and the sexes.

In Turkey, medicinal plants are cultivated only in a small fragment of the total area of farmlands, however, the sector produces significant income in this small area. The mounting interest for natural components in medicine, food and cosmetics industry enhances the importance of medicinal plants. In the past few years, significant increase could be observed not only in the use of traditional medicinal plants but also new plant species were introduced into cultivation.

Insects have very great ecological roles which are of benefit to people such as pollination of many plants, recycling of plant nutrients and breakdown of waste and organic matter. Without insects these natural processes would be greatly slowed down and life would become very difficult. Medicinal plants offers lots of food for insects. Accordingly, there is a great importance in the sustainability of biodiversity of insects. Unfortunately, spite of their increasing importance, the fauna of the cultivated medicinal plants is almost unknown. In some studies, have been mentioned out to reveal the importance and species richness of Elateridae in medicinal plants of Turkey (Yüksel, 1970, Lodos, 1998, Kesdek et al., 2006, Gülperçin & Tezcan, 2009, 2010b, 2012a, 2012b, 2014a, 2014b, 2014c, 2015, Özgen et al., 2012) and the knowledge of this subject has been increased. According to preliminary observations and literature data, it became clear that Elateridae species occur on several

medicinal plants in Turkey, frequently in high density. Preliminary observations suggested that some species might eventually cause considerable damage.

Medicinal plants are one of the agricultural ecosystems that provide the environment for nutrition, shelter, protection and reproduction of insects. There is also a great importance in terms of the sustainability of biological diversity. The species belonging to the family Elateridae (Insecta: Coleoptera) found in the insect groups of the medicinal are generally found on flowers, leaves, trunk and roots of medical plants.

There are many species that attract attention from the agricultural side (Lodos, 1998) and especially *Agriotes*, *Ampedus*, *Adrastus* and *Synaptus* species are predominant in medicinal plants (Laibner, 2000). These species are *Cardiophorus discicollis*, *C. nigratissimus*, *Drasterius bimaculatus*, *Peripontius terminatus* and *Synaptus filiformis*.

The studies on the species belonging to the family Elateridae have been increasing in recent years in Turkey as well as in the world. The aim of this article is to evaluate the studies on the species belonging to the family of Elateridae in medicinal plants in Turkey and aim to holding future studies.

MATERIAL AND METHODS

For the investigation of the studies on the species belonging to the family of Elateridae found in Turkish medicinal plants and to collect information about this subject Önder et al. (1982, 1986) and Tezcan et al. (2013) based on the publications in the bibliography, the works prepared by local and foreign researchers were used (Yüksel, 1970; Lodos, 1998; Gülperçin & Tezcan, 2009, 2010b, 2012a, 2012b, 2014a, 2014b, 2014c, 2015; Kesdek et al., 2006; Löbl & Smetana, 2007; Mertlik & Platia, 2008; Özgen et al., 2012).

RESULTS AND DISCUSSION

At the end of the study, 32 species belonging to 14 genera belonging to the family Elateridae were found in medicinal plants in Turkey and these species are given in Table 1.

Table 1. Species attached to the family of Elateridae found in Turkish medicinal plants.

Species	
<i>Adrastus anatolicus</i> Platia & Schimmel, 1991	<i>Cardiophorus rotundicollis</i> Frivaldszky, 1845
<i>Adrastus limbatus</i> (Fabricius, 1777)	<i>Cardiophorus sacratus</i> Erichson, 1840
<i>Adrastus montanus</i> (Scopoli, 1763)	<i>Cardiophorus vestigialis</i> Erichson, 1840
<i>Aeoloderma crucifer</i> (Rossi, 1790)	<i>Dicronychus rubripes</i> (Germar, 1824)
<i>Agriotes brevis</i> Candéze, 1863	<i>Drasterius bimaculatus</i> (Rossi, 1790)
<i>Agriotes gurgistanus</i> (Faldermann, 1835)	<i>Hemicrepidius hirtus</i> (Herbst, 1784)
<i>Agriotes kraatzi</i> Schwarz, 1891	<i>Melanotus crassicornis</i> (Erichson, 1841)
<i>Agriotes lineatus</i> (Linnaeus, 1767)	<i>Melanotus cuneiformis</i> Baudi di Selve, 1871
<i>Agriotes obscurus</i> (Linnaeus, 1758)	<i>Melanotus fusciceps</i> (Gyllenhal, 1817)
<i>Agriotes paludum</i> Kiesenwetter, 1859	<i>Melanotus villosus</i> (Geoffroy, 1785)
<i>Agriotes sputator</i> (Linnaeus, 1758)	<i>Peripontius concolor</i> (Desbrochers des Loges, 1875)
<i>Ampedus elegantulus</i> (Schönherr, 1817)	<i>Peripontius omissus</i> (Buysson, 1889)
<i>Athous vittatus</i> (Fabricius, 1792)	<i>Peripontius terminatus</i> (Erichson, 1841)
<i>Cardiophorus discicollis</i> (Herbst, 1806)	<i>Prosternon tessellatum</i> (Linnaeus, 1758)
<i>Cardiophorus megathorax</i> Faldermann, 1835	<i>Selatosomus latus</i> (Fabricius, 1801)
<i>Cardiophorus nigratissimus</i> Buysson, 1891	<i>Synaptus filiformis</i> (Fabricius, 1781)

Table 2. Species belonging to the family of Elateridae on medicinal plants in Turkey.

<i>Family of Plant</i>	<i>Plant</i>	<i>Species of Elateridae</i>
Apiaceae	<i>Petroselinum crispum</i> Miller, 1925	<i>Agriotes brevis</i>
Asteraceae	<i>Centaurea</i> sp.	<i>Cardiophorus vestigialis</i> , <i>Drasterius bimaculatus</i>
	<i>Cirsium</i> sp.	<i>Drasterius bimaculatus</i>
	<i>Matricaria</i> sp.	<i>Agriotes paludum</i> , <i>Cardiophorus rotundicollis</i>
	<i>Onopordum</i> sp.	<i>Cardiophorus discicollis</i> , <i>C. megathorax</i> , <i>C. nigratissimus</i> , <i>C. sacratus</i> , <i>Melanotus fusciceps</i>
Brassicaceae	<i>Sinapis</i> spp.	<i>Aeoloderma crucifer</i> , <i>Agriotes lineatus</i> , <i>A. obscurus</i> , <i>A. sputator</i> , <i>Cardiophorus discicollis</i> , <i>Melanotus crassicollis</i> , <i>Peripontius terminatus</i>
	<i>Humulus lupulus</i> Linnaeus, 1753	<i>Adrastus anatolicus</i> , <i>A. limbatus</i> , <i>A. montanus</i> , <i>Hemicrepidius hirtus</i> , <i>Selatosomus latus</i> , <i>Synaptus filiformis</i>
Capparaceae	<i>Capparis</i> sp.	<i>Melanotus fusciceps</i>
Cistaceae	<i>Cistus</i> sp.	<i>Cardiophorus vestigialis</i> , <i>Peripontius omissus</i> , <i>P. terminatus</i>
Cyperaceae	<i>Cyperus rotundus</i> Linnaeus, 1753	<i>Melanotus fusciceps</i> , <i>M. villosus</i>
Equisetaceae	<i>Equisetum</i> sp.	<i>Synaptus filiformis</i>
Ericaceae	<i>Rhododendron</i> sp.	<i>Cardiophorus discicollis</i>
Euphorbiaceae	<i>Euphorbia</i> sp.	<i>Cardiophorus megathorax</i> , <i>C. nigratissimus</i> , <i>Peripontius concolor</i>
Fabaceae	<i>Spartium junceum</i> Linnaeus, 1763	<i>Cardiophorus discicollis</i>
	<i>Glycyrrhiza glabra</i> Linnaeus, 1753	<i>Cardiophorus nigratissimus</i>
Geraniaceae	<i>Pelargonium</i> sp.	<i>Athous vittatus</i> , <i>Melanotus cuneiformis</i>
Lamiaceae	<i>Sideritis</i> sp.	<i>Drasterius bimaculatus</i>
	<i>Mentha</i> sp.	<i>Synaptus filiformis</i>
Papaveraceae	<i>Papaver somniferum</i> Linnaeus, 1753	<i>Agriotes sputator</i> , <i>Melanotus villosus</i>
Polygonaceae	<i>Rumex acetosella</i> Linnaeus, 1753	<i>Adrastus anatolicus</i> , <i>Agriotes lineatus</i> , <i>A. obscurus</i> , <i>A. sputator</i>
Scrophulariaceae	<i>Verbascum</i> sp.	<i>Drasterius bimaculatus</i> , <i>Peripontius terminatus</i> , <i>Prosternon tessellatum</i> , <i>Selatosomus latus</i>
Styracaceae	<i>Styrax</i> sp.	<i>Drasterius bimaculatus</i> , <i>Peripontius concolor</i> , <i>P. terminatus</i>
Urticaceae	<i>Urtica</i> sp.	<i>Agriotes brevis</i> , <i>A. gurgistanus</i> , <i>A. kraatzi</i> , <i>Ampedus elegantulus</i> , <i>Cardiophorus megathorax</i> , <i>C. nigratissimus</i> , <i>C. vestigialis</i> , <i>Dicronychus rubripes</i> , <i>Hemicrepidius hirtus</i> , <i>Peripontius terminatus</i> , <i>Synaptus filiformis</i>

Table 1 shows that *Agriotes* (7), *Cardiophorus* (6) and *Melanotus* (4) have the highest number of species. *Aeoloderma*, *Ampedus*, *Athous*, *Dicronychus*, *Drasterius*, *Hemicrepidius*, *Prosternon*, *Selatosomus* and *Synaptus* have been represented by one species.

Studies have also been carried out on the identification of the plant species on which these species are present and nourished, and many of the sources have noted the fact that the species are found on the plants and that they are nourished. The information obtained in this context is given in Table 2.

When studied according to the collected samples from 32 species in the investigated studies, it was found that there were 11 species in *Urtica* genus, 7 species in *Sinapis* genus, 6 species in *Humulus* genus, 5 species in *Onopordum* genus, 4 species in *Rumex* and *Verbascum* genera. From other medicinal plants, it has been determined 3 species in plants belonging to *Cistus*, *Euphorbia* and *Styrax* genera, two species in plants belonging to *Centaurea*, *Cyperus*, *Matricaria*, *Papaver* and *Pelargonium* genera; one species in *Capparis*, *Cirsium*, *Equisetum*, *Glycyrrhiza*, *Mentha*, *Petroselinum*, *Rhododendron*, *Spartium* and *Sideritis* genera are found in Turkish medicinal plants.

This distribution are examined according to plants family, **Brassicaceae** family (13 species) attracts attention. **Urticaceae** family (11 species), **Asteraceae** family (10 species), **Polygonaceae** and **Scrophulariaceae** (4 species), **Cistaceae**, **Euphorbiaceae** and **Styracaceae** (3 species), **Cyperaceae**, **Fabaceae**, **Geraniaceae**, **Lamiaceae** and **Papaveraceae** (2 species) and **Apiaceae**, **Capparaceae**, **Equisetaceae** and **Ericaceae** (1 species) follow this family respectively.

According to Gülperçin & Tezcan (2010a), 39 species have been added to the Elateridae fauna of Turkey which is represented by 462 species and total number of species has increased to 501. In this study, the number of species entering the evaluation 32, this value constitutes 6,39 % of Turkish Elateridae fauna and medicinal plants constitute an important living place for other living groups as well as for species affiliated with this family.

The distribution of the 32 species found in the medicinal plants in Turkey are given in Table 3.

When Table 3 is examined, it is determined that the species belonging to Elateridae family are found mostly in the Aegean Region (31 species), Mediterranean Region (26 species) and Central Anatolia Region medicinal plants (26 species). This regions are followed by the Black Sea (25 species), Marmara (24 species), Eastern Anatolia (19 species) and Southeast Anatolia Region (14 species) respectively.

When the information on the first reported species in Turkey is examined according to ten year periods, the results obtained are given in Figure 1.

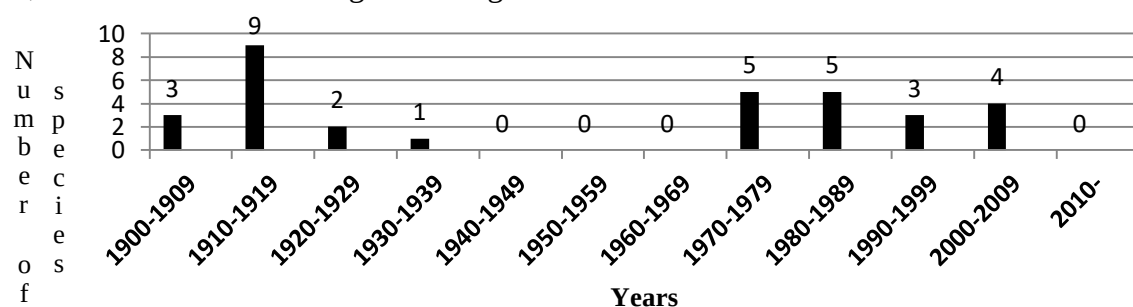


Fig 1. The first reported years from Turkey according to ten year periods of examined species (n= 110).

When Figure 1 was examined, the first reports on this subject were started with Heyden et al. (1906)'s works between 1900-1909 years, it is understood that Sahlberg, 1912-1913, Schenkling, 1925-1927, İyriboz, 1934, Yüksel, 1970 and Gül-Zümreoğlu, 1972 continued with their works. Noted that there were no significant additional records until 1980's and then it has been seen that this number has risen with the Guglielmi & Platia, 1985, Lodos, 1998, Kabalak & Sert, 2005, Kesdek et al., 2006 and Löbl & Smetana, 2007's works.

Table 3. Distribution of the species belonging to the family Elateridae found in Turkish medicinal plants according to geographical regions: 1. Mediterranean Region, 2. Eastern Anatolia Region, 3. Aegean Region, 4. Southeastern Anatolia Region, 5. Central Anatolia Region, 6. Black Sea Region, 7. Marmara Region.

<i>Species of Elateridae</i>	<i>Geographical Regions</i>						
	1	2	3	4	5	6	7
<i>Adrastus anatolicus</i> Platia & Schimmel, 1991	+	+	+	+	+	+	+
<i>Adrastus limbatus</i> (Fabricius, 1777)	+	+	+			+	+
<i>Adrastus montanus</i> (Scopoli, 1763)	+		+		+	+	+
<i>Aeoloderma crucifer</i> (Rossi, 1790)	+		+	+	+	+	+
<i>Agriotes brevis</i> Candéze, 1863	+		+		+		+
<i>Agriotes gurgistanus</i> (Faldermann, 1835)		+	+		+	+	+
<i>Agriotes kraatzi</i> Schwarz, 1891	+		+				
<i>Agriotes lineatus</i> (Linnaeus, 1767)	+	+	+		+	+	+
<i>Agriotes obscurus</i> (Linnaeus, 1758)	+	+			+		
<i>Agriotes paludum</i> Kiesenwetter, 1859	+	+	+	+	+	+	+
<i>Agriotes sputator</i> (Linnaeus, 1758)	+	+	+	+	+	+	+
<i>Ampedus elegantulus</i> (Schönherr, 1817)	+		+		+	+	
<i>Athous vittatus</i> (Fabricius, 1792)		+	+			+	
<i>Cardiophorus discicollis</i> (Herbst, 1806)	+		+	+	+	+	+
<i>Cardiophorus megathorax</i> Faldermann, 1835	+	+	+	+	+	+	+
<i>Cardiophorus nigratissimus</i> Buysson, 1891	+	+	+	+	+	+	
<i>Cardiophorus rotundicollis</i> Fivaldszky, 1845			+				
<i>Cardiophorus sacratus</i> Erichson, 1840	+	+	+	+	+	+	
<i>Cardiophorus vestigialis</i> Erichson, 1840	+	+	+	+	+	+	+
<i>Dicronychus rubripes</i> (Germar, 1824)	+		+		+	+	+
<i>Drasterius bimaculatus</i> (Rossi, 1790)	+	+	+	+	+	+	+
<i>Hemicrepidius hirtus</i> (Herbst, 1784)			+		+	+	+
<i>Melanotus crassicollis</i> (Erichson, 1841)	+		+				+
<i>Melanotus cuneiformis</i> Baudi di Selve, 1871	+		+				+
<i>Melanotus fusciceps</i> (Gyllenhal, 1817)	+	+	+	+	+	+	+
<i>Melanotus villosus</i> (Geoffroy, 1785)	+	+	+		+	+	
<i>Peripontius concolor</i> (Desbrochers des Loges, 1875)	+	+	+		+	+	+
<i>Peripontius omissus</i> (Buysson, 1889)	+		+		+	+	+
<i>Peripontius terminatus</i> (Erichson, 1841)	+		+	+	+	+	+
<i>Prosternon tessellatum</i> (Linnaeus, 1758)		+	+		+	+	+
<i>Selatosomus latus</i> (Fabricius, 1801)		+	+	+	+		+
<i>Synaptus filiformis</i> (Fabricius, 1781)	+	+	+	+	+	+	+
Total species	26	19	31	14	26	25	24

CONCLUSION

As our lifestyle is now getting techno-savvy, we are moving away from nature. While we cannot escape from nature because we are part of nature. As herbs are natural products they are free from side effects, they are comparatively safe, eco-friendly and locally available. Traditionally there are

lot of herbs used for the ailments related to different seasons. There is a need to promote them to save the human lives.

These herbal products are today are the symbol of safety in contrast to the synthetic drugs, that are regarded as unsafe to human being and environment. Although herbs had been priced for their medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security. It's time to promote them globally.

When the above findings are evaluated as a whole, there are 32 species belonging to Elateridae family in Turkish medicinal plants and these species constituted 6.39 % of Turkey Elateridae fauna represented by 501 species; these species are predominantly on plants belonging to **Brassicaceae** (13 species), **Urticaceae** family (11 species), **Asteraceae** family (10 species), family members. It was understood that the total number of species in the Aegean and Mediterranean Region medicinal plants were 31 (96.87 %) and 26 (81.25 %) respectively. Other regions follow these regions.

When the information on the first reported species in Turkey is examined according to ten year periods it is seen that it is important break through with the studies aiming to reveal species in medicinal plants especially from the year 2000. The protection of the insects, which are very important for biodiversity in Turkish medicinal plants cultures; as well as the sustainability of its ecological functions and the preservation of the biological wealth of Turkey and its future transportation.

REFERENCES

- Guglielmi A., Platia, G., 1985. Contributio alla conoscenza degli Elateridi di Grecia e Turchia. *Fragmenta Entomologica*, Roma, 18 (1): 169-224.
- Gül-Zümreoğlu, S., 1972. İzmir Bölge Zirai Mücadele Araştırma Enstitüsü Böcek ve Genel Zararlılar Kataloğu, 1928-1969 (I. Kısım). İstiklal Matbaası, İzmir, 119 s.
- Gülperçin, N., Tezcan, S., 2009. Faunistic notes on the species of Elateridae (Coleoptera) in Izmir province of Turkey. *Munis Entomology & Zoology*, 4 (2): 519-526.
- Gülperçin, N., Tezcan, S., 2010a. Türkiye Elateridae (Insecta: Coleoptera) Faunasının Dağılım Kataloğu. Meta Basım, Bornova, İzmir, VIII+63 s.
- Gülperçin, N., Tezcan, S., 2010b. Contribution to the knowledge of the Agrypninae (Coleoptera, Elateridae) fauna of Turkey. *Linzer Biology Beitrage*, 42 (2): 1405-1412.
- Gülperçin, N., Tezcan, S., 2012a. Contribution to the knowledge of the **Cardiophorus** Eschscholtz, 1829 (Coleoptera, Elateridae, Cardiophorinae) fauna of Turkey. *Linzer Biology Beitrage*, 44 (1): 449-464.
- Gülperçin, N., Tezcan, S., 2012b. Contribution to the knowledge of the Elaterinae (Coleoptera, Elateridae) fauna of Turkey. *Linzer Biology Beitrage*, 44 (2): 1087-1110.
- Gülperçin, N., Tezcan, S., 2014a. Contribution to the knowledge of the Dicronychus BRULLÉ 1832 (Coleoptera, Elateridae, Cardiophorinae) fauna of Turkey. *Linzer Biology Beitrage*, 46 (1): 675-682.
- Gülperçin, N., Tezcan, S., 2014b. Contribution to the knowledge of the Melanotinae (Coleoptera, Elateridae) fauna of Turkey. *Linzer Biology Beitrage*, 46 (2): 1491-1498.
- Gülperçin, N., Tezcan, S., 2014c. Contribution to the knowledge of the Denticollinae and Negastrinae (Coleoptera, Elateridae) fauna of Turkey. *Linzer Biology Beitrage*, 46 (2): 1499-1507.
- Gülperçin, N., Tezcan, S., 2015. Two new records and some additional notes on Elateridae (Coleoptera) fauna of Turkey. *Linzer Biology Beitrage*, 47 (1): 545-567.
- Heyden, L. V., Reitter, E. Weise, J., 1906. *Catalogus Coleopterorum Europae, Caucasi et Armeniae Rossicae*. Berlin, 774 s.
- İyriboz, Ş. N., 1934. Şekerpancarı Hastalıkları. Resimli Ay Matbaası, 164 s.
- Kabalak, M., Sert, S., 2005. Ankara İli Elateridae (Coleoptera) familyası türleri üzerinde faunistik çalışmalar. *Türkiye Entomoloji Dergisi*, 29 (1): 49-60.
- Kesdek, M., Platia, G., Yıldırım, E., 2006. Contribution to the knowledge of click beetles fauna of Turkey (Coleoptera: Elateridae). *Entomofauna Zeitschrift für Entomologie*, 35: 417-432.
- Laibner, S., 2000. Elateridae of the Czech and Slovak Republics. Kabourek Publishing, 292 pp.
- Lodos, N. 1998. Türkiye Entomolojisi VI. Ege Üniversitesi Ziraat Fakültesi Yayınları No: 529, 2-50.
- Löbl, I., Smetana, A., 2007. *Catalogue of Palearctic Coleoptera*. Volume 4. Elateroidea, Derodontoidea, Bostrichoidea, Lymexyloidea, Cleroidea, Cucujoidea. Apollo Books, Stenstrup, 935 pp.
- Mertlik, J., Platia, G., 2008. Catalogue of the family Cebrionidae, Elateridae, Lissomidae, Melasidae and Throscidae from Turkey. *Elateridarium*, 2: 1-40.

- Önder, F., Karsavuran, Y., Önder, E. P., Önuçar, A., Tutkun, E., 1982. Türkiye Entomoloji ve Zirai Zooloji Bibliyografyası (1595-1978). T. C. Tarım ve Orman Bakanlığı, Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü, Bitki Koruma Derneği Yayınları No: 1, 505 s.
- Önder, F., Karsavuran, Y., Önder, E. P., Tutkun, E., Tezcan, S., 1986. Türkiye Entomoloji ve Zirai Zooloji Bibliyografyası (1979-1983). Tübitak Yayınları No: 631, Tübdok Seri No: 43, 129 s.
- Özgen, İ., Gözüaıcık, C., Gülperçin, N., 2012. Some additional notes about Elateridae (Coleoptera) fauna in Adıyaman, Diyarbakır and Şanlıurfa provinces of Turkey. Munis Entomology & Zoology, 7 (1): 226-228.
- Sahlberg, J., 1912-1913. Coleoptera Mediterranean Orientalia, quae in Aegyptia, Palaestina, Syria, Caramania atque in Anatolia occidentali anno 1904. Ofversigt af Finska Vetenskaps-Societetens Förhandlingar, 60 (13): 127-132.
- Schenkling, S., 1925-1927. Coleopterorum Catalogus (Pars 80 & 88). W. Junk, Berlin W. 15, 636 s.
- Tezcan, S., Tezcan, F., Gülperçin, N., 2013. Türkiye Entomolog, Akarolog ve Nematologlarının Bibliyografik Yayın Listeleri (1984-2011). Türkiye Entomoloji Derneği Yayınları No: 12, Meta Basım, Bornova, İzmir, 534 s.
- Yüksel, H., 1970. Türkiye’de Bulunan Telkurtları (Elateridae) Familyası Türleri Üzerinde Sistematik Çalışmalar. Atatürk Ü. Yayınları No. 85, Zir. Fak. Yay. No: 32, Arş. Ser. No: 12, Erzurum, 66 s.

GENOTYPIC VARIATION IN SEEDS CHARACTERS IN SOME GENOTYPES OF *Citrullus colocynthis* UNDER WATER STRESSES

EL MADIDI Said, BENMOUMOU Abedlhamid

Laboratory BVRN, Department of Biology, Faculty of Sciences, Ibn Zohr University,
Agadir, Morocco

Corresponding author email: s.elmadidi@uiz.ac.ma

Abstract

Citrullus colocynthis (L.) Schrad (2n = 22), closely related to domesticated watermelon (*Citrullus lanatus* var. *lanatus*), is a very drought-tolerant perennial herbaceous species in the Cucurbitaceae family. This plant is a drought tolerant species with a deep root system, widely distributed in the Sahara-Arabian desert in Africa and the Mediterranean region. *C. Colocynthis* is a rich source of functionally important bioactive compounds and therapeutics such as polyphenols, glycosides, triterpenes and cucurbitacins and its fruit has been widely used for the treatment of many diseases including diabetes, rheumatism, paronychia, ulcer and cancer. 12 accessions of *C. colocynthis* collected from different regions from Morocco, were studied for their tolerance to water deficiency stresses. The 12 accessions were analysed in tow field experiments, one under well irrigated (WI) and one under limited irrigated conditions (LI). The experimental design was a randomized complete block with three replications. Data were recorded on 15 randomly selected fruit for each plot. A total of 45 fruits were scored for each genotype. No fertilizer or insecticide spraying was applied at any time for the duration of the trial. Genotypic differences for drought susceptibility were highly significant and significant genotype × water treatment interactions were found also highly significant. Heritability and genotypic advance are moderate to high for the characters measured.

Keywords: *Citrullus colocynthis*, genotypic variation, seeds characters, water stresses

INTRODUCTION

Citrullus colocynthis (L.) Schrad (2n = 22), closely related to domesticated watermelon (*Citrullus lanatus* var. *lanatus*) (Chomicki and Renner 2015), is a very drought-tolerant perennial herbaceous species in the Cucurbitaceae family. It is a wild native plant growing in arid areas, widely distributed in the Sahara-Arabian desert in Africa and the Mediterranean region (Si et al, 2010; Wang et al, 2014). *C. Colocynthis* is a rich source of functionally important bioactive compounds and therapeutics such as polyphenols, glycosides, triterpenes and cucurbitacins and its fruit has been widely used for the treatment of many diseases including diabetes, rheumatism, paronychia, ulcer and cancer (Hussain et al., 2014; Marzouk et al. 2010). *C. colocynthis* being an exceptionally hardy plant with a potential for use as biodiesel feedstock (Giwa et al., 2010 ;Bello and Mekanju, 2011). The plant was also shown to be rich in nutritional value with high protein contents and important minerals as well as edible quality of seed oil (Hussain et al., 2014).

MATERIAL AND METHOD:

12 accessions of *C. colocynthis* collected from different regions from Morocco. Two field experiments were carried out during the growing seasons 2015-16 and 2016-17 at the experimental farm of Sidi Bibi (30° 15' 00" N , 9° 30' 36" W), following a randomized block experimental design with 3 replicates. Two irrigation levels were imposed to determine variability on drought tolerance of cultivars. T1 treatment (Well irrigated) received full irrigation which plants received sufficient water to maintain soil water content close to pot capacity, While T2 (limited irrigated) treatment received 50 % of T1. The measured traits are: fruit weight per plant (FW), seed weight per plant (SW), seed number per plant (SN), weight of 100 seeds per plant (W100), and oil content of seed (OC). The lipid components of the *C. Colocynthis* seeds were subsequently extracted with n-hexane using a Soxhlet apparatus at 45-50 °C for 6-8 h until the extraction was completed.

The crushed seeds (approximately 400 g) of *Citrullus colocynthis* were placed in a Soxhlet apparatus and then extracted with 300 mL n-hexane for 7 hours on a water bath. lipid fraction was extracted in a Soxhlet apparatus for 16 h at 60°C. The solvent was evaporated, and the lipid fraction residues were weighed. Data were analyzed by the GLM procedure of SAS with the following statistical model:

$$Y_{ijk} = \mu + A_i + B_j + A \times B_{ij} + C_k + E_{ijk}$$

Where : A_i : Effects of accessions, B_j : Effects of experiments , $A \times B_{ij}$: Effects of and interactions C_k : Effects of Blocs

Statistics analysis was carried out using computer software SAS version 9.3 (SAS Institute Inc. 2010)

The phenotypic variance in each trial: $\sigma^2_P = \sigma^2_G + \frac{\sigma^2_E}{n}$. The Broad-sense heritability was estimated by : σ^2_G / σ^2_P . The expected genotypic advance (genotypic gain) was estimated by:

$\Delta G_i = i H \sigma_P$ where i = selection differential, the value is 1.40 at 20 % selection intensity. The relative genotypic gain $RA G_i$ is obtained by dividing the genotypic gain by the mean of the character measured (as percent of mean). Statistics analysis was carried out using computer software SAS version 9.3 (SAS Institute Inc. 2010)

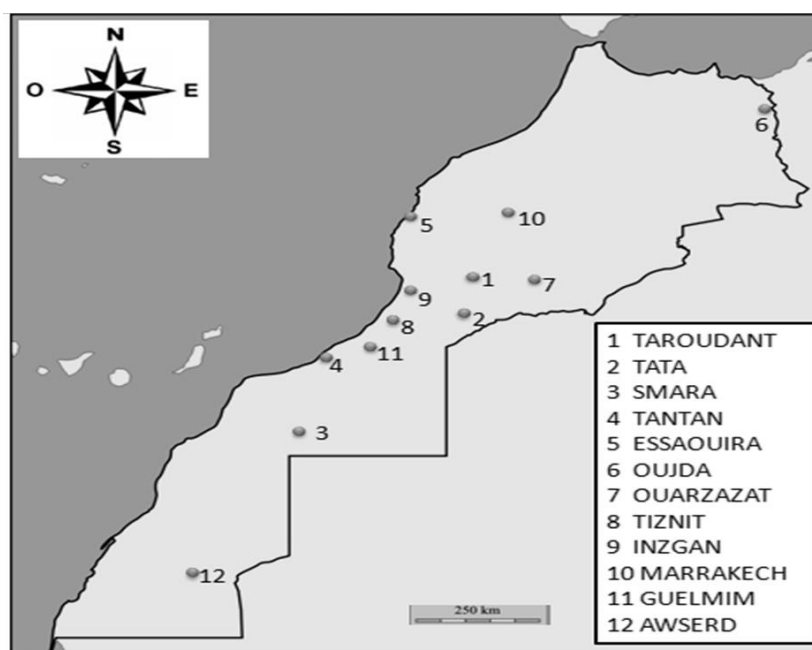


Figure1: Different localities of sampling accessions

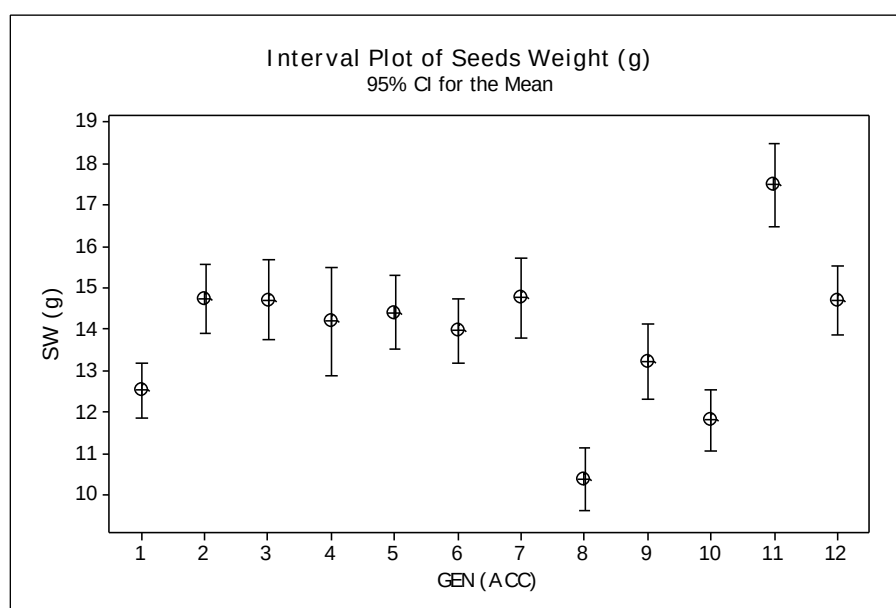
RESULTS AND DISCUSSION

The data recorded for morphological and agronomic characteristics showed considerable variability among the accessions (Table 1). The fruits weight per plant varied between 79 to 482, seed number from 37 to 682 per plant, seed weight from 1,94 to 38,25 grams per plant and seed oil content from 16.94 to 34.85 % of seed weight. The coefficients of variation (CV) estimates ranged from 6.21 % to 43 %.

Table 1: Descriptive statistics for the measured characters.

Characters	Mean	Min	Max	SD	CV (%)
FW	178,50	79,00	482,00	63,92	35,81
SN	222,15	37,00	682,00	90,68	40,82
SW	11,97	1,94	38,25	5,12	42,81
W100	5,32	0,00	9,40	0,80	15,12
OC	24.75	16.94	34.85	1.78	6.21

FW : fruit weight, **SN** : seed number, **SW** : seed weight, **W100** : weight of 100 seeds and **OC** : oil content

**Figure 2:** Variation of c.colocynthis seeds weight for different accessions.**Table 2:** Results of ANOVA for the characters analyzed.

Source of variation	DL	FW	SN	SW	W100	OC
ACC	11	7,25 ***	11,35 ***	13,20 ***	33,63 ***	25,69 ***
EXP	1	11,11 ***	5,34 **	6,60 **	11,24 ***	2,84 *
ACC x EXP	11	5.35 **	6.05 **	7.84 ***	5.92 **	2.68 *
ERROR	96					
TOTAL	119					

DF: degree of freedom, ***, **, * : was significant at 0,001, 0.01 and 0.05 level respectively.

The analysis of variance revealed that differences between the two irrigation levels (the effect of irrigation) were highly significant for FW, SN, SW, W100 and significant for OC. The effect of accessions was highly significant for all the traits studied (Table 2). This indicates the existence of a

high degree of genotypic variability in the material studied. Genotypes x trials interactions were highly significant for FW,SN,SW,W100, indicating that differences among mean values of cultivars varied with irrigated conditions.

Table 3: The Broad-sense heritability and relative genotypic gain.

Character	H^2 (WI)	H^2 (LI)	$R\Delta G_i$ (WI)	$R\Delta G_i$ (LI)
FW	0.41	0.39	24.68%	19.45%
SN	0.45	0.33	12.60%	9.33 %
SW	0.43	0.26	10.23%	7.21 %
W100	0.33	0.29	14.73%	12.71 %
OC	0.62	0.53	18.23 %	13.47%

WI : well irrigated, LI : limited irrigated

The heritability in the broad sense is defined as the proportion of phenotypic variance that is attributable to an effect for the whole genotype, comprising the sum of additive, dominance, and epistatic effects (Nyquist 1991; Falconer and Mackay 1996). Heritability is a key parameter in quantitative genetics because it determines the response to selection. The values of heritability and relative genotypic gain under stress were lower to those obtained in well-watered conditions (table 3). The broad sense heritability estimates ranged from 0.33 to 0.62 in (WI) and from 0.26 and 0.53 in (LI), suggesting that genotypic components may play an important role in the improvement of these traits and genetic advance could be effectively used in selection on the basis of phenotypic performance. Selection for drought tolerance is one way of reducing the impacts of water deficit on crop yield. Direct selection under stress has been considered ineffective, because the broad-sense heritability (H) under stress is assumed to be lower than in non-stress environments (Edmeades et al., 1999; Fukai and Cooper, 1995). However, other researchers suggest that direct selection under drought stress can produce yield gains without reducing yield potential and selection for yield under stress is the most effective approach to identifying drought-tolerant genotypes combining high yield potential with high levels of drought tolerance. (Kumar et al., 2007 ; Atlin et al., 2004).

ACKNOWLEDGEMENTS

This work is supported financially by the University Ibn Zohr. We are grateful to the management of the experimental center Sidi Bibi for their invaluable help in installing and monitoring the two trials.

REFERENCES

- Atlin, G.N., Lafitte, R., Venuprasad, R., Kumar, R., Jongdee, B., 2004. Heritability of rice yield under stress, correlations across stress levels, and effects of selection: implications for drought tolerance breeding. In: Poland, D., Sawkins, M., Ribaut, J.-M., Hoisington, D. (Eds.), *Proceedings of a Workshop on Resilient Crops for Water-Limited Environments*, Cuernavaca, Mexico, 24–28 May 2004. CIMMYT, Mexico D.F., pp. 85–87
- Bello, E., & Makanju, A. (2011). Performance evaluation of Egusi melon (*Citrullus colocynthis* L.) seeds oil biodiesel. *Journal of Emerging Trends in Engineering and Applied Sciences*, 2(5), 741-745.
- Chomicki G, Renner SS. Watermelon origin solved with molecular phylogenetics including Linnean material: another example of museomics. *New Phytologist* 2015 ; 205: 526–532.
- Edmeades, G. O., Chapman, S. C., and Lafitte, H. R. 1999. Selection improves drought tolerance in tropical maize populations: I. Gains in biomass, grain yield, and harvest index. *Crop science*, 39(5), 1306-1315.

- Elsheikh Y. A., Man Z., Bustam M. A., Yusup S., Akhtar F. H, and Ayyaz M. 2014. "Preparation and characterization of citrulus colocynthis oil biodiesel: optimization of alkali-catalyzed transesterification," The Canadian Journal of Chemical Engineering, vol. 92, no. 3, pp. 435–440, 2014
- Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics. 4th Edn., Benjamin Cummings, England, ISBN-10 : 0582243025.
- Fukai, S. and Cooper, M., 1995. Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crops Res.* 40, 67–86.
- Giwa, S., Abdullah, L. C., & Adam, N. M. (2010). Investigating "Egusi"(Citrullus colocynthis L.) seed oil as potential biodiesel feedstock. *Energies*, 3(4), 607-618.
- Hussain, A. I., Rathore, H. A., Sattar, M. Z., Chatha, S. A., Sarker, S. D., & Gilani, A. H. (2014). Citrullus colocynthis (L.) Schrad (bitter apple fruit): A review of its phytochemistry, pharmacology, traditional uses and nutritional potential. *Journal of ethnopharmacology*, 155(1), 54-66.
- Kumar, R., Venuprasad, R., and Atlin, G. N. 2007. Genetic analysis of rainfed lowland rice drought tolerance under naturally-occurring stress in eastern India: heritability and QTL effects. *Field Crops Research*, 103(1), 42-52.
- Marzouk B., Marzouk Z., Haloui E., Fenina N., Bouraoui A. et Aouni, M. Screening of analgesic and anti-inflammatory activities of Citrullus colocynthis from southern Tunisia. *Journal of ethnopharmacology*. 2010; 128(1): 15-19.
- Menon, K., Sood, N., & Rao, N. K. (2016). Study of morpho-agronomic diversity and oil content in desert gourd ('Citrullus colocynthis'(L.) Schrad.). *Australian Journal of Crop Science*, 10(7), 1000-1006.
- Nyquist, W.E. 1991. Estimation of heritability and prediction of selection response in plant population. *Crit Rev Plant Sci.* 10, 235-322.
- SAS Institute. 2010. Statistical analysis Software version 9.3 user's manual., Cary, NC: SAS Institute Inc. USA.
- Si Y, Dane F, Rashotte A, Kang K., Singh N K. Cloning and expression analysis of the Ccrboh gene encoding respiratory burst oxidase in Citrullus colocynthis and grafting onto Citrullus lanatus (watermelon). *Exp Bot* 2010; 61 (6): 1635-1642
- Wang Z, Hu H, Goertzen LR, McElroy JS, Dane F. Analysis of the Citrullus colocynthis Transcriptome during Water Deficit Stress. *PLoS ONE* 2014; 9(8): e104657

EFFECT OF DIFFERENT TREATMENTS ON SEED GERMINATION OF *Dorystoechas hastata* (Lamiaceae), A MEDICINAL AND AROMATIC PLANT FROM ANTALYA

Ceren SELİM¹, Songül SEVER MUTLU²

¹Department of Landscape Architecture, Faculty of Architecture, Akdeniz University, Antalya, Turkey

²Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

*Corresponding author email: cerenselim@akdeniz.edu.tr

Abstract

Seed germination is defined as a critical stage in the life cycle of plants and often controls population dynamics, with major practical implications. Determinations of seed germination protocols are important in terms of species survival and future conservation efforts. The purpose of this study was to investigate the effects of different treatments on seed germination in a relict endemic medicinal and aromatic plant from Antalya (Turkey), *Dorystoechas hastata* Boiss. & Heldr. ex Benth. In this study, three experiments were performed for determining germination characteristics of *D. hastata* species seeds which were collected from 16 different populations in its natural distribution area in Konyaaltı, Kemer, Korkuteli, Kumluca districts of Antalya on May-July 2006. Populations altitude ranged from sea level to 2000 meters. The first experiment performed as a preliminary germination test and made without any pre-treatment. In the second germination experiment, the effect of 1-month cold stratification (moist, +4°C) against control (untreated seeds) were investigated. In the third experiment, treatments included; 3-month cold stratification (moist, +4°C), soaking for 24 h in water and control (untreated seeds). Experiments were conducted in a completely randomized design with 3 replications, under greenhouse conditions. In each replication, 30 seeds were used for 16 locations. Counting number of germinating seeds began from the first day and was done till the end of the experiment (30 days) and germination percentage was recorded weekly during the study period. The obtained data were analysed using the variance analysis method in the GLM procedure of the SAS Statistical Program. The averages were compared with the least significant difference (LSD) test preserved according to the Fisher method and the LSD (0,05) values at the application level were calculated. Pearson correlation test was performed for determining the relationship between altitude and germination range. Germination activity was not observed in pre-germination test. Our findings indicate that in the second germination experiment, cold stratification, location and interaction of cold stratification and location were statistically important in germination rates at 0.01% significance level. As a result of the correlation analysis, there was a negative correlation between the localization of the locations and the germination rate ($r = -0,41$ ($<.0001$)), and as the altitude increased, germination decreased. In the third germination experiment, pre-treatments (soaking for 24 h in water, 3-month cold stratification), locations and interaction of pre-treatments and location were statistically important in germination rates at 0.01% significance level. Similar to the previous germination experiment, there was a negative correlation between the localization of the locations and the germination rate ($r = -0,53$ ($<.0001$)). The data presented in this study provide useful information for the conservation of this relict endemic species of Antalya, which was under human pressure and not been cultivated.

Keywords: Cold stratification, germination, pre-treatment, relict endemic

INTRODUCTION

Turkey is one of the countries with the richest biological diversity of Europe and Middle East and shows a small continental character in this respect. Richness in plant diversity is due to climatical, geomorphological and topographical variation over short distances. While there are more than 12.000 gymnosperm and angiosperm plant species in Europe (Barthlott et al., 1999, European Union 2011), this number is about the same in Turkey with last records and nearly %34 of Turkey's flora consists of endemic species (Avcı 2005, Yılmaz 2013). It was known that Mediterranean Region took first place when the distribution of endemic plants in Turkey was investigated (Ekim et al., 2000). Turkey's flora with this high endemism rate, is also rich in medicinal and aromatic plants. This high level of endemism imposes an even greater responsibility to adequately protect and prevent the disappearance of these species.

Lamiaceae family which has 250 genera and 6500 species in it, contains many medicinal plant species which are well known and economically important such as thyme, sage, peppermint, lavender and sweet basil. Species in this family are important in supplying raw materials to various branches of industry, especially chemistry, food, cosmetics and agricultural struggle. This family 6rd major family in genus with 45 genera and 550 species after Graminae, Asteraceae, Apiaceae (Umbelliferae), Brassicaceae (Cruciferae), Fabaceae (Leguminosae) and 3rd major family in species after Asteraceae and Fabaceae. 245 of these species are endemic and endemism rate of the genus was %44.7 (Donmez et al., 2011). The genus of *Dorystoechas* has consists *Dorystoechas hastata* Boiss.&Heldr. Benth which was monotypic relict endemic to Antalya province of Turkey.

Seed is production material in generative propagation technique and germination is mainly an important stage which determines reproduction success in plants (Bu et. al., 2008). Germination studies in rare and endemic species have great importance in identification of protection techniques of these taxa (Lorite et al., 2007). Germination of seeds is the only way to preserve genetic diversity in populations of these taxa that was under the risk of extinction (Blanca et. al., 1998; Moreno-Saiz et. al., 2003). There are a number of internal and external factors that affect the germination of seed which play an important role in inducing germination of seeds or entering the dormancy period. It has been reported that this effect is usually caused by changes in the levels of plant hormones (Güneş, 2000; Unal et al., 2004). While plant hormones such as oxine, cytokinin and gibberellin stimulate germination, the presence of abscisic acid may prevent germination (Unal et al., 2004, Palavan-Unsal 1993, Unyayar 1995, Kabar 1997). Gibberellic acid (GA₃) plays an important role at the beginning of germination and provides the mobilization of storage nutrients by removing the effect of abscisic acid (ABA) in the dormant seeds. It has been stated that cold stratification reduces the ABA level and increases the gibberellic acid level to germinate (Okogami and Teuri, 1996). Pre-treatments as cold stratification and GA₃ application could removed the inhibitor in embryos which were occurred by physical or physiological factors and promote germination (Güneş, 2000). Cold-stratification, application of potassium nitrate, exposing the seeds to different light intensity, wavelength and duration of light and application of different plant hormones are useful ways to breakdown of physiological seed dormancy and increase the germination success. Pre-treatments such as scarification, stratification, mechanically nicking of seed coats, application of sodium hydrochloride are ways of breakdown of physical seed dormancy (Teimouri et al., 2013, Baskin and Baskin, 1998). It was known that especially in medicinal and aromatic plants germination of seeds were inhibited by eteric fat content which cause allelopatric effect. Kadis et al (2010) suggested that seeds should be removed from calyx quickly after has been collected and germination experiments should be done at least three monts after the seed collection time. Also it was pointed out that identification of germination characteristics of species belonging to Lamiaceae family has importance for the survival of these species and the development of protection strategies (Kadis et al., 2010). The objective of this study was to investigate the effects of different pre-treatments on seed germination in a relict endemic medicinal and aromatic plant *Dorystoechas hastata* Boiss. & Heldr. ex Benth which is located in only Antalya (Turkey) and protected as Vulnerable status in IUCN Red List Catogories.

MATERIAL AND METHODS

Plant Material and Site of Seed Collection

D. hastata is a medicinal aromatic plant species with its dense volatile and aromatic oil content and being consumed as herbal tea by local inhabitants (Meriçli and Meriçli., 1986; Valant-Vetschera et al., 2003). *D. hastata* is the only species in genus of *Dorystoechas* which is monotypic in the Lamiaceae family (Hedge, 1982). The member of this monotypic genus is a woody shrub with its 40-100 cm. All of its parts are densely aromatic, leaves are lanceolate, hastate, often soft pile, rough and edges are small lobed. It's florescence is between May and July. It's an East Medeterranean element which ranges near rock and friganas between 650-2000 m attribute (Hedge, 1982). *D.hastata*'s rich chemical content brings pharmacological studies with it. It has a big potential in the medical and

perfumery industry because of its high antioxidant and essential oil content (Karagözler et al., 2008; Öztürk, 1990; Valant-Vetschera 2003; Venturella et al 1988; Başer 1994; Uluben et al 2004; Erkan et al., 2011). Also it was emphasized that although this species is a monotypic endemic species in Lamiaceae, it was collected uncontrolledly from nature and this situation endangered the future of the species. In this content, cultivation of this species is suggested as immediate as possible.

D. hastata seeds were collected from 16 different populations in its natural distribution area in Konyaaltı, Kemer, Korkuteli, Kumluca districts of Antalya on May-July 2006 from ripe fruits. Populations altitude ranged from sea level to 2000 meters. For every of 16 population, one genotype was selected to represent the population and its seeds were collected. Genotype codes names, locations, seed collection time were shown in Table.1. The collected seeds are immediately removed from their calyx and were stored in paper bags in the dark at room temperature until germination tests were started.

Table 1. Locations of *D.hastata* populations where seeds were collected, location codes, seed collection dates and altitudes.

Collection Location	Code	Altitude	Seed collection date
Hisarcandır	H	964	06.06.2016
Termessos(Gulluk)	GU	974	14.06.2016
Tunektepe	TN	96	23.05.2016
Beldibi	BL	16	18.05.2016
Kesmebogazi	K	109	18.05.2016
Altinyaka	A	1137	13.06.2016
Goyruk	AY	62	18.05.2016
Alakir	AL	1059	21.06.2016
Golcuk	GL	1112	13.06.2016
Sogutcumasi	SO	1400	21.06.2016
Beycik	BY	1009	30.05.2016
Hacisekiler	HC	233	23.05.2016
Tahtali	T	1113	13.06.2016
Feslikan	F	1862	15.07.2016
Sivridag	S	1204	17.06.2016
Ucoluk	UC	1053	21.06.2016

Germination Experiment

Three germination experiments were conducted in three different times under green house conditions in Antalya. Seeds were sown in bedding trays containing the mixture of peat moss, perlite and vermiculite (3:1:1). All seeds were sterilized by fungicide (Metalaxyl and Fludioxonil) solution and washed three times with sterilized distilled water. Experimental treatments were assessed every week for one month. A seed was considered to have germinated when the emerging radicle elongated to 2 mm. Germinated seeds were counted and recorded. Bedding trays were kept in a pre-germination room at constant temperature (28°C) and 75% humidity for 9 days. After these conditions bedding trays were kept in heated green house (daytime 23°C±1, night time 17°C) conditions and germination was observed.

First germination experiment was conducted on February 2017 and any pre-treatments were used. First experiment was designed with 3 replications in a completely randomized block design. In the second germination experiment, the effects of exposing seeds to 1-month cold stratification (moist, +4°C) against control(untreated dry seeds) in germination were investigated on May 2017. Second experiment was designed 3 replications and 2 factors (1-month cold stratification and control) in a completely randomized design. Third germination experiment was conducted in a completely randomized design with 3 replications and 3 factors (3-month cold stratification, soaking for 24h in water and control). In each replication 30 seeds were used.

Statistical Analysis

The data were analysed using analysis of variance procedures with PROC GLM (SAS release 8.0; SAS Institute, Cary, NC). Means were separated using Fisher's protected least significant difference ($P < 0.001$) procedure. Least significant difference values are reported for application level at the 0.05 level. The PROC CORR procedure was used to perform correlation analyses to understand the amount and way of correlation among germination and altitude.

RESULTS AND DISCUSSION

In first experiment, germination was not observed. It was thought that seeds might need to be cooled for germination. Second and third experiments mainly conducted to understand the effect of different period cold-stratification on germination success. Findings of second and third germination experiments were shared within the scope of this study.

II. Germination Experiment

Germination rates of seeds and statistical analyses of second germination experiment were given in Table. 2. It was found that cold stratification, location and interaction of cold stratification X location were significant at %0.01 level. No germination was observed in any location's control seeds. When the results of the interaction between location and cold stratification was examined, 1 month cold-stratification pre-treatment increased the germination rate compared to control seeds in which were from Tunektepe location (%66.66). In Beldibi location, 1 month cold-stratification pre-treatment increased the germination rate by % 26.66 compared to control seeds. In Kesmebogazi location, cold-stratification the germination rate by %13.33 compared to the control seeds. Similar situations have also been identified in the Alakir and Kesmebogazi locations. In Alakir location, germination rate increased by %20 with 1 month cold-stratification application and in Hacisekiler location this rate is %33.33 compared to the control seeds. In other locations, there was no significant difference in germination between applications. As a result of the correlation analysis, there was a negative correlation between altitude of the locations and the germination rate ($r = -0,41$ ($< .001$)). As the altitude was increased, the germination rate was decreased. It was thought that there should be a possibility of longer cold-stratification need for germination. Because only the seeds of locations were germinated which were from sea level to 500 m altitude except for Alakir population. Unal et. al. (2004) studied different pre-treatments (GA_3 doses, cold-stratification, different germination temperatures) on seed germination of some *Origanum* species, which is another member of Lamiaceae family. They recorded that seed which were treated cold-stratification have high germination percentage than other pre-treatments. The highest percentage of germination among species was found in the seeds of *Origanum solymycin*, which was distributed at low altitudes, while the lowest percentage of germination was detected in the seeds of *Origanum husnucan-baseri*. Young and Young (1992) recorded that cold-stratification was the most useful way to observe higher germination ratio in *Salvia* species, which is close to *Dorystoechas* in Lamiaceae family, than other pre-treatments. Subasi and Guvensen (2010) recorded that cold-stratification at +5 °C for 45 days in an endemic species of *Salvia smyrnaea* seeds showed higher germination percentage (66%) than untreated seeds. Kadis et al. (2010) stated that cold-stratification enhanced germination in *Salvia willeana* species. Similar results have been found in other species belonging to the Lamiaceae family (Yücel and Altınöz, 2001; Hilooglu et. al., 2016; Subasi and Guvensen, 2010).

Table.3. The germination ratio, mean and standard deviation values of control seeds and treated with 1 month cold-stratification.

Genotype code	Altitude	Control (Percentage of germination %)	1 month cold-stratification (Percentage of germination %)
H	964	0	0
GU	974	0	0
TN	96	0	66,66
BL	16	0	26,66
K	109	0	13,33
A	1137	0	0
AY	62	0	0
AL	1059	0	20
GL	1112	0	0
SO	1400	0	0
BY	1009	0	0
HC	233	0	33,33
T	1113	0	0
F	1862	0	0
S	1204	0	0
UC	1053	0	0
Mean		0	9,99
Min.		0	0
Max.		0	66,66
Std.		0	18,69
Significance			
Location		**	
Location	X Cold-stratification (interaction)	**	
In P= 0.01 level significant			

III. Germination Experiment

Germination rates of seeds and statistical analyses of third germination experiment were given in Table. 3. It was found that pre-treatments (3 months cold stratification and soaking for 24 h in water), location and interaction of pre-treatments X location were significant at %0.01 level. When the results of the interaction between location and pre-treatments was examined, 3 months cold-stratification application (%33.33) increased the germination rate compared to control seeds (%13.33) in Beldibi location. In Kesmebogazi location, control seeds (untreated) have more germination rate than the seeds which were applied pre-treatments. In Alakir location, application of cold-stratification (%13.33), was caused the maximum germination than soaking for 24 h in water (%3.33) and control (%3.33). Application of 3 months cold-stratification (%43.33) to seeds from Hacisekiler location was increased the germination rate than control (%16.66) and 24 h in water (%36.66) application. In Tunektepe location, application of 24 h in water (%40) was increased the germination rate than control (%26.66) and cold-stratification (%10) application. Similarly, Afzalifar et. al., (2015) recorded soaking in water for 24h significantly enhanced germination percentage and germination rate of *Satureja khuzistanica* and *Satureja rechingeri* (Lamiaceae). Shalaby et al. (1997) has been reported that seeds of different Echinacea species needed different stratification periods to overcome the dormancy. As a result of the correlation analysis, there was a negative correlation between altitude of the locations and the germination rate ($r = -0,53$ ($<.001$)). As the altitude was increased, the germination rate was decreased. These findings also supported with previous studies (Unal et. al., 2004; Young and Young, 1992; Subasi and Guvensen, 1992). Similar to previous germination experiments, seeds from locations where germination is observed are close to sea level and below 500 meter altitude, except for the Alakır population, It was thought that there should be a possibility of longer than 3 months cold-stratification need for germination.

Table.3. The germination ratio, mean and standard deviation values of contro seeds and treated with pre-treatments.

Genotype code	Altitude	Control (Percentage of germination %)	3 month cold-stratification (Percentage of germination %)	Soaking for 24 h in water (Percentage of germination %)
H	964	0	0	0
GU	974	0	0	0
TN	96	26,66	10	40
BL	16	13,33	33,33	6,66
K	109	13,33	6,66	0
A	1137	0	0	0
AY	62	0	0	0
AL	1059	3,33	13,33	3,33
GL	1112	0	0	0
SO	1400	0	0	0
BY	1009	0	0	0
HC	233	16,66	43,33	36,66
T	1113	0	0	0
F	1862	0	0	0
S	1204	0	0	0
UC	1053	0	0	0
Mean		4,58	6,66	5,42
Min		0	0	0
Max		26,66	43,33	40
Std		8,24	13,16	12,99
Significance				
Location		**		
Location X Pre-treatment (interaction)		**		
In P= 0.01 level significant				

The success of seed germination may require breaking morphological, physiological, morphophysiological, physical and combinational dormancy depending on the species (Baskin and Baskin, 2004; Fang et al., 2006). In the second experiment 1 month cold-stratification in Tunektepe location (%66.66), in third experiment 3 month cold-stratification in Hacisekiler location (43,33) had the highest germination rates. Yılmaz (2006) studied different pre-treatments (24 h darkness, 12 h darkness/12 h light germination media, distilled water, different doses of (%0.05, %1, %2, %3) NaCl, KNO₃, HCl, H₂CO₄) on seed germination of *D. hastata*. The highest percentage of germination among pre-treatments was %9,75 with %0,5 NaCl application. The germination rates obtained as a result of this study are much higher than previously reported in the literature.

CONCLUSION

D. hastata had been able to maintain its genetic and morphological diversity up to now, although it was a relict local endemic species. It is necessary to start cultivation and breeding program of the *D. hastata* as a potential of ornamental and medicinal and aromatic plant. Also, *D. hastata* is the only species which represents *Dorystoechas* genus and there was not any species in Lamiaceae members which is endemic to Antalya and distributed from sea level to 2000 meters altitude. Species has not been cultivated yet and its uncontrolled collection from natural populations has endangered its future. It was recommended that immediate domestication of this species has to be started. In this context, it is very important to study propagation and cultivation techniques of this species.

No literature has been found to investigate the effects of cold-stratification and soaking in water for 24 h pre-treatments on germination in *D. hastata* seeds. When all the findings were evaluated together, application of cold-stratification increased germination in seeds of some locations. It was stated that altitude of locations were determinative in germination of seeds. Correlation analyses between altitudes of locations and percentage of seed germination results confirmed this hypothesis. The lack of germination in the seeds of high altitude (especially over 1000 m) suggests that longer cold-stratification periods may be needed. Besides, seed viability test (Tetrazolium test) absolutely should be done before new germination experiments and it was suggested that more comprehensive

germination experiments should be carried out by trying different combinations of pre-treatments such as different periods of cold-stratification, different doses of GA₃ application, soaking in water for different periods and combinations of them in order to determine germination characteristics and increase germination rate of seeds in *D. hastata*.

REFERENCES

- Afzalifar, M., Ghorbani ghohzdi, H., Pezhmanmehr, M., Hadian, J. 2015. Seed germination improvement of *Satureja khuzistanica* and *S. rechingeri* (Lamiaceae) as valuable endemic medicinal species from Iran. International Journal of Agronomy and Agricultural Research (IJAAR). Vol. 7, No. 2, p. 93-99. ISSN: 2223-7054 (Print) 2225-3610 (Online)
- Avci, M., 2005. Çeşitlilik ve endemizm açısından Türkiye'nin bitki örtüsü. İstanbul Üniversitesi Edebiyat Fakültesi Coğrafya Bölümü, Coğrafya Dergisi, 13: 27–55.
- Barthlott, W., Biedinger, N., Braun, G., Feig, F., Kier, G., Mutke, J., 1999. Terminological and methodological aspects of the mapping and analysis of global biodiversity. Acta Botanica Fennica, 162: 103-110.
- Baser, K.H.C., 1994. Essential Oils of Labiatae from Turkey-Recent Results. Lamiales Newsletter, Royal Botanic Gardens, 3: 6-11.
- Baskin, J.M., Baskin, C.C., 2004. A classification system for seed dormancy, Seed Science Research, 14: 1 16.
- Baskin, C.C., Baskin, J.M., 1998. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego, CA, Academic Press.
- Blanca, G.M., Cueto, Martinez-Lirola, M.J., Molero, J., 1998. Threatened Vascular Flora of Sierra Nevada (Southern Spain) Biol. Conservation. 85/269-285.
- Bu, H., Du, G., Chen, X., Xu, X., Liu, K., Wen, S., 2008. Community wide germination strategies in an alpine meadow on the eastern Qinghai-Tibet plateau: phylogenetic and life-history correlates. Plant Ecology, 195/87–98.
- Donmez, A., 2001. A new Turkish species of *Salvia* L. (Lamiaceae). Botanical Journal of the Linnean Society 137, 413–416.
- Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., Adıgüzel, N., 2000. Türkiye Bitkileri Kırmızı Kitabı (Yayın no: 18). Ankara: TTKD ve Van 100. Yıl Üniversitesi.
- Erkan, N., Akgonen, S., Ovat, S., Goksel, S., Ayranci, E., 2011. Phenolic compounds profile and antioxidant activity of *Dorystoechas hastata* L. Boiss et Heldr. Food Research International, 44: 3013-3020.
- European Union, 2011. European Red List of Vascular Plants. http://ec.europa.eu/environment/nature/conservation/species/redlist/downloads/European_vascular_plants.pdf. Son erişim tarihi: 30.03.2015.
- Fang, S., Wang, J., Wei, Z., Zhu, Z., 2006. Methods to break seed dormancy in *Cyclocarya paliurus* (Batal)Iljinskaja. Scientia Horticulturae 110(3):305-309. DOI:10.1016/j.scienta.2006.06.031
- Gunes, T., 2000. *Arctium minus* (Hill.) Bernh. Tohumlarında Gibberellik Asit Uygulmasının Çimlenme Ve α - Amilaz Enzim Aktivitesi Üzerine Etkisi. Gazi Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 13, 3, p:589-597.
- Hedge, I.C., 1982. *Dorystoechas* Boiss. & Heldr. ex Benth. Rev. Flora of Turkey. Volume: 7. Editor: Davis, D. Edinburgh: Edinburgh University Press 22: 461-462.
- Hilooglu, M., Yucel, E., Kandemir, A., Sozen, E., 2016. Endemik *Teucrium leucophyllum* (Lamiaceae) tohumlarında in vitro çimlendirme çalışmaları. Sinop Uni J Nat Sci 1(1): 53 – 61. ISSN: 2536-4383.
- Kabar, K., 1997. Comparison of Reversal of Absciscic Acid-Induced Inhibition of Seed Germination and Seedling Growth of Some Gramineae and Liliaceae Members by Kinetin and Gibberellic Acid. Turk.J.Bot., 21, p:203-210.
- Kadis, C., Kounnamas, C., Georgiou, K., 2010. Seed germination and conservation of endemic, rare and threatened aromatic plants of Cyprus. Israel Journal of Plant Sciences. 58: 251-261.
- Karagözler, A.A., Erbag, B., Emek, Y.C., Uygun, D.A., 2008. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. Food Chemical, 111: 400-407.
- Lorite, J., Ruiz-Girela, M., Castro, J., 2007. Patterns of seed germination in Mediterranean mountains: study on 37 endemic or rare species from Sierra Nevada, SE Spain. Candollea. 62/5-16.
- Merikli, F., Merikli, A.H., 1986. The essential oil of *Dorystoechas hastata*. Planta. Med., 52: 506.
- Moreno-Saiz, J.C., Dominguez F., Sainez-Ollero, H., 2003. Recent Progress In Conservation of Threatened Spanish Vascular Flora: A Critical Review. Biol. Conservation. 113/419-431.
- Okogami, N., Terui, K., 1996. Differences in the rates of metabolism of various Triacylglycerols during Seed germination and the subsequent growth of seedlings of *Dioscorea tokoro* perennial Herb. Plant cell physiol. 37 (3). 273-277.
- Ozturk, N.K., 1990. *Dorystoechas hastata* Uçucu Yağının Bileşimi (Yüksek Lisans Tezi). Anadolu Üniversitesi Sağlık Bilimleri Enstitüsü Farmakognozi Anabilimdalı.
- Palavan-Unsal, N., 1993. Bitki Büyüme Maddeleri. İst. Üniv. Basımevi ve Film Merkezi, Üniversite Yayın No: 3677, İstanbul, p:357.
- Shalaby, A.S., Agina, E.A., El-Gengaihi, S.E., El-Khayat, A.S., Hindawy, S.F., 1997. Response of Echinacea to some agricultural practices. Journal of Herbs, Spices and Medicinal Plants 4, 59–67.
- Subasi, U., Guvensen, A., 2010. Seed germination studies on rare endemic *Salvia smyrnaea* Boiss. (Lamiaceae). Biological Diversity and Conservation. 3/3 (2010) 126-132. ISSN 1308-8084 Online; ISSN 1308-5301 Print.

- Teimouri, M.S., Koocheki, A., Mahallati, M.N., 2013. Seed germination and breaking of seed dormancy techniques for endemic *Hymenocrater platystegius* Rech.f. of Khorasan Razavi province, Iran. International Journal of Agriculture and Crop Sciences, 6 (12): 885-889.
- Uluben, A., Mericli, A.H., Mericli, F., 2004. Diterpenes and Norditerpenes from the Roots of *Dorystoechas hastata*. Pharmazie, 59-4201, 301-3.
- Unal, O., Gokceoglu, M., Topcuoglu, S.F., 2004. Antalya Endemiği *Origanum Türlerinin* Tohum Çimlenmesi ve Çelikle Çoğaltılması Üzerinde Araştırmalar. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 17(2): 135-147.
- Unyayar, S., 1995. *Phanerochaete chrysosporium* ME446'da Kültür Periyoduna Bağlı Olarak İndol-3-Asetik Asit (IAA), Gibberellik Asit (GA₃), Absisik Asit (ABA) ve Zeatin Üretimi ve Biyolojik Aktivitelerinin Tayini. İnönü Üniv. Fen Bil. Enst. Doktora Tezi, p:163.
- Valant-Vetschera, K., Roitman, J.N., Wollenweber, E., 2003. Chemodiversity of exudate flavonoids in some members of the Lamiaceae, Biochemical Systematics and Ecology, 31: 1279-1289.
- Venturella, P., Venturella, G., Marino, M.S., Mericli, A.H., Cubukcu, B., 1988. Phytochemical Investigation of the Labiatae *Dorystoechas hastata*. Giornol Botany, 122: 291-294.
- Yilmaz, S., 2013. Türkiye'nin keşfedilmeyi bekleyen doğal hazinesi; Tıbbi ve aromatik bitkiler. Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü, Antalya.
- Young, J.A., Young, C.G., 1992. Seeds of woodylants in North America. Bioscorides Press, Port-End, Oregon.
- Yilmaz, G., 2006. *Dorystoechas hastata*'nın (Lamiaceae) biyolojik ve ekolojik özellikleri. Anadolu Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı Yüksek Lisans Tezi. Eskişehir.
- Yucel, E., Yilmaz, G., 2009. Effects of Different Alkaline Metal Salts (NaCl, KNO₃), Acid Concentrations (H₂SO₄) and Growth Regulator (GA₃) on the Germination of *Salvia cyanescens* Boiss. & Bal. Seeds. Gazi University Journal of Science 22(3)/123-127.

VEGETATIVE PROPAGATION OF A RELICT ENDEMIC MEDICINAL AND AROMATIC PLANT OF ANTALYA-TURKEY, *Dorystoechas hastata* Boiss. & Heldr. EX BENTHAM (LAMIACEAE)

Ceren SELİM¹, Songül SEVER MUTLU²

¹Department of Landscape Architecture, Faculty of Architecture, Akdeniz University, Antalya, Turkey

²Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

Corresponding author email: cerenselim@akdeniz.edu.tr

Abstract

Dorystoechas hastata Boiss. & Heldr. ex Benth (Lamiaceae) is a monotypic relict endemic Mediterranean element naturally grown in Antalya province of Turkey (Hedge, 1982) and protected as Vulnerable status in IUCN Red List Categories. It has a big potential in the medical and perfumery industry due to its high antioxidant content and essential oils (Karagözler et. al., 2008). Because of grazing, unregulated and heavy harvest pressure in natural populations, immediate domestication of this species is suggested (Öztürk, 1990). The objective of this study was to determine the effect of different Indole butyric acid (IBA) concentrations on rooting of *D. hastata* cuttings.

In this study five different concentrations of IBA (1000, 2000, 3000, 4000 ppm and control (0 ppm IBA)) treatments on rooting characteristics of *D. hastata* semi-hardwood cuttings taken in March, 2017 was investigated. The study was carried out in green house using perlite as growing medium. Experimental design was randomized complete block design with 6 replications and each replication included 10 cuttings. Percentage of rooting(%), root number(root number/per cutting), root length (average root length/per cutting), wet and dry leaf weight, wet and dry shoot weight were determined 12 weeks after the initiation of study. The obtained data were analysed using the variance analysis method in the GLM procedure of the SAS Statistical Program. The averages were compared with the least significant difference (LSD) test preserved according to the Fisher method and the LSD (0,05) values at the application level were calculated. The statistical analyses results showed that differences between IBA and control (0 ppm IBA) groups were not statistically significant in terms of all measured characters of rooting. It was determined that mean rooting rate in *D. hastata* was 91.33% and this ratio varied between 80% and 96.66%, but these differences were not significant. Although no statistically significant difference was found between the applications, it was stated that there was a proportional increase in the measured parameters with respect to the increasing number of IBA doses, especially in root number. Identification of propagation techniques of an endangered relict endemic species which have not yet been cultivated and collected unconsciously from nature has importance in terms of ensuring continuity of the species in the future. Generally, it has been determined that the species exhibits high rooting success without a need for any IBA concentration.

Keywords: Plant growth regulators(PGRs), Indole butyric acid (IBA), cutting, rooting

INTRODUCTION

Native species, which have been best adapted to the ecological characteristics of the region for centuries, show better resistance to biotic and abiotic stress conditions of the region compared to classical species and varieties. In order to create sustainable and less maintenance landscapes and increase the diversity of plant material, the interest and demand to native species in outdoor ornamental plants sector is increasing (Sever Mutlu et al. 2013). In this context, determining the appropriate propagation techniques of the species is one of the important step in researches of the usage of native species and genotypes in landscape designs. There are many reasons for propagating plants. Ensuring the sustainability of genetic diversity, making multiple plants from a single plant, rejuvenating plants from old ones and production for commercial reasons can be defined as the reasons for the propagation of plants. Propagation with cuttings is one of the vegetative propagation techniques which stem, branch and leaf parts of the plant prepared by cutting from the mother plant and placed in the appropriate growth medium in order to obtain a new plant. The success and rate of rooting in cuttings are determined by environmental and vegetative factors and their interactions (Davies, 1995; Sever Mutlu et. al., 2013; Leakey, 1983). Plant hormones, especially auxins, are the

most important factors to determine rooting (Blakesley et al. 1991, Sever Mutlu et al 2013). Plant growth regulators are not effective substances that allow each plant to produce with cuttings but they can be defined as rooting aids. They play an auxiliary role in shortening the rooting time of cuttings and increasing the rooting rate (Ürgeç, 1982; Babaoğlu and Kalyoncu, 2011). Auxin promote root formation in cuttings (Çimen 1988, Babaoğlu and Kalyoncu 2011) and also known as rooting hormone which is effective in division and growth of meristematic cells, formation and differentiation of stem cells and [mobilization](#) of reserve nutrients to rooting site (Blakesley et al 1991, 1988; Hartman et al 1997, Sever Mutlu et al. 2013). Currently, the most commonly used synthetic auxin to promote rooting is indole butyric acids (IBA) (Burak 1991, Babaoğlu and Kalyoncu 2011). It has been reported in many studies which investigate the effect of auxin on root number and quality that IBA shorten the rooting period and induces to have quality roots and strong shoot growth in a shorter time (Stefancic, 2005; Wang et. al., 2011; Sever Mutlu et. al., 2013; Özcan et. al., 2013; Ayanoğlu et. al., 2000; Kara, 2011; Kara et. al., 2011; Arslanoğlu ve Albayrak, 2011; Kumar ve Sreeja, 1996; Elhaak et. al., 2014; Ünal, 2004).

Anatolia is situated at the junction of four biodiversity gene centers which are totally 34 in worldwide. In this context, it shows a small continental character, with 12.000 plant taxa, still a great number of new species are being discovered progressively and nearly %35 endemism rate (Turkey Ministry of Environment, 2015). Turkey is one of the world's most important countries in terms of endemic plants. Especially, Mediterranean region constitutes most of the endemic plant species distributed in Turkey (Ekim et al., 2000). Flora of Turkey is also rich in medicinal and aromatic plants. Lamiaceae (formerly Labiatae) which includes many economically important medicinal and aromatic plant species, is one of the largest (sixth) Angiosperm family, contains 245 genera and 7886 species (The Plant List, 2013; Celep and Dirmenci, 2017). *Dorystoechas hastata* Boiss.&Heldr. Benth. is a monotypic species in the genus of *Dorystoechas* in Lamiaceae family and relict endemic to Antalya province of Turkey. It is a woody shrub with 40-120 cm height and all of its parts are densely aromatic. Due to its antioxidant and aromatic, volatile oil and content (Valant-Vetschera, 2003; Meriçli and Meriçli, 1986; Özcan et al., 2016; Karagözler et al., 2008; Erkan et al., 2011; Venturella et al., 1988; Uluben et al., 2004; Başer, 1994), this species has a big potential of being raw material in medicinal and perfumery industry. Also species is consumed by local inhabitants as tea. It's an East Mediterranean element which ranges near rock and fringes between 650-2000 m altitude (Hedge, 1982) in Konyaaltı, Kemer, Korkuteli and Kumluca districts' boundaries. It was not cultivated yet and collected uncontrollably from nature. This threatens the future existence of the plant and immediate cultivation and agriculture of the plant is suggested (Öztürk, 1990). This study aims to determine the effect of different IBA doses in *Dorystoechas hastata* cuttings upon the growth of seedling root. In the scope of the study, it has been determined that there is no scientific publication about the propagation of the *D. hastata* species with cuttings and this makes the research unique.

MATERIAL AND METHODS

In the research, *Dorystoechas hastata* grown in Hacisekiler location in Konyaaltı district was used as study material. Semi-hardwood cuttings were obtained in the form of 3-5 internodes in 18-20 cm length on 13th March 2017. The study was carried out in Akdeniz University, Department of Landscape Architecture, Ornamental Plant Research Greenhouse in Antalya. Perlite was used as rooting medium and fogging irrigation system was used for cuttings. 5 doses of IBA (1000, 2000, 3000, 4000, 5000 ppm) and control (0 ppm) as factors. Crystal structure IBA (Merck) was first dissolved in 96 % alcohol, and then decreased to 50 %. The obtained cuttings were planted in pots after being waited in the hormone solutions for 5 seconds. Control cuttings were soaked in distilled water. Experimental design was randomized complete block design with 6 replications and each replication included 10 cuttings. 12 weeks after the initiation of study cuttings were carefully removed and washed, percentage of rooting(%), root number(root number/per cutting), root length (average root length/per cutting) were determined. Then the roots and shoots were separated, weighted and recorded their wet weights. They dried at 72°C for 48 hours to determine their dry weights.

The obtained data were analysed using the variance analysis method in the GLM procedure of the SAS Statistical Program. The averages were compared with the least significant difference (LSD) test preserved according to the Fisher method and the LSD (0,05) values at the application level were calculated.

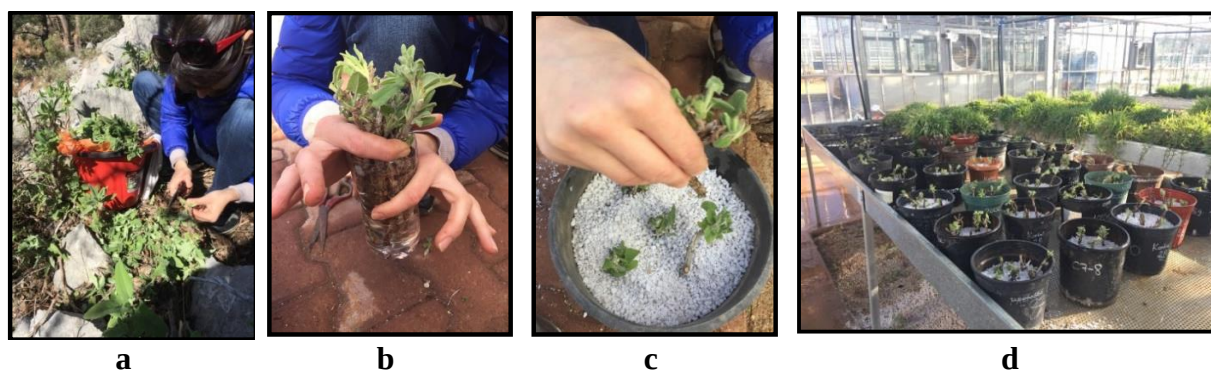


Figure 1. Different steps of cutting propagation experiment a) General view of propagation with cuttings from Hacisekiler location, b) Cuttings were soaked into rooting hormone, c) Cuttings were planted in pots, d) Cuttings in pots after application in greenhouse).

RESULTS AND DISCUSSION

Data on propagation of *D. hastata* with cuttings and statistical analysis results are presented in Table 1. and visuals from the IBA applications are shown in Figure 2. As a result of the statistical analysis, differences between IBA applications and control (0 ppm IBA) group were not statistically significant. The mean rooting rate of *D. hastata* was 91.33% and this ratio was varied between 80% and 96.66%. Otherwise, different doses of IBA have been applied to *Lavandula angustifolia* which is another member of Lamiaceae family, the highest rooting rate has been reported at 2000 ppm IBA applied cuttings (Kumar and Sreeja, 1996).

Table 1. Effect of different pre-treatments application to *D.hastata* cuttings on rooting, mean, minimum, maximum and standard deviation values.

Treatments	RL*	RN	RR(%)	RWW	RWW/C	RDW	RDW/C	CWW	CWW/C	CDW	CDW/C	LSWW	LSWW/C	LSDW	LSDW/C
Control	9,18	12,18	96,66	4,19	0,918	0,99	0,21	55,62	11,73	17,58	3,70	11,21	2,37	4,15	0,88
1000 ppm	7,85	17,09	80	4,65	1,12	1,03	0,26	48,60	12,86	14,64	3,87	10,26	2,63	3,31	0,85
2000 ppm	8,91	16,44	93,33	4,33	0,98	1,15	0,25	54,07	11,83	16,71	3,67	11,14	2,37	3,67	0,78
3000 ppm	7,66	20,4	96,66	6,29	1,31	1,12	0,23	54,32	11,25	15,95	3,30	10,94	2,26	3,71	0,76
4000 ppm	7,65	18,52	90	5,26	1,22	0,86	0,19	60,38	13,61	17,27	3,89	10,22	2,34	3,15	0,70
Mean	8,25	16,93	91,33	4,94	1,11	1,03	0,23	54,60	12,25	16,43	3,68	10,76	2,39	3,60	0,79
Min.	4,57	8	60	0,91	0,18	0,44	0,11	36,15	7,23	11,26	2,25	5,87	1,46	1,97	0,49
Max.	11,7	26,8	100	11,91	2,97	1,77	0,44	97,97	19,59	27,79	5,79	18,52	4,04	5,86	1,46
Std	1,76	5,87	12,52	2,80	0,65	0,36	0,08	11,99	3,18	3,72	0,99	2,48	0,60	0,85	0,19

*RL: Root length, RN: Root number, RR: Rooting rate, RWW: Root wet weight, RWW/C: Root wet weight/cutting, RDW: Root dry weight, RDW/C: Root dry weight/cutting, CWW: Cutting wet weight, CWW/C: Cutting wet weight/cutting, CDW: Cutting dry weight, CDW/C: Cutting dry weight/cutting, LSWW: Leaf-shoot wet weight, LSWW/C: Leaf-shoot wet weight/cutting, LSDW: Leaf-shoot dry weight, LSDW/C: Leaf-shoot dry weight/cutting

When all the applications were evaluated together, the root length values varied between 11.7 and 4.57 cm and the overall average was 8.25 cm. The average root number values per cutting showed variation between 8 and 26.8 and the overall average was 16.92. The average wet root weight per cutting is 1.11 grams and the average dry root weight per cutting is 0.23 grams. Average wet weight per cutting is 12.25 g and average dry weight per cutting is 3.69 grams. The measured leaf-shoot weight per cutting is 2,39 grams. Average leaf-shoot dry weight per cutting was measured as 0.79 grams.

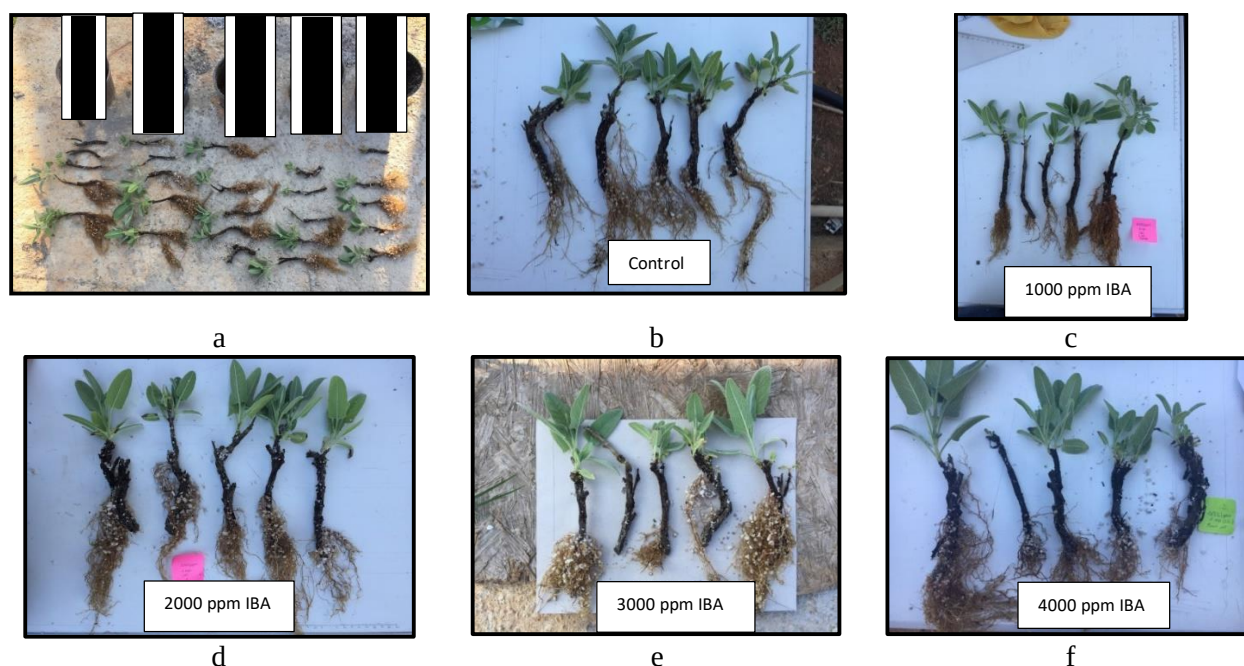


Figure 2. a) Control-1000ppm-2000ppm-3000ppm-4000ppm IBA applied cuttings, b) Control cuttings, c) 1000 ppm. IBA applied cuttings, d) 2000 ppm. IBA applied cuttings, e) 3000 ppm. IBA applied cuttings, f) 4000 ppm IBA applied cuttings.

Although no statistically significant difference was found between the applications as a result of the rooting experiment, it was found that the root number increased in direct proportion to the increasing IBA doses in terms of the measured parameters. In general terms, it was determined that the species has showed high rooting success without any IBA application.

Ünal et al. (2004) investigated the effects of different IBA concentrations (0, 100 ppm, 500 ppm and 1000 ppm) on rooting of *Origanum husnucan-baseri*, *Origanum bilgeri*, *Origanum minutiflorum* and *Origanum saccatum* species which were endemics of Antalya. Cuttings of *Origanum solymicum*, *Origanum bilgeri* and *Origanum saccatum* species have showed rooting but it was determined that in terms of rooting rate between the control and IBA application, and for both applications there is no significant difference between the species. These results show parallelism with the results obtained from our study.

Özcan et. al. (2013) investigated the effect of different growing media (peat, perlite, field soil) and different doses of IBA (Control, 500, 1000, 2000, 4000 ppm) on root number, length, rate and quality of *Lavandula hybrida* semi-hardwood cuttings. It was found that in terms of root number, 4000 ppm IBA applied cutting in field soil had the best result. In terms of root length and quality, the longest and top quality rooted cuttings were found in peat:perlite mixture and 4000 ppm. IBA application. Concordantly, Ayanoğlu et. al. (2000) investigated the effect of different IBA doses (Control, 1000, 2000, 4000 ppm) on rooting in *Lavandula stoechas* cuttings. It was observed that the rooting percentage, root length and number of roots increased due to the increase in IBA concentrations (Ayanoğlu et. al., 2000). Kara et. al., (2011) searched the effect of different propagation times (march, june, september) and IBA doses (Control, 500, 1000, 2000, 4000 ppm) on rooting of *Rosemary officinalis*, *Hyssopus officinalis* and *Salvia officinalis* which are other members of Lamiaceae family. It was stated that the highest rooting rate (85%, 82.3% and 81%, respectively), the maximum number of roots (28.8, 21.6 and 10.6 numbers/plant, respectively) and the longest root length (7.1, 6.1 and 5.1 cm, respectively) was found in March and 4000 ppm IBA applied cuttings (Kara et. al., 2011). These findings are in line with the findings of the rooting experiment of *D. hastata* cuttings.

CONCLUSION

Although *D. hastata* is a local relict endemic species, it has been able to preserve its genetic and morphological diversity until today. Cultivation and breeding program should be initiated as a potential of ornamental and medicinal-aromatic plant. In this context, it is important to study the propagation techniques of the species. It has been determined that the species can be propagated by semi-hardwood cuttings taken in early spring. Although no statistically significant difference was found between different IBA applications, it was observed that the root number increased in direct proportion to the increasing IBA doses. Adventive root formation is an important step in propagation with cuttings (Sever Mutlu et. al., 2013). Kevers et. al. (1997) stated that it was a complex consecutive physiological period which is control on auxins, peroxidases, cytokinins, ethylenes, phenolic compounds and interactions of them. Although the usage of IBA alone in our study resulted a significant increase in adventitious root formation, this increase was not statistically significant. In future studies, it may be possible to obtain significant results by testing different phenolic compounds which promote root formation (indole-3-acetic acid, hydroxy cinnamic acid, naphthalene acetic acid, etc.) and combinations of them.

REFERENCES

- Aslanoğlu, F., Albayrak Ö., 2011. Farklı IBA Dozlarının Biberiye (*Rosmarinus Officinalis* L.) ve Lavanta (*Lavandula Angustifolia* Spica) Gövde Çeliklerinin Köklenmesi Üzerine Etkileri. 9. Tarla Bitkileri Kongresi, 12–15 Eylül 2011, Bursa, 1390–1393.
- Ayanoğlu, F., Mert, A., Kaya, A. 2000. Hatay Florasında Yetişen Karabaş Lavantanın (*Lavandula stoechas* subsp. *stoechas* L.) Çelikle Köklendirilmesi Üzerine Farklı Lokasyonların ve Hormon Dozlarının Etkisi. Turk J. Agric. Forest., 24: 607–610.
- Babaoğlu, D., Kalyoncu, İ.H., 2011. M9 Elma Anacının Yeşil Çelikle Çoğaltılması Üzerine Değişik Nem ve Indol Butirik Asit (IBA) Uygulamalarının Etkileri. *Selçuk Tarım ve Gıda Bilimleri Dergisi*, 25 (1): 46-52.
- Başer, K.H.C. 1994. Essential Oils of Labiatae From Turkey-Recent Results. *Lamiales Newsletter*, Royal Botanic Gardens, 3: 6-11.
- Blakesley, D., Weston, G.D., Hau, S.F., 1991. The Role of Endogenous Auxin in Root. *Plant Growth Regulation*, 10(4): 341–353.
- Burak, M., 1991. Meyvecilikte Büyüme Düzenleyici Maddelerin Kullanım İmkanları. *Derim*, 8(4):174- 186s. Antalya.
- Celep F, Dirmenci T. 2017. Systematic and Biogeographic overview of Lamiaceae in Turkey. *Nat. Volatiles & Essent. Oils*, 2017; 4(4): 14-27.
- Çimen, İ., 1988. Meyvecilikte Büyüme Düzenleyicilerin Kullanımı. *Derim*, Antalya, 5 (3): 134- 142.
- Davies, P.J., 1995. Plant hormones. *Physiology, Biochemistry and Molecular Biology*. 2 nd Edition. Springer Science and Business Media.
- Elhaak, M.A., Matter, M.Z., Zayed, M.A. Gad, D.A., 2015. Propagation Principles in Using Indole-3-Butyric Acid for Rooting Rosemary Stem Cuttings. *J Horticulture*, 2: 121. doi: 10.4172/2376-0354.1000121.
- Erkan N, Akgonen S, Ovat S, Goksel G, Ayranci E. 2011. Phenolic compounds profile and antioxidant activity of *Dorystoechas hastata* L. Boiss et Heldr. *Food Research International* 44 (2011) 3013–3020.
- Hartmann, H.T., Kester, D.E., Davies, J.F.T., Geneve, R.L., 1997. Plant propagation: Principles and Practice, Sixth Edition. Upper Saddle River, NJ, USA: Prentice-Hall.
- Hedge, I.C. (1982). *Dorystoechas* Boiss. & Heldr. ex Benth. *Rev. Flora of Turkey* (Volume: 7). Editor: Davis, D. Edinburgh: Edinburgh University Press 22. Pp: 461-462.
- Kara, N., 2011. Uçucu Yağ Üretimine Uygun Lavanta (*Lavandula sp.*) Çeşitlerinin Belirlenmesi ve Mikroçoğaltım Olanaklarının Araştırılması, SDÜ, Fen Bil. Enstitüsü Doktora Tezi, Isparta.
- Kara, N., Baydar, H., Erbaş, S., 2011. Farklı Çelik Alma Dönemleri ve IBA Dozlarının Bazı Tıbbi Bitkilerin Köklenmesi Üzerine Etkileri. *Derim Dergisi*, 28(2): 71–81.
- Karagözler AA, Erbağ B, Emek YÇ, Uygun DA. 2008. Antioxidant activity and proline content of leaf extracts from *Dorystoechas hastata*. *Food Chemical*, 111: 400-407.
- Kevers, C., Hausman, J.F., Faivre-Rampant, O., Evers, D., Gaspar, T., 1997. Hormonal control of adventitious rooting: progress and questions. *J. Appl. Bot.* 71:71–79.
- Kumar, N., Sreeja, K.V., 1996. Effect of Growth Regulator On the Rooting Ability of Lavender (*Lavandula angustifolia* Mill). *Indian Perfumer*, 40: 93–94.
- Leakey, R.R.B., 1983. Stock Plant Factors Affecting Root Initiation in Cuttings of *Triplochiton scleroxylon* K.Schum. an Indigenous Hardwood of West Africa. *J.of.Hort. Sci.*, 58(2): 277-290.
- Meriçli F, Meriçli AH. 1986. The essential oil of *Dorystoechas hastata*, *Planta. Med.*, 52, 506.

- Özcan, İ.İ., Arabacı, O., Öğretmen, N.G., 2013. Lavanta (*Lavandula hybrida*)'nın Köklenmesi Üzerine Farklı Hormon Dozları ve Köklendirme Ortamlarının Etkisi. V. Süs Bitkileri Kongresi Bildiriler Kitabı, Cilt 2, 529-534 s., ISBN: 978-605-4672-71-4.
- Özcan, M.M., Chalchat, J.C., Figueredo, G., Bağcı, Y., Dural, H., Savran, A., Al-Juhaimi, F.Y., Ozcan, M.M., 2016. Chemical Composition of The Essential Oil of The Flowers and Leaves of Calba Tea (*Dorystoechas hastata* Boiss & Helder. ex Benth). Journal of Essential Oil Bearing Plants, 19:3, 782785, DOI: 10.1080/0972060X.2014.981597
- Öztürk NK, 1990. *Dorystoechas hastata* Uçucu Yağının Bileşimi (Yüksek Lisans Tezi). Anadolu Üniversitesi Sağlık Bilimleri Enstitüsü Farmakognozi Anabilimdalı.
- Sever Mutlu, S., Yıldız, F., Selim, C., 2013. Hayıt (*Vitex agnus-castus* L.) bitkisinin çelikle üretimine bitki büyüme düzenleyicilerin etkisi. V.Süs Bitkileri Kongresi Bildiriler Kitabı, Cilt 1, 499-504 s., ISBN: 978-605-4672-71-4.
- Stefancic, M, Stampar, F., Osterc, G., 2005. In uence of IAA and AIB on root development and quality of Prunus 'GiselA 5' leafy cuttings. Ljubljana, Slovenia. *HortScience* 40(7): 2052-2055.
- The Plant List. 2013. Version 1.1. Published on the Internet; <http://www.theplantlist.org/> (accessed 1st January).
- Turkey Ministry of Environment, 2015. Ulusal Biyolojik Çeşitlilik Stratejisi ve Eylem Planı. <http://www.nuhungemisi.gov.tr/DosyaRaporSunum/Belgeler/bb951776e87440ce842b-d90ae82b6381.pdf> Son erişim tarihi: 30.03.2015.
- Uluben, A., Meriçli, A.H., Meriçli, F., 2004. Diterpenes and Norditerpenes from the Roots of *Dorystoechas hastata*. *Pharmazie*, 59-4201, 301-3
- Ünal, O., Gökçeoğlu, M., Topçuoğlu, Ş.F., 2004. Antalya Endemiği *Origanum Türlerinin* Tohum Çimlenmesi ve Çelikle Çoğaltılması Üzerinde Araştırmalar. *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi*, 17(2): 135-147.
- Ürgeç, S 1982. Orman ağaçları ıslahı. İstanbul Üniversitesi Orman Fakültesi Yayın No: 293, İstanbul.
- Valant-Vetschera, K., Roitman, J.N., Wollenweber, E., 2003. Chemodiversity of exudate flavonoids in some members of the Lamiaceae. *Biochemical Systematics and Ecology*, 31, 1279-1289.
- Venturella P, Venturella G, Marino MS, Meriçli AH, Çubukçu B. 1988. Phytochemical Investigation of the Labiatae *Dorystoechas hastata*. *Giornol Botany*, 122: 291-294.
- Wang., X.L., Zhao, Z., Quan, J.E., 2011. Indole-3-Butyric acid on rooting and endogenous plant hormones in tetraploid and diploid pseudoacacia harwood cuttings. *Phyton-International Journal of Experimental*, 80: 93-100.

EFFECTS OF SALICYLIC ACID APPLICATIONS ON ESSENTIAL OIL COMPOSITION AND SALT STRESS TOLERANCE IN SWEET BASIL (*Ocimum basilicum* L.)

Şengül Nacar KARAMAN¹, Emel Dıraz YILDIRIM¹, Halime KAHVECİ¹

¹Kahramanmaraş Sütçü İmam Univ. Faculty of Science and Letters, Biology Dept. Kahramanmaraş, Kahramanmaraş

Corresponding author email: sengulk@ksu.edu.tr

Abstract

Plant hormones and growth regulators are used against the negative effects of salinity on plants. It has been widely reported that salicylic acid (SA) could reduce salt stress in plants. In this study, we reviewed the emerging role of SA in enhancing salt tolerance in plants. For this purpose, 0,05 and 0,025 mM SA were applied to the basil (*Ocimum basilicum* L.) seeds. After seedlings having 5-6 leaves, they were watered with saline solution gradually increasing to 100 mM. The plants were harvested before flowering and their growth parameters and essential oil composition were investigated. SA applications increased the yield parameters and quality under control and salt stress. Essential oil composition of dried leaves were analyzed using Solid Phase Micro Extraction method (SPME). Linalool (41,08%) was main component in the control group whereas methy eugenol (79,18%) was found to be high in salt application. Both 0,05 and 0,025mM SA applications increased linalool content (11,19 and 23,99% respectively) under salt stress. The results propose that a regulatory role of SA on the basil plants for salt tolerance.

Keywords: *Ocimum basilicum*; salt stres; salicylic acid, linalool

INTRODUCTION

Salinity is an important abiotic stress which reduces the productivity and quality of agricultural areas and also negatively affecting plant growth and development (Kaya and Inan 2017). Shortage of water in arid and semi-arid regions throughout the world necessitates utilisation of marginal waters in agricultural production fields. Such water, saline, brackish, or treated effluents usually contain high levels of salts, which may restrict plant productivity (Bernstein et al.,2010). Plant species and varieties differ in tolerance to salinity and ability to sustain physiological functions and yield production under stress. The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Yildirim and Taylor, 2005, Said-Al Ahl and Mahmoud 2010). Exogenous hormone and plant growth regulators (PGR) are used to eliminate to negative effects of salinity in plants. Polyamines, brassinosteroids, jasmonic acid, salicylic acid are examples of compounds commonly studied for this purpose. SA produce different anti-stress mechanisms under osmotic stress. It is well known exogenous application of SA under salt stress is widely effective in many metabolic process as plant growth, development, ion uptake and transport, photosynthesis ratio, stoma conductivity and transpiration.

The most important species of *Ocimum* genus (Lamiaceae family) is *O. basilicum* L.; this species, usually named sweet basil, is considered economically useful because of their basic natural characteristics as essential oil producers (Lawrence, 1993, Karaman et al., 2008, Telci et al., 2005). *O.basilicum* is a popular culinary herb used in food, oral care products and perfumery. In this study basil was preferred because it is an important culinary herbs in the world and can be easily grown and commercially produced in greenhouses (Koca and Karaman 2015). The aim of this study was to evaluate the effect of SA application on production of secondary metabolites and salt stress tolerance of sweet basil plants.

MATERIAL AND METHODS

Sweet basil seeds obtained from Vilmorin trade mark (purple leaf) were sterilized with 1% hypochlorite solution for 2–3 min then washed thoroughly with tap water. The seeds were soaked in 0,05 and 0,025 mM SA solutions for 24 h (Karaman et al., 2008). In the experiment, 4 seeds were planted in each pot. After emerge from the seedlings, they were allowed to grow in a single seedling in each pot. When the seedlings have two real leaves, they were fertilized every two weeks with 100 ppm (according to N) liquid manure until harvest. When the seedlings have five real leaves, they were watered with saline solution at two days intervals gradually increasing 25 to 100 mM. The plants irrigated with 100 mM NaCl for two weeks. The basil plants were harvested with roots in the pre-flowering period and the leaves were dried at room temperature for volatile components analysis.

Isolation of the Volatile Oil Components

Dried leaves of the plant were analysed by solid-phase micro extraction (SPME) method. The SPME fibres coated with polydimethylsiloxane were supplied by Agilent. 0.05 g dried plant material was put into glass bottle and placed into ultrasonic bath and waited for 30 min at 70 °C. Then SPME syringe (Supelco 57346-U) needle pricked to silicone rubber septum of the glass bottle and the volatiles trapped. After 15 mins syringe injected to the GC-MS. The extracted compounds were thermally desorbed at 250 °C for 1 min in the injector port of a gas chromatograph and left in the injection block for 15 minutes to clean the fibre.

Gas Chromatography–Mass Spectrometry (GC – MS) Analysis

GC–MS analyses were conducted in the Plant Physiology Laboratory in the Department of Biology of Kahramanmaraş Sutcu Imam University. GC/MS analyses were performed with Agilent GC – 6890 II series coupled with Agilent 5975C Mass Spectrometer. Column: HP – 88, 100 m x 250 µm x 0.20 µm film thickness. Temperature programmed: increased from 70°C (1 min) to 230°C (20 min) at a rate of 10°C/min. The injection temperature: 250°C. Injection volume: 1.0 µL. Carrier gas: He. Injection mode: split (20:1). MS interface temp.: 250 °C; MS mode: EI; detector voltage: 70 eV; mass range: 35 – 400m/z; scan speed (amu/s). The components of the oil were identified by mass spectra with those of pure authentic samples and NIST08, Willey7n.1 and HPCH1607 libraries reference compounds.

RESULTS AND DISCUSSION

According to GC analyses of dried basil leaves, 12 components were detected with FID dedector. - Linalool, methyl eugenol and eugenol were determined as main components (Table-1). Linalool (41,08%) was main component in the control group. Lower linalool content observed at 0.05 mM SA application (29,21%) than control group. Although methyl eugenol was the main component (37.62%) and following linalool at 0.05mM SA treatments, low SA concentrations (0.025mM) had no effect on the linalool content without salt conditions. Interestingly methyl eugenol (79,18%) was found to be high compound in salt application. As seen in Table-1, both 0,05 and 0,025mM SA treatments increased linalool content (11,19 and 23,99% respectively) under salt stress, and methyl eugenol content began to fall at SA treatments under salt stress. However, higher methyl eugenol contents were observed at these applications under salt stress than the control group, and methyl eugenol was found as main component at all treatments under the salt stress. Methyl eugenol ratio was 79.17, 58.80 and 41.60 % for NaCl, SA 0.05+NaCl and SA 0.025+NaCl respectively.

Table 1 The ratio of essential oil composition of *O.basilicum* plants obtained from different SA and NaCl applications.

Treatment	1,8 cinelol	linalool	Betafar masene	e caryoph ylene	Bornyl acetate	Gama cadine	α - terpineol	Cis- calemen	Methyl eugenol	Epi- acadinol	Spathul enol	Eugenol
Retention Time	14,18	17,86	18,32	18,81	19,46	19,93	20,57	21,69	24,86	25,54	25,80	26,97
Control	4,04	41,08	-	1,87	3,81	4,81	5,46	1,69	7,56	2,64	2,40	16,31
Sa 0,05 mM	2,66	29,21	1,84	1,11	1,40	3,08	2,67	0,87	37,62	1,28	1,68	14,60
Sa 0,025 mM	6,91	40,52	-	-	1,31	4,65	6,69	1,12	13,81	1,44	1,54	15,89
Salt (NaCl)	1,65	5,71	1,45	-	-	1,16	1,73	0,75	79,17	0,67	0,48	3,98
Sa 0,05 mM+NaCl	2,26	11,19	1,51	0,87	0,76	2,28	2,189	0,30	58,80	0,84	0,73	17,17
Sa 0,025mM+ NaCl	4,39	23,99	-	1,12	0,88	3,45	4,77	0,66	41,61	1,09	1,13	12,67

As a result there was a pronounced difference in the volatile composition of *O. basilicum* content due to different SA applications. Our results supported by Gharib (2006) report that, SA application increased the eugenol level at basil plants. Also a high percentage of eugenol in the SA treated herb is of particular interest in basil, which could constitute new sources of antioxidant phenolics in the diet (Gharib 2006). According to our results, the application of 0.05 mM SA showed similar effect in terms of linalool and methyl eugenol exchange, as in salt stress, although the major change in salt stress was observed. We can say that 0.025 mM SA treatments showed lower effect than 0.05mM SA. Miura and Tada (2014) reported that the important characteristics of SA applications were applied concentration and the method of application, such as foliar spray and hydroponic culture. These methods depend on the plant species; therefore, contradictory results can be reported. Generally, low concentrations or the transient application of SA promotes plant tolerance to abiotic stresses, and high concentrations or the continual application of SA induce inhibitory effects on plant growth and reduce tolerance.

In this research a regulatory role of SA on the basil plants were obtained under salt stress. Similar to our work, Kulak (2016) reported that linalool content was lower than eugenol content of basil plants under drought stress. Same results about linalool and eugenol exchange under salt stress reported by Tarchoune et al., (2013). Baydar and Çoban (2017) reported that NaCl caused differences in essential oil composition. Menthol and menthone, the main components of the peppermint oil, reduced significantly when NaCl concentration rose to 150 mM. They also reported that NaCl at high concentrations negatively affected plant growth, essential oil content and quality of the essential oil.

CONCLUSION

In conclusion, it was determined that SA treatments may change the ratio of volatile components of basil plants according to applied concentrations. Generally higher concentrations can affect stress. In salt stress, SA application can play a protective role the plant. In our study, it was observed that the synthesis of methyl eugenol increased by changing the linalol biosynthesis pathway in salt stress. SA treatments improved the components ratio of the basil plants, especially 0.025 mM SA application gave better results than 0.05mM SA. However, further research is needed to elucidate the possible role of SA on the plant.

ACKNOWLEDGEMENTS

The data presented in this paper is obtained from the project financially supported by Kahramanmaraş Sütçü İmam University (Scientific Research Project No: 2015/3-13YLS).

REFERENCES

- Baydar, N. G., Çoban, Ö., 2017. Tuz Stresinin Nane (*Mentha piperita* L.)’de Büyüme ile Uçucu Yağ Miktarı ve Bileşenleri Üzerine Etkileri. *Turkish Journal of Agriculture-Food Science and Technology*, 5(7), 757-762.
- Bernstein, N., Kravchik, M., Dudai, N., 2010. Salinity-induced changes in essential oil, pigments and salts accumulation in sweet basil (*Ocimum basilicum*) in relation to alterations of morphological development. *Annals of Applied Biology*, v.156, p.167-177.

- Fatemi, R., Aboutalebi, A., 2012. Evaluation of the interaction of salinity and salicylic acid on sweet basil (*Ocimum basilicum*) properties. *Annals of Biological Research*, v.3, p.5106-5109.
- Gharib, F. A., 2006. Effect of salicylic acid on the growth, metabolic activities and oil content of basil and marjoram. *Int. J. Agric. Biol.*, 4, 485-492.
- Lawrence BM 1993. A planning scheme to evaluate new aromatic plants for the flavor and fragrance industries. In: Janick J, Simon (eds.) *New crops*. New York: Wiley, p.620-627.
- Karaman, S., Kirecci, O.A, Ilcim, A., 2008. Influence of polyamines (spermine, spermidine and putrescine) on The Essential Oil Composition of Basil (*Ocimum basilicum* L.). *Journel of Essential Oil Research*, 20(4), 288-292.
- Kaya, A., İnan, M., 2017. Tuz (NaCl) stresine maruz kalan reyhan (*Ocimum basilicum* L.) bitkisinde bazı morfolojik, fizyolojik ve biyokimyasal parametreler üzerine Salisilik Asidin etkileri. *Harran Tarım ve Gıda Bilimleri Dergisi*, 21(3), 332-342.
- Koca, N., Karaman, Ş., 2015. The effects of plant growth regulators and L-phenylalanine on phenolic compounds of sweet basil. *Food chemistry*, 166, 515-521.
- Kulak, M., 2016. Su stresi ve salisilik asit ön uygulamalarının fesleğen (*Ocimum basilicum* L.)'in fizyolojik parametreleri ve protein içeriğine etkileri. Doktora Tezi, KSÜ, Kahramanmaraş
- Miura .,Tada Y, 2014 Regulation of water, salinity, and cold stress responses by Salicylic Acid. *Front. Plant SCI*, 5,4-8
- Said-Al Ahl, H. A. H., & Mahmoud, A. A., 2010. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean Journal of Applied Sciences*, 3(1), 97-111.
- Telci, İ., 2005. Reyhan (*Ocimum basilicum* L.) genotiplerinde uygun biçim yüksekliklerinin belirlenmesi, Gazi Osmanpaşa Üniv. Ziraat Fak. Dergisi, 22 (2): 77- 83.
- Tarchoune, I., Baâtour, O., Harrathi, J., Cioni, P. L., Lachaâl, M., Flamini, G., Ouerghi, Z., 2013. Essential oil and volatile emissions of basil (*Ocimum basilicum*) leaves exposed to NaCl or Na₂SO₄ salinity. *Journal of Plant Nutrition and Soil Science*, 176(5), 748-755.
- Yildirim, E., Taylor A.G. Effect of biological treatments on growth of bean plants under salt stress . *Annu. Rep. Bean Improv. Coop.*, 48 (2005), pp. 176-177

THE REVIEW OF ARTICLES OF THE STUDYING ON MYRTLE IN THE WORLD

Ayşe EVEREST¹, Uysal YENİPINAR²

¹Mersin University, Science Faculty, Biology Department, Çiftlikköy-Mersin

²Mersin Üniversitesi, Turist Rehberliği Bölümü, Çiftlikköy-Mersin

Corresponding author email: ayseeverest@mynet.com

Abstract

The researchs on the natural aromatical material; *Myrtus communis* L. (mersin) was studied from 1970 to 2018' s. But the using of mersin plant depends on ancient times (MÖ.3 century) in the world. Mersin (*Myrtus communis*) plant is growing in Mersin city and a Mediteranean element. It was planned the using to be an endustrial material and the marketing and advertising sector with different technicals of this plant. Articles was revising, about the spreading and the production of this plant in the world.

REVIEW

In human history; the epic of Gilgamesh and the Roma Olympics have all lived with myrtle and died with myrtle (Rhizopoulou, 2004, Elinç et al., 2013, Köse and Kandemir, 2016).

Mersin plant in Turkey is seen in the Mediterranean climate and patches of the Mediteranean Region and althouth the local names “murt” and “hambeles” was known, “myrtle” named for each location (city, town, village, etc.) such as inOrdu,also Afyon settlements investigation it has been undertaken in the name-that is grown. Atılgan (2013), mersin's litters brought by Çorum: Iskilip rivers-this feature has been investigated.

In fact, the Mediterranean climate is visible in distant countries such as California, Florida in USA, some parts of Australia, Samsun, Sinop, Kahramanmaraş; Andırın patches in Turkey and as originally resemble the Mediterranean 's river valleys can be seen in all of the non-Mediteranean phytogeographicregion same Çoruh valley.

It grows as an ornamental plant like roses at the flow points in South Australia. According to Missouri Botanical Garden, there is no insect and disease problem, roots can be sheltered even in poorly drained places. The main distribution area is western Europe and the flowering period is shown as May-July period. It likes a lot of sunshine, partly likes shade, it needs medium water, is tolerant to drought, it is a green kind that smells good.

In Mersin: Silifke Göksu Delta Conversation Area, as it is in the 5-10 meter sand dunes of Israel, Jordan, but also grown the under forests of pine (*Pinus brutia*). As in the 3rd century BC, it was crown, but in 20rd century, we were regarded as popular ornamental-wreath, plants in the years 1990 and 2000, as it is the plant of northern Europeans. Today the filling material of these wreaths is probably *Ceratonia siliqua* (keçiboynuzu) and *Ficus nitida* (Israel Kauçuğu)are filling. In fact the Mediteranean plant, Degtyareva et al. İn 1970, were Studies on some properties of an antibacterial preparation from myrtle.

Some animals; the marten, the ant, the insects and the birds also love this plant. But it will known a good insectisit plant. As these local names also describe, the leaves of this plant, which loves the slopes, are collected in the form of sediments in the pits. However, there are also reports that pharmacists have diarrhea very often. The black little fruity wilderness in the east is called "gıcık", which is a word used in the Arabic cities.

All of the phrases of myrtl that are still used in Mersin are evidence of her being a plant for many years. In old Mersin houses there were citrus trees in the garden and definitely myrtle plants at the door entrance. It was something to be expected to become a tree with white flowers, and this figurine took its place from time to time with inedible and nonfragrant, spiny, flowering celery;

Boungavillea plants. This plant, like Morocco and Peru, which missionaries spread lovingly to the world was settlement in Mersin.

Mersin University campus is based on the maquis vegetation where the citrus gardens are located. It is a visual transfer of the people who are interested in planting the university in the farm village of Çiftlikköy; “yörük” village, which is about 110 meters. In Muğla and Mut, their nurses are using for the sweet sleep by the leaves and flowers in the hot water, with citrus and mentha. In Antakya, newborn children and the deceased are washed with the myrtle plant, and the tradition is still continuing in Mersin. Although the wild is referred to as the “hambeles” (in Karaisalı, “murt”), it has been tested and approved in human history as a long-lived and durable plant in the myrtle gene flow (Albaladejo, et al., 2009)

Its vegetative distribution, with *Quercus ilex* in Antalya and *Quercus infectoria* in Mersin. One of the most beautiful features of the work done for *Cistus*, *Quercus* and *Myrtus* (Mersin) is that Mersin is found to be faster than *Quercus* (Fioretto, et al., 2005). In environmental light factors chlorophyll production was not found statistically significant in terms of sugar in light and shadow leaves (Mendes, et al., 2001).

Appendino et al., 2003, identified from leaves of myrtle (*Myrtus communis*); myrtucommulone showed significant antibacterial activity against multidrug-resistant clinically relevant bacteria, while semimyrtucommulone was less active. Also, in 2006; Appendino et al., showed an investigation of the polar glycosidic fraction from the leaves of myrtle afforded four galloylated nonprenylated phloroglucinol glucosides (3a–d) related to the endoperoxide hormone G3 (4) in terms of structure and biogenesis. Despite their close similarity, significant antibacterial activity was shown only by one of these compounds (3b, gallomyrtucommulone B), while the G3 hormone (4) was inactive

Vacca et al. (2003), The results of a study on the evolution of phenolic compounds, colour and antioxidant activity in two industrial red myrtle liqueurs during storage in bottles under different bottle headspace (constant or increasing) and exposure to light are reported. They significantly decreased during storage in the bottles with increasing headspace, while values remained almost constant in the others. Lubello et al (2004), Agronomic results indicate no major limitations to the use of a tertiary effluent as an irrigation source in an ornamental plant nursery. The nutrient content of the tertiary effluent was able to maintain good plant growth as well as fertigated water for most of the tested species.

Tuberosa et al. (2007), find that anthocyanin qualitative composition was the same in all selections, but varied strongly in its total amount. Only one was the selection with the characteristics most suitable for industrial use. Tiryaki et al. (2009), The results suggest that the use of myrtle leaves oil is an innovative and useful tool as an alternative to the use of chlorine or other synthetic disinfectants in fruits and vegetables, especially for organic products

Many studies on it are usually done by Mediterranean countries. These; Italy and the islands (Montoro et al, 2006; Barboni et al, 2010), Spain (Mendes et al., 2001; Caravaca et al, 2005), Portugal, France, Greece (Chryssavgi et al, 2008), Tunisia (Masseoud et al 2005, 2012; Jamouss et al., 2005 Wannes, 2010; 2013), Libya (Elfellah et al., 1984), Iraq (Muhaned et al., 1989), Egypt and Turkey is. The studies of fruit color polymorphism and polynization also *Bambus terrestris* effects have attracted attention.

Öztürk, 1970; Demir, 2014; Sepici 2000; Yıldırım, 2012 and Tuncer, 2012 postgraduate studies were completed on this plant in Turkey. The volatile oil ratios are measured at different harvest times in İzmir; Bornova. Plant leaves are contain for tannin, essential oil, bitter substances, also wound healing and antioxidant (Öztürk, 1970; Tuncer, 2012).

Studies have been undertaken in 2007 and conservation strategies have been investigated while antifungal and insecticide effects were investigated in 1970 (Kashman et al., 1974; Erlachin and Erciyas, 1978; Akgul and Bayrak, 1989) and 1990s (Aronne and Russo, 1997).

The enzymatic dynamics of leaf microcirculation (2000) and cellulose and lignin degradation / dynamics (2005), as well as soil biology and biochemical properties, have been studied closely (Sepici et al., 2000;

Fungal diseases, healing fungiitis, gingivitis, boil healing, asthma, sugar regulating, blood-thinning and soothing effects are known and used in public. According to Demir (2014), it can be argued that the *Myrtus communis* L. fruits may reduce the free radicals and oxidative stress. While it has high phenolic content and antioxidant capacited also antioxidant and antimicrobial effected plant have been suggested to be consumed freshly or processed materials (Aksay, 2005; Tuberosa et al., 2010; Mimica-Dukić et al., 2010; Messaoud et al., 2012).

Söke and Elmacı, 2015; in the processing of candies of black and white myrtle fruits showed statistically significant results in terms of the appearance of using corn syrup and sucrose.

This beautiful Mediterranean ornamental plant, identified as mirtenol, limonene, myrtenyl acetate, alpha terpinol, alpha-pinene, 1,8-cineole, has found phenolic compounds of Yeğin and Uzun (2015) the amounts of epicatechin-3-O-gallate (ECG) and flavonol were found to be higher than those of olive compounds.

Again, Uzun (2016), two different locations in Antalya; examining the yield and quality characteristics of those growing on the coast and in the spring; a culture of white myrtle type (Hambeles) with 3 black myrtle types (Yakup, Yumaklar, Işlangıç) was studied. The highest average fruit weight in black berries was determined as Yakup type (0.76 g) on the plateau, and Yumaklar type (0.92 g) on the shore. There was no difference between the types in terms of fruit breaking force. Must/ juice ratios were varied between 29.6% and 35.0%. Malic acid was found higher in fruits than in others. As the essential oil, maximum 1.8 cineol and α -pinene components were detected in black and white myrtle leaves.

Bozdoğan et al., (2017) compiled the informations on different uses of *Myrtus communis* L., one of the important species in the natural vegetation of the Mediterranean Region; the aesthetic and functional qualities of the crops are particularly preferred in landscape architecture studies due to their resistance to drought, and they are used in the context of breeding technology in order to clean up contaminated soils and in landscape restoration.

This seasonal spice used as an animal feed and used as a skin tanning and dyeing material (Doğan et al., 2003), which has traditionally been used in medicine for a long time since the treatment of various diseases in the medical field, sherbet, marmalade and jam making and diet supplement. Attention has been paid to the interest of state institutions by drawing attention to the protection of this species, which has a high habitat value due to its contribution to wild life. Insecticidal use includes fly, moth, bean bit (Bozdoğan et al., 2017). Ayvaz et al. (2010) also found protective effects against some adult *Acanthoscelides obtectus*, although less than *Satureja thymbra* and *Origanum otites*.

Mersin, which is known by the name of the city of Mersin; in short, with the idea of rebuilding knowledge to improve the use of commercial space by using new technological methods,

Mersin Metropolitan Municipality, Mersin University and Mersin (İçel) Art Club in cooperation with a workshop held on January 5, 2018.

Myrtle seedlings cultivated by Alata Horticultural Research Institute and Regional Directorate of Eastern Mediterranean Forestry Research Institute in Mersin, which have proved by seedling and cuttings procedure and distributed free for the farmers formany years, constituted a step for myrtle 's (mersin's) peace to the wild enviroment again. We expect that the myrtle shrubs like Mersin parks and gardens that decorate their gardens will also take their place in Mersin.

REFERENCES

- Alım, E., Uzun, H.İ. 2017. Siyah mersin (*Myrtus communis* L.)'de gibberellik asit (GA3) uygulamalarının meyve kalitesi ve çekirdeksizlik üzerine etkisi, *Derim*, 34:2 (113-121).
- Asif, M., Afaq, S.H., Tariq, M., Masoodi, A.R. 1979. Chromatographic analysis of *Myrtus communis* fixed oil. *European Journal of Lipid Science and Technology*, 81, 473–474.
- Avcı, A.B., Bayram, E. 2008. Mersin Bitkisi (*Myrtus communis* L.)'nde Farklı Hasat Zamanlarının Uçucu Yağ Oranlarına Etkisi, *Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü Dergisi*, 12:3(178-181).
- Ayvaz, A., Osman Sagdic,O., Karaborklu, S., Ozturk. I. 2010. Insecticidal Activity of the Essential Oils from Different Plants Against Three Stored-Product Insects, *Journal of Insect Science* 10:21 (1-13).
- Aydın, C., Özcan, M.M.2007. Food EngineeringDetermination of nutritional and physical properties of myrtle (*Myrtus communis* L.) fruits growing wild in Turkey, *Journal of food engineering*.79:453–458.

- Akgül A. ve Bayrak A. 1989. Mersin Bitkisi (*Myrtus Communis* L.) Yapraklarının Uçucu Yağ Verimi ve Yağların Bileşimi, Doğa Tu Tar. ve Ör. 13: 2 (143-147).
- Aksay, S. 2005. Murt (*Myrtus communis* L.) Meyvesinin Farklı Ekstraktlarının Toplam Fenolik Madde Miktarı ve Antioksidan Özellikleri Dergi park; Çukurova Tarım ve Gıda Bilimleri Dergisi, 31:2 (43-45).
- Aronne, G., Russo, G. 1997. Carnivorous mammals as seed dispersers of *Myrtus communis* (Myrtaceae) in the Mediterranean shrublands, Official Journal of the Societa Botanica Italiana, 131: 3 (189-195).
- Albaladejo, R.G., Carrillo, L. F., Aparicio, A., Fernández-Manjarrés JF., González-Varo, J.P. 2009. Population genetic structure in *Myrtus communis* L. in a chronically fragmented landscape in the Mediterranean: can gene flow counteract habitat perturbation? Plant biology, 11: 3 (442-453).
- Barboni, T., Cannac, M., Massi, L., Perez-Ramirez, Y., Chiaramonti, N. 2010. Variability of polyphenol compounds in *Myrtus communis* L. (Myrtaceae) berries from Corsica. Molecules, 15, 7849-7860.
- Bradesi, P., Tomi, F., Casanova, J., Costa, J., Bernardini, A.F. 1997. Chemical composition of myrtle leaf essential oil from Corsica (France), Journal of Essential Oil Research, 9: 283-288.
- Boelens, M., Jimenez, R. 1992. The chemical composition of Spanish myrtle oils, Part II. Journal of Essential Oil Research, 4: 349-353.
- Benkhayal FA, Musbah E, Ramesh S, El-Ageeli DWH. 2009. Pathological studies on the effect of phenolic compounds extracted from *Myrtus communis* in diabetic rats. *Tamilnadu Journal of Veterinary and Animal Sciences*, 5: 155-156.
- Bozdoğan, E., Güler, S., Burgut, A. 2017. *Myrtus communis* L. türünün farklı kullanım alanları açısından değerlendirilmesi, Bahçe 46:1 (73-77).
- Barboni, T., Venturini N., Paolini J., Desjobert J.M., Chiaramonti N., Costa J. 2010. Characterisation of volatiles and polyphenols for quality assessment of alcoholic beverages prepared from Corsican *Myrtus communis* berries, *Food Chemistry*, 122: 1304-1312.
- Cannas, S., Molicotti, P., Usai, D., Maxia, A., Zanetti, S. 2014. Antifungal, anti-biofilm and adhesion activity of the essential oil of *Myrtus communis* L. against *Candida* species. *Natural Product Research*, 28 : 2173-2177.
- Chryssavgi, G., Vassiliki, P., Athanasios, M., Kibouris, T., Komaitis, M. 2008. Essential oil composition of *Pistacia lentiscus* and *Myrtus communis* L.: Evaluation of antioxidant capacity of methanolic extracts, *Food Chemistry*, 107: 1120-1130.
- Caravaca, F., Alguacil, M.M., Hernández, J.A., Roldán, A. 2005. Involvement of antioxidant enzyme and nitrate reductase activities during water stress and recovery of mycorrhizal *Myrtus communis* and *Phillyrea angustifolia* plants, *Plant Science*, 169: 1 (191-197).
- Çakır A. 2004. Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (Sea Buckthorn) and *Myrtus communis* L. from Turkey, *Biochemical Systematics and Ecology*, 32: 809-816.
- Demir, G.M. 2014. Streptozotocinle indüklenen Diyabetli ratlar üzerinde mersin bitkisi (*Myrtuscommunis* L.) meyvesinin su ekstreşi etkilerinin İncelenmesi, Atatürk Üniversitesi, Sağlık bilimleri enstitüsü, Eczacılık biyokimya anabilim dalı, yüksek lisans tezi, Erzurum.
- Doğan, Y., Başlar, S., Mert, H., Ay, G. 2003. Plants Used As Natural Dye Sources In Turkey. *Economic Botany*, 57:4 (442-453).
- Elfellah M.S., Akhter M.H. Khan, MT. 1984. Anti-hyperglycaemic effect of an extract of *Myrtus communis* in streptozotocin-induced diabetes in mice, *Journal of Ethnopharmacology*, 11: 3 (275-281).
- Erlaçın S, Erciyas E. 1978. *Myrtus Communis* L. (Mersin Bitkisi) Yapraklarının Tanen Yönünden İncelenmesi, Doğa II, 75-79.
- Elinç, Z. K., Kaya, L.G., Elinç, H. 2013. Efes Antik Kenti Yamaç Ev-i'nin Peristil Bahçesi Restitüsyonu ile Antik Dönem Bahçe Mimarisi'nin İncelenmesi, *IJSES*, 3:2 (99-110).
- Eliuz, Erdoğan A.E., Gökşen, G., Everest, A., Ayas, D. 2014. *Myrtus* Bitkisinden konjuge Linoleik Asitle Yapılandırılmış fonksiyonlu yağ Üretimi, KOSGEB; Ar-ge inovasyon ve endüstriyel Teknopark projesi raporu.
- Jamoussi, B., Romdhane, M., Abderraba, A., Ben Hassine, B., EL Gadri, A. 2005. Effect of harvest time on the yield and composition of Tunisian myrtle oils, *Flavour and fragrance journal*, 20: 274.
- Fioretto, A., Papa, S., Curcio E., Sorrentino, G., Fuggi, A. 2000. Enzyme dynamics on decomposing leaf litter of *Cistus incanus* and *Myrtus communis* in a Mediterranean ecosystem *Soil Biology and Biochemistry*, 32, 13, 1847-1855.
- Fioretto, A., Di Nardo, C., Papa, S., Fuggi, A. 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem, *Soil Biology and Biochemistry*, 37: 6 (1083-1091).
- Gortzi, O., Lalas, S., Chinou, I., Tsaknis, J. 2008. Reevaluation of Bioactivity and Antioxidant Activity of *Myrtus communis* Extract before and after Encapsulation in Liposomes. *European Food Research and Technology*, 226:583-590.
- Hacıseferoğulları, H., Musa Özcan, M., Arslan, D., Ünver, A. 2012. Biochemical compositional and technological characterizations of black and white myrtle (*Myrtus communis* L.) fruits *J Food Sci Technol*. 49(1): 82-88.
- Karaman, Ö., Cebe, G. E. 2016 Diyabet ve Türkiye de antidiyabetik olarak kullanılan bitkiler, *Ankara Ecz. Fac. Derg., j. Phac. Pharm.*, 40: 3 (47-61)
- Köse, M., Kandemir, A. 2016. Başbakanlık Osmanlı Arşivi'nde botanik üzerine belgeler (1797-1926), *Bağbahçe Bilim Dergisi*, edergi.ngbb.org.tr
- Kashman Y, Rotstein A, Lifshitz A. 1974. Isolation and Antibacterial Activity of Acylphloroglucinols from *Myrtus communis*, *Antimicrobial Agents and Chemotherapy*, 6: 539-542.

- Montoro P, Tuberoso CI, Piacente S, Perrone A, De Feo V, Cabras P, Pizza C. 2006. Stability and antioxidant activity of polyphenols in extracts of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. Journal of Pharmaceutical and Biomedical analysis, 41: 1614-1619.
- Messaoud, C., A. Laabidi A., Boussaid, M. 2012. *Myrtus communis* L. Infusions: The Effect of Infusion Time on Phytochemical Composition, Antioxidant and Antimicrobial Activities, Journal of Food Science, 77: 9 (941–947).
- Montoro, P., Tuberoso, C.I.G., Piacente, S., Perrone, A., De Feo, V., Cabras P., Pizza, C. 2006. Stability and Antioxidant Activity of Polyphenols in Extracts of *Myrtus communis* L. Berries Used for the Preparation of Myrtle Liqueur, Journal of Pharmaceutical and Biomedical Analysis, 41:1614–1619.
- Muhaned K.,Al-HindawiIhsan H.S., Al-DeenMay H.A., NabiMudafar, Ismail,A. 1989. Anti-inflammatory activity of some Iraqi plants using intact rats,Journal of Ethnopharmacology, 26: 2 (163-168).
- Messaoud, C.,Zaouali, Y., Ben A., Salah, Khoudja, M.L., Boussaid, M. 2005. *Myrtus communis* in Tunisia: variability of the essential oil composition in natural populations, Flouvor and Fragrance Journal, 20: 6 (577–582).
- Mendes M.M.,Gazarini, L.C., Rodrigues, M.L.2001. Acclimation of *Myrtus communis* to contrasting Mediterranean light environments - effects on structure and chemical composition of foliage and plant water relations,Environmental and Experimental Botany, 45: 2(165-178).
- Mahboubi, M., Bidgoli, F.G. 2010. In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of *Candida albicans*, Phytomedicine, 17: 771-774.
- Messaoud, Y., Zaouali,A., Ben Salah,M. L. Khoudja,M. Boussaid. 2005. *Myrtus communis* in Tunisia: variability of the essential oil composition in natural populationsflouvor and fragrance journal, 20: 6 (577–582).
- Mimica-Dukić, N., Bugarin, D., Grbović, S., Mitić-Ćulafić, D., Vuković-Gačić, B., Orčić, D., Jovin, E., Couladis, M. 2010. Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents, Molecules, 15: 2759-2770.
- Malekpour A, Dehghani S, Zahedi S, Eskandari F. 2012. Effects of the hydro-ethanolextract of *Myrtus communis* L. on blood glucose level and histopathologicalchanges in alloxan-induced diabetic rats,Middle-East Journal of Scientific Research, 12: 517-522.
- Sepici A, Gurbuz I, Cevik C, Yesilada E. 2004. Hypoglycaemic effects of myrtle oil innormal and alloxan-diabetic rabbits,Journal of Ethnopharmacology, 93:311-318.
- Sepici-Dincel A, Acikgoz S, Cevik C, Sengelen M, Yesilada E. 2007. Effects of in vivo antioxidant enzyme activities of myrtle oil in normoglycaemic and alloxandiabetic rabbits,Journal of Ethnopharmacology,110: 498-503.
- Sepici A. 2000. Türkiye’ de Halk Arasında Diabetes Mellitus Hastalığının TedavisindeKullanılan Mersin Uçucu Yağı (*Myrtii oleum*) Üzerine Biyokimyasal Çalışmalar, Doktora Tezi, Gazi Üniversitesi Sağlık Bilimleri Enstitüsü, Ankara, 121s.
- Sıcak, Y., Çolak, Ö.F., İlhan, V., Sevindik, E., Alkan, N. 2017. Köyçeğiz Yöresinde Halk Arasında Yaygın Olarak Kullanılan Bazı Tıbbi ve Aromatik Bitkiler, Anadolu Doğa Bilimleri Dergisi, 4:2 (70-77).
- Sağiroğlu, M., Topuz, T., Ceylan, K., Turnas, M. 2013. An Ethnobotanical survey from Yahyalı (Kayseri) and Tarsus (MERSİN), AÜ Fen- Edebiyat Dergisi, 2: 13-26.
- Sargın, S. A. 2015. Ethnobotanical survey of medicinal plants in Bozyazı district of Mersin, Turkey, Journal of ethnopharmacology, 173:105-126.
- Şan, B., Karakurt, Y., Dönmez, F. 2015. Effects of Thidiazuron and Activated Charcoal on in vitro Shoot Proliferation and Rooting of Myrtle (*Myrtus communis* L.), Tarım Bilimleri Dergisi, 21: 2, dergipark.ulakbim.gov.tr
- Söke, P., Elmacı, Y. 2015. Siyah ve Beyaz Mersin (*Myrtus communis* L.) Meyvelerinin Şekerlemeye İşlenmesi, Akademik Gıda, 13: 1 (35-41).
- Tuberoso C.I.G., Rosa, A., Bifulco, E., Melis M.P., Atzeri, A., Pirisi, F. M. Dessi MA. 2010. Chemical composition and antioxidant activities of *Myrtus Communis* L. Berriesextracts,Food Chemistry, 123: 1242-1251.
- Ozek, BDA. 2000. Chemical composition of Turkish myrtle oil, Journal of Essential Oil Research, 12: 541-544.
- Öztürk, M. 1970. İzmir Yöresindeki *Myrtus communis* L.’ Nin Eko-fizyolojisi Hakkında Bir İnceleme, Ege Üniversitesi, Fen Bilimleri Enstitüsü, Doktora Tezi, İzmir.
- Tumen I, Senol FS, Orhan IE. 2012. Inhibitory potential of the leaves and berries of *Myrtus communis* L. (myrtle) against enzymes linked to neurodegenerativediseases and their antioxidant actions. International journal of Food sciences andNutrition, 63: 387-392.
- Rhizopoulou, S., 2004. Plant Culture: Symbolic Plant(s) of the Olympic Games, Journal of experimental Botany, 55: 403 (1601–1606).
- Tunçer, P. 2012. Mersin Bitkisinin (*Myrtus communis* L.) Broiler Rasyonlarında Kullanım İmkanlarının Araştırılması, Afyon Kocatepe Üniversitesi Sağlık Bilimleri Enstitüsü doktora tezi, Hayvan Besleme ve Beslenme Hastalıkları ABD, Afyonkarahisar, 96s.
- Uzun, H.İ., Aksoy, U., Gözlekçi, Ş., Yeğin, A.B., Selçuk, N. 2016. Siyah mersin (*Myrtus communis* L.)’in değişik ekolojilerde verim ve kalite özellikleri üzerine araştırmalar, Derim, 33 :2 (159-174).
- Wannes, W. A., Marzouk, B. 2013. Differences between myrtle fruit parts (*Myrtus communis* var. *italica*) in phenolics and antioxidant contents. Journal of Food Biochemistry, 37: 585-594.
- Wannes, W.A., Mhamdi, B., Sriti, J., Marzouk, B. 2010. Glycerolipid and fatty acid distribution in pericarp, seed and whole fruit oils of *Myrtus communis* var. *italic*, Industrial Crops and Products, 31: 77-83.

- Yeğin, A.B., Uzun, H.İ. 2015. Mersin (*Myrtus communis* L.) meyvelerinin fenolik bileşik içerikleri, *Derim*, 32:1 (81–88).
- Yıldırım, H. 2012. Adana ve Mersin Ekolojik Koşullarında Yetişen Mersin Bitkisinde (*Myrtus communis* L.) Bazı Bitkisel ve Pomolojik Özellikler ile Yaprak Uçucu Yağ Bileşenlerinin Belirlenmesi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü yüksek lisans tezi, Bahçe Bitkileri ABD, Adana, 110 s.
- Yıldırım, H., Kargı, S.P., Karabıyık, Ş. 2013. Adana ve Mersin Ekolojik Koşullarında Doğal Olarak Yetişen Mersin (*Myrtus communis* L.) Bitkileri Üzerinde Bir Araştırma, *Alatarım*, 12:16 (1–9), dergipark.ulakbim.gov.tr
- www.tubives.com
- www.gazeteimece.com/guncel/mersin-sus-bitkisi-merkezi-olacak-h860.html
- <https://www.antalyaborsa.org.tr/yonetim/pdf/1552015121725.pdf>
- <https://doa.ogm.gov.tr/SitePages/OGM/OGMDefault.aspx>
- http://www.ihsanozturk.com/mersin_murt_hambeles-91.html
- <http://iseo2012.fc.ul.pt/addendum-abstract-book.html>
- <https://www.antalyaborsa.org.tr/yonetim/pdf/1552015121725.pdf>
- https://arastirma.tarim.gov.tr/yalovabahce/Belgeler/bahce/sayilar/Bahce_46_1_2017_ozel_sayi.pdf
- APPENDINO, G., MAXIA, L., BETTONI, P., LOCATELLI, M., VALDIVIA, C., BALLERO, M., STAVRI, M., GIBBONS, S., STERNER, O. 2006. ANTIBACTERIAL GALLOYLATED ALKYLPHLOROGLUCINOL GLUCOSIDES FROM MYRTLE (*MYRTUS COMMUNIS*). *J. NAT. PROD.*, 69 (2): 251–254
- Appendino, G., Bianchi, F., Minassi, A., Sterner, O., Ballero, M., Gibbons, S. 2002. Oligomeric Acylphloroglucinols from Myrtle (*Myrtus communis*), *J. Nat. Prod.*, 65 :3 (334–338).
- Angioni A, Pirisi F, Caboni P, D'Aquino S, Fadda A and Schirra M, Effects of cold storage on quality traits of Sardinian myrtle (*Myrtus communis* L.) berries and their alcoholic extracts. *J Agric Sci Technol B* 1: 790–798(2011).
- Angioni A and Schirra M, Long-term frozen storage impact on the antioxidant capacity and chemical composition of Sardinian myrtle (*Myrtus communis* L.) berries. *J Agric Sci Technol B* 1: 1168–1175 (2011).
- Aidi Wannes W, Mhamdi B and Marzouk B, Variations in essential oils and fatty acid composition during *Myrtus communis* var. *italica* fruit maturation. *Food Chem* 112: 621–626 (2009).
- Angela Fadda, Amedeo Palma, Salvatore D'Aquino, Maurizio M. Effects of Myrtle (*Myrtus communis* L.) Fruit Cold Storage Under Modified Atmosphere on Liqueur Quality, 41, 1, 2017
- Angioni, A. and Schirra, M. 2011. Long-term frozen storage impact on the antioxidant capacity and chemical composition of sardinian myrtle (*Myrtus communis* L.) berries. *J. Agric. Sci. Technol. B* 1, 1168–1175.
- Angioni, A., Pirisi, F., Caboni, P., D'Aquino, S., Fadda, A. and Schirra M. 2011. Effects of cold storage on quality traits of Sardinian myrtle (*Myrtus communis* L.) berries and their alcoholic extracts. *J. Agric. Sci. Technol. B* 1, 790–798.
- Brand-Williams, W., Cuvelier, M.E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT – Food Sci. Technol* 28, 25–30.
- Belisario, A., Lio, G.M., Pane, A., Cacciola, S.O. Phytophthora iranica, a new root pathogen of myrtle from Italy, Plant disease, 1993 - cabdirect.org
- Bell, E.K. - 2011 Australian Council of University Art and Design A gust of myrtle trees and other curious proposals: engaging studio-focussed students with history and theory ACUADS Conference 2011
- BOAS, I.H. A.A.C.I. 2013. forest products research in australia, *Journal Australian Forestry*, 1, 1936: 2 (21–24)
- DEGTYAREVA, A. P.; POCHINOK, V. YA.; GORPINENKO, L. YA. 1970. Studies on some properties of an antibacterial preparation from myrtle Trudy Gosudarstvennogo Nikitskogo opytного Botanicheskogo Sada 1970 No.45 pp.146–150 ref.14
- Claudio Lubello, Riccardo Gori, Francesco Paolo Nicese, Francesco Ferrini. 2004. Municipal-treated wastewater reuse for plant nurseries irrigation *Water Research*, 38, Issue 12, 2939–2947
- Denys J. Charles 2012. Myrtle, Antioxidant Properties of Spices, Herbs and Other Sources : 409–414
- Fadda A and Mulas M, Chemical profile changes during myrtle (*Myrtus communis* L.) fruit development and ripening. *Sci Hort* 125: 477–485 (2010).
- Hacseferoullar H, Özcan MM, Arslan D and Ünver A, Biochemical compositional and technological characterizations of black and white myrtle (*Myrtus communis* L.) fruits. *J Food Sci Technol* 49: 82–88 (2012).
- Fadda, A. and Mulas, M. 2010. Chemical changes during myrtle (*Myrtus communis* L.) fruit development and ripening. *Sci. Hortic.* 125, 477–485.
- Fejýt, C., Franke, L., Appendino, G. and Werz, O. 2005. Identification of molecular targets of the oligomeric nonprenylated acylphloroglucinols from *Myrtus communis* and their implication as anti-inflammatory compounds. *J. Pharmacol. Exp. Ther.* 315, 389–396.
- Haciseferoğullari, H., özcan, M., Arslan, D. and ünver, A. 2012. Biochemical compositional and technological characterizations of black and white myrtle (*Myrtus communis* L.) fruits. *J. Food Sci. Technol.* 49, 82–88.
- KONG Jing-si, CHEN Ji-wu, WANG Bang-zheng, YAO Lei. 2011. Antibacterial comparison and GC/MS analysis of three aromatic plants *Science and Technology of Food Industry*; 11.
- XIE Jian-ying. 2013. Antioxidant activity of extracts from Myrtle root in Vitro *Food Science and Technology*, 12.

- KIRALAN, M., BAYRAK, A., ABDULAZIZ, O.F., ÖZBUCAK, T. 2012. ESSENTIAL OIL COMPOSITION AND ANTIRADICAL ACTIVITY OF THE OIL OF IRAQ PLANTS, NATURAL PRODUCT RESEARCH, FORMERLY NATURAL PRODUCT LETTERS, 26, 2012:2.
- Ludovina R. Galego, José P. Da Silva, Valentim R. Almeida, Maria R. Bronze, Luís V. Boas. 2011. Preparation of novel distinct highly aromatic liquors using fruit distillates, *Int. Jour. Of Food Sci +Tech.*, 46:1 (67-73).
- Lamia, H.; Mohsen, H.; Larbi, K. M. 2010. Evaluation of salinity tolerance in myrtle (*Myrtus communis*) during germination and the seedling stage. 88 :10 (893-900).
- Montoro P, Tuberoso CIG, Perrone A, Piacente S, Cabras P and Pizza C, Characterisation by liquid chromatography–electrospray tandem mass spectrometry of anthocyanins in extracts of **Myrtus communis** L. berries used for the preparation of myrtle liqueurs. *J Chromatogr A* 1112: 232–240 (2006).
- Mulas M and Cani MR, Germplasm evaluation of spontaneous myrtle (*Myrtus communis* L.) for cultivar selection and crop development. *J Herbs Spices Med Plants* 6: 31–49 (1999).
- Montoro P, Tuberoso CIG, Piacente S, Perrone A, De Feo V, Cabras P, *et al*, Stability and antioxidant activity of polyphenols in extract of *Myrtus communis* L. berries used for the preparation of myrtle liqueur. *J Pharmaceut Biomed Anal* 41: 1614–1619 (2006).
- Mulas M, Francesconi AHD and Perinu B, Myrtle (*Myrtus communis* L.) as a new aromatic crop: cultivar selection. *J Herbs Spices Med Plants* 9: 127–131 (2002).
- Mulas, M., Fadda, A., Angioni, A.. 2013. Effect of maturation and cold storage on the organic acid composition of myrtle fruits, *Journal of the Science of Food and Agriculture*, 93: 1(37–44)
- Tuberoso CIG, Barra A and Cabras P, Effect of different technological processes on the chemical composition of myrtle (**Myrtus communis** L.) alcoholic extracts. *Eur Food Res Technol* 226: 801–808
- Tuberoso CIG, Barra A, Angioni A, Sarritzu E and Pirisi FM, Chemical composition of volatiles in Sardinian myrtle (*Myrtus communis* L.) alcoholic extracts and essential oils. *J Agric Food Chem* 54: 1420–1426 (2006).
- Tuberoso CIG, Melis M, Angioni A, Pala M and Cabras P, Myrtle hydroalcoholic extracts obtained from different selections of *Myrtus communis* L. *Food Chem* 101: 806–811 (2007).
- Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi FM, *et al*, Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. *Food Chem* 123: 1242–1251 (2010).
- Tiryaki, G., Şahika, G., Gönül, A., Karapinar, M. 2009. Efficacy of myrtle oil against *Salmonella Typhimurium* on fresh produce *International Journal of Food Microbiology*, 130, 2, 31 147-150.
- Thomas, G. 1999. The role of research and education in site management at the Lemba Experimental Village, Cyprus, *Conservation and Management of Archaeological Sites*, 3:1-2.
- Turkyılmaz, B., Koca, I. 2017. yabani olarak yetişen bazı meyvelerin ursolik ve oleanolik asit içeriği, *international-congress-on-medicinal-and-aromatic-plants-natural-and-healthy-life-proceedings-book.pdf* may
- Tuberoso, C.I.G., Melis, M., Angioni, A., Pala, M., Cabras P. 2007. Myrtle hydroalcoholic extracts obtained from different selections of *Myrtus communis* L. 2007. *Food Chemistry*, 101: 2 (806-811).
- Usai, M., Mulas, M., Marchetti, M. 2015. Chemical composition of essential oils of leaves and flowers from five cultivars of myrtle (*Myrtus communis* L.) *Journal of Essential Oil Research*, 27, 6, 2015 (465-476).
- Vacca, V., Piga, A., Del Caro, A., Paolo A. M., Fenu, P.A.M., Agabbio, M. 2003. Changes in phenolic compounds, colour and antioxidant activity in industrial red myrtle liqueurs during storage, *Molecular Nutrition & Food Research*, 47: 6 (442–447)

PHARMACOGNOSTIC FEATURES OF THE AERIAL PARTS OF THE MEDICINAL plant *Echinops spinosus* L.

Amel BOUZABATA¹

¹Faculty of Medicine, City of Zaafrania BP 205, Annaba, BP23000. Algeria.

Corresponding author email: amelbouz2009@gmail.com

Abstract

The genus *Echinops* (Asteraceae family, Echinopeae class) comprises ca. 120 species, and is native to Africa, the Middle East, Europe, and Asia. In Algeria, this genus is represented by the very common species *Echinops spinosus* L., also known as «tesskra», which is used as a diuretic, hypoglycemic, for liver disorders, and for post-partum care and for its stomach effects. The aim of this work is to generate pharmacognostic parameters of discriminating *Echinops* powders. The present study reports the detailed pharmacognostic characters of the different parts of *E. spinosus*, using microscopic methods, coupled with statistical techniques, to analyze the partition of the structure observations. This study revealed that microscopic analysis, coupled with statistical analysis, could provide a simple platform for medicinal plant identification, particularly for the quick identification of a drug powder, and may be helpful in developing pharmacopoeial standards in traditional medicine.

Keywords: *Echinops*, Algeria, pharmacognostic characters, microscopic analysis, statistics.

INTRODUCTION

The genus *Echinops* (Asteraceae family, Echinopeae class) comprises ca. 120 species, and is native to Africa, the Middle East, Europe, and Asia (Halim et al., 2011). *Echinops spinosus* L. is very common in Algeria, where it is known as «tesskra», and is used as a diuretic, hypoglycemic, and for its stomachic effect, against liver disorders, and for post-partum care (Bellakhdar et al., 1991; Baghdad et al., 2016). Two subspecies are established: *E. spinosus* ssp. *eu. spinosus* Maire (var. *chaetocephalus* Pomel), and *E. spinosus* ssp. *bovei* (Boiss.) Maire (var. *pallens* Maire.), which is also known as *E. bovei* Boiss. (Dobignard and Chatelain, 2011).

Many studies on *E. spinosus* have been carried out in different research fields. However, there is no report on its botanical investigation. The only related study available focused on the microscopic identification of *E. albicaulis* Kar. & Kir. (Kiyekbayeva et al., 2017). Therefore, microscopic analyses were attempted for the purpose of identifying characteristic features of *Echinops spinosus* subsp. *bovei* (Boiss.) Murb., which could serve as morphological markers for authentication.

MATERIAL AND METHODS

Sampling: *Echinops spinosus* subsp. *bovei* (Boiss.) Murb. was provided from El Tarf district, situated in the North Eastern Algeria, during May, 2017. The voucher specimens of the plant was deposited at the herbarium of the conservatory and Botanical Garden, Geneva under reference number G00403753. The identification of the species was carried out by Dr. G. Debelaire, by correlating the morphological characters with those described in the literature.

The different plant parts were dried in the sun. The dried and cured organs were crushed into a powder and the powder suspended in water and subjected to microscopic evaluation. The organoleptic characters of the dried powder, like colour, odour, and taste, and the microscopic characters were evaluated according to the standard WHO guidelines (WHO, 1999).

Mounting: the powdered material was placed on the slides. Lactic acid (1-3 drops) was added and the materials stirred with a fine pointed needle to distribute the testing agent evenly. The mixture was covered with a glass slip, and any excess liquid exuding from under the cover slip was removed

by blotting around its edges gently with filter paper. Lactic acid provided a yellow color for all the lignified elements, and a red-orange color for the secretion products, such as the resins and essential oil. Each sample was observed with an Amscope microscope B120b-9M equipped with Amscope 3.7.7303 software for digital cameras (2003-2016), including 16 observations for leaf sample [1L-16L], 19 observations for flower samples [17F-35F], and 15 observations for root sample [36R-50R].

Statistical Analysis: Statistical tests used in this study were analyzed using SPSS Statistics 17.0 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA, 2008). Multivariate tests were used to determine the complex relationship among variables. Fourteen microscopic features were analyzed by correspondence analysis. Analyses examined the relationships between the fifty observations and the associations between variables in two dimensions. Additionally, similar microscopic observations were identified from their positions, with respect to the axes, and the underlying features in the noticed pattern. Finally, the observed patterns were explained based on key features in the microscopic observation.

RESULTS AND DISCUSSION

The diagnostic features of the leaf powder are given in Fig. 1. A greenish powder, it has an aromatic odour and a slightly bitter and aromatic taste.

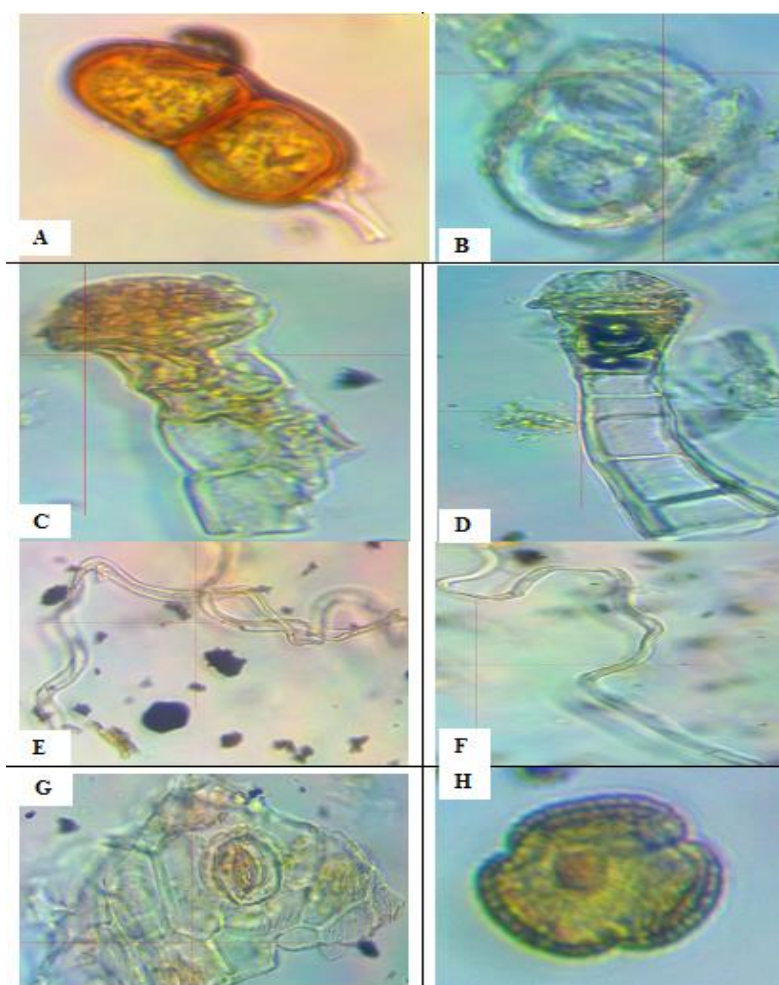


Fig. 1. The diagnostic features of *E. spinosus* leaves.

A: glandular trichome with unicellular stalk and a bicellular gland, B: glandular trichome, C, D: glandular trichome, E, F: Long covering trichome, uniseriate, unicellular, G: fragment of epidermis with cuticular striation and anomocytic stomata, H: tricoporate pollen grain

The leaf powder is characterized by the abundance of glandular trichomes having a unicellular stalk and bicellular head. In addition, different types of glandular trichomes were observed: *i)* with a uniseriate stalk and unicellular gland, *ii)* with a biseriate stalk and multicellular gland. This powder is also characterized by the presence of large covering trichome. Cells of the epidermis are polygonal, showing cuticular striations and anomocytic stomata. The amount of stomata index underlying ten observations on a 1 mm² area of the upper epidermis is 29.60 ± 5.64 .

Pollen grains are fairly small, spherical, with three pores; the exine is finely warted. Prisms of calcium oxalate, and very small cluster crystals of calcium also occur.

The diagnostic features of the flower powder are presented in Fig. 2. A light greenish powder, with a characteristic aromatic odour and a slightly bitter and aromatic taste.

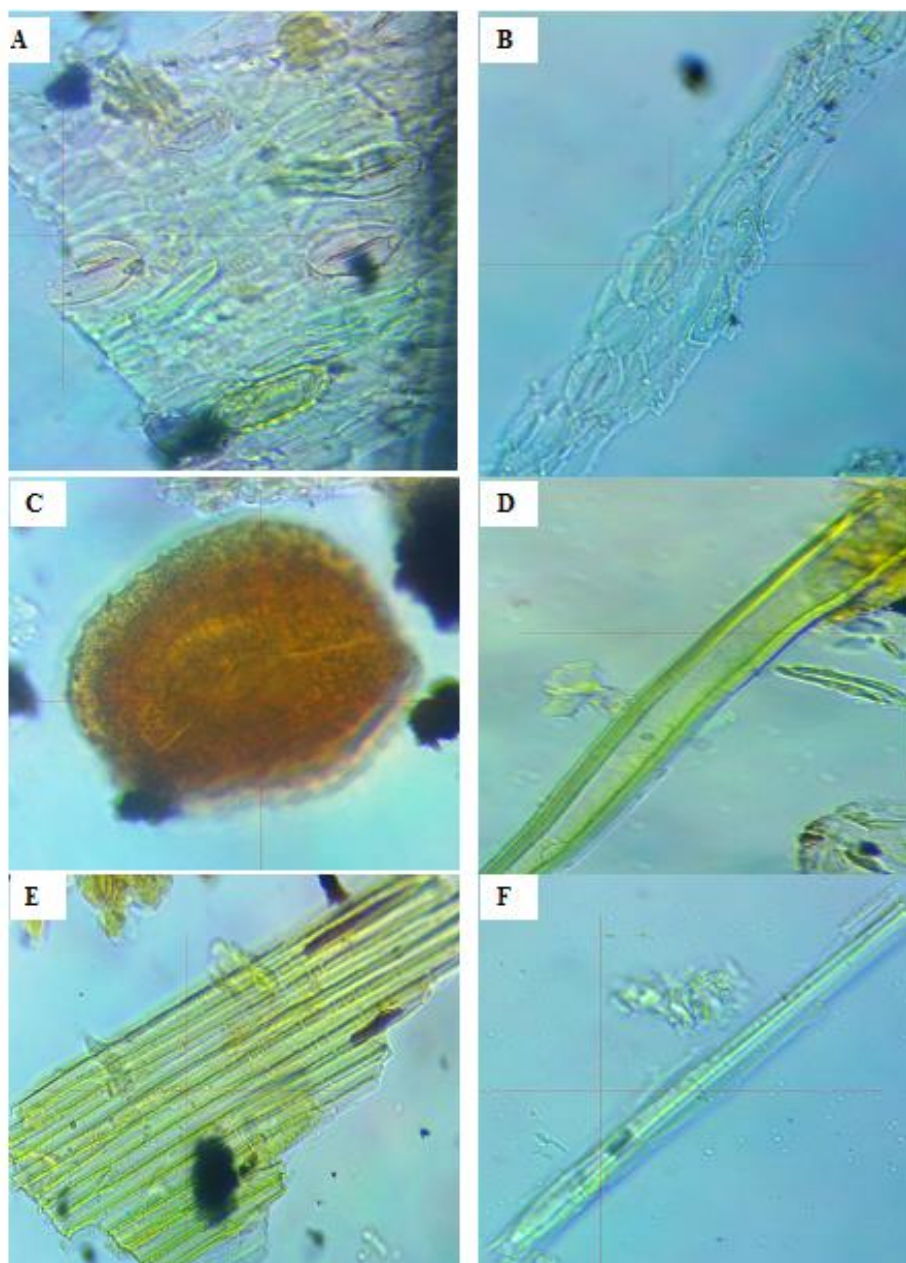


Fig. 2. The diagnostic features of *E. spinosus* flowers.

A, B: inner epidermis from near the central region of a bract in surface view showing anomocytic stomata, *C:* oil resin cell, *D:* covering trichome uniseriate, unicellular, *E:* part of a group of fibrous cells, *F:* acicular crystals of calcium oxalate.

The flower powder is characterized by the abundance of a group of fibrous cells, cells containing oleo-resin and a small covering trichome. Moreover, acicular crystals of calcium oxalate and large covering trichome were observed, which are rare morphological elements. Cells of the epidermis are elongated showing stomata from the margin region. Large trichome is also present rarely.

The diagnostic features of the root powder are presented in Fig. 3. A brownish powder, with a characteristic aromatic odour and an astringent taste.

The diagnostic characters are:

The very abundant sclereids vary considerably in size and shape, and two types can be distinguished: one type is rectangular to ovale in outline and heavily thickened with a small lumen. However, the very abundant ones are more elongated with moderately thickened and striated walls with numerous pits and are developed as *fibrous sclereids*.

Covering trichomes also occur, are unicellular, thin-walled, shortly conical with a swollen base, and are numerous. Occasionally, long covering trichomes, uniseriate and unicellular, were observed. Fairly abundant crystals of calcium oxalate, in acicular form, were found scattered. Very occasional fragments of cork in surface view, the cells are thin-walled and polygonal form. Very occasional groups of small, lignified vessels with spiral or annular thickening were noted.

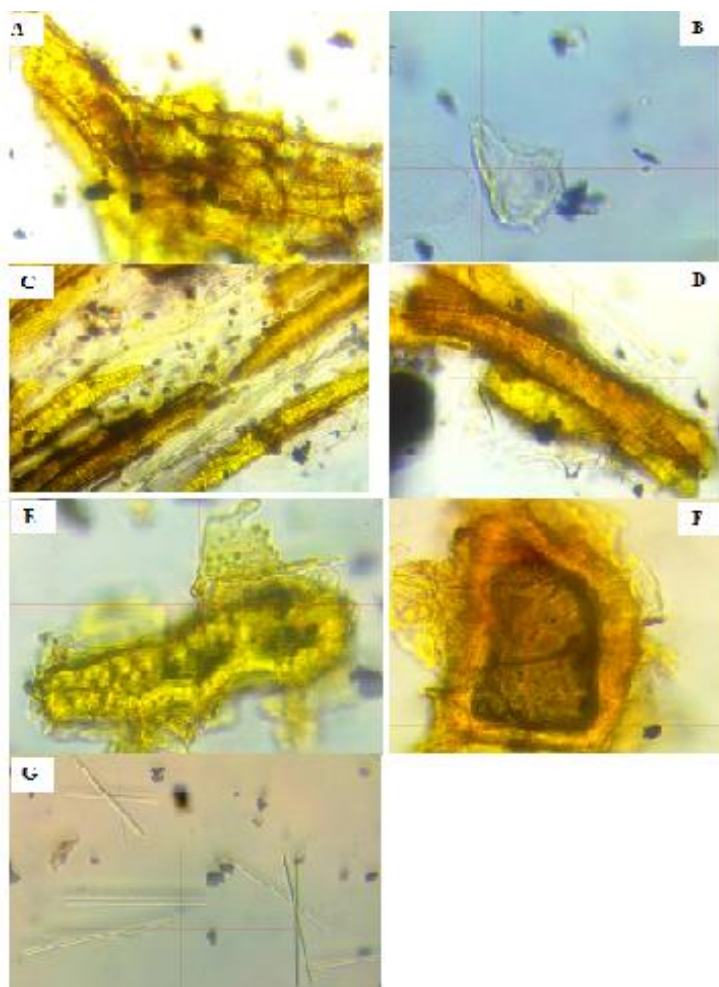


Fig. 3. The diagnostic features of *E. spinosus* roots.

A: fragment of cork, B: conic covering trichome, C: part of a group of sclereids fibres, D: an isolated fibrous sclereid, E- F: an isolated sclereid, G: acicular calcium oxalate crystals.

On the other hand, for multivariate analysis, the two axes explained 22.26% of the observed variations. The X axis and the Y axis accounted for 25.3% and 19.22% of the variations, respectively. The coefficients represent the correlation with the two axes. The sclereid fibers were positively linked on the first factor (0.600), but weakly related to the second factor (0.090). In contrast, it was observed that the fiber and stomata showed a positive link on the second factor, and were loaded weakly on the X axis (0.488/0.425 vs. 0.001/0.025) (Fig. 4). In addition, the long covering trichomes showed a positive link on the X and Y axes (0.227/0.373).

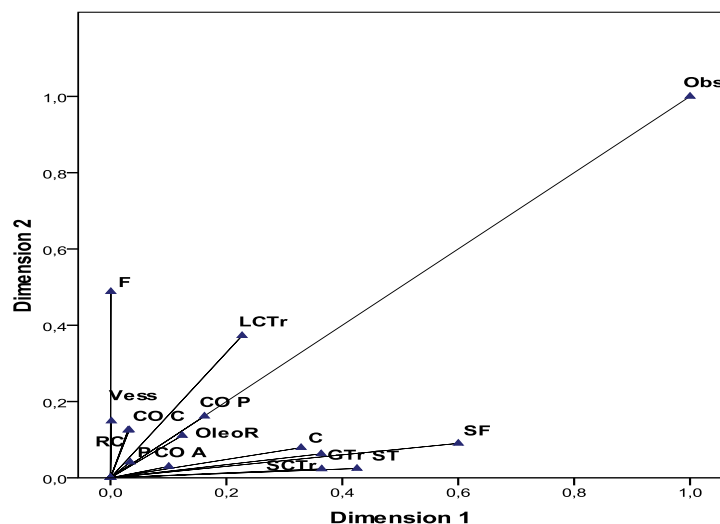


Fig. 4. Projections of fourteen analyzed parameters in the two axes (X and Y).

CO: cork, **Vess:** vessels, **Oleo R:** oleo resin, **LCTr:** long covering trichomes, **SCTr:** short covering trichomes, **GTr:** glandular trichomes, **PO:** pollen, **F:** fibers, **CO P:** prisms of calcium oxalate, **CO C:** clusters of calcium oxalate, **CO A:** acicular of calcium oxalate, **St:** stomates, **SF:** sclereid fibers, **Rcell:** cells of resin, **Obs:** Observation.

The two-dimensional projection of the matrix is plotted in Fig. 5, and the position of observation depicted underlying the analyzed parameters. Two groups have been distinguished on the basis of the presence of sclereid fibers. Group I is characterized by the lack of sclereids and included 39 observations [1L, 40R, 3L, 37R, 39R, 41R, 6L, 4L, 2L, 13L, 8L, 12L, 10L, 5L, 14L, 11L, 9L, 16L, 15L, 32F, 25F, 33F, 50R, 31F, 22F, 7L, 17F, 34F, 20F, 21F, 23F, 26F, 24F, 18F, 27F, 35F, 19F, 29F, 30F, and 28F]. Group II is characterized by the presence of a sclereids, including 11 observations 46R, 44R, 43R, 42R, 36R, 38R, 45R, 48R; 47R, 48R, and 49R. However, two subgroups were distinguished in Group II, related to the presence or the absence of fiber. In this way, the fibers were absent in all observations of the subgroup I [1L, 40R, 3L, 37R, 39R, 41R, 6L, 4L, 2L, 13L, 8L, 12L, and 10L].

According to the World Health Organization (WHO, 1999), the macroscopic and microscopic description of a plant is the first step to establish the identity and the degree of purity of such materials and should be carried out before any tests are undertaken (Sharma and Pracheta, 2013). This study is an attempt to establish the diagnostic characteristics of **E. spinosus L.** The present microscopic observation of different parts of the plant, coupled with statistical analysis provides useful information for developing the quality control parameters for the crude drug. In the first step, the microscopic studies of the leaf powder showed the presence of covering trichome in different shape and size; and glandular trichomes.

These results are in agreement with Kiyekbayeva et al. (2017) who identified long covering trichome in the lower epidermis of *E. albicaulis* Kar. & Kir. leaf. The stoma type was also identified in the lower epidermis as an anomocytic type. In addition, needle-shaped crystals were also found in the form of rafids, and was also identified in the leaf of *E. spinosus* L. However, no glandular trichome was identified in the leaf of *E. albicaulis*, which may serve as a microscopic marker for identification.

In the second step of this study, microscopic features of the flowers and roots were identified. In this determinations, specific characters were found: special cells with oleoresin in flowers, and sclereids in the roots with different shape and size.

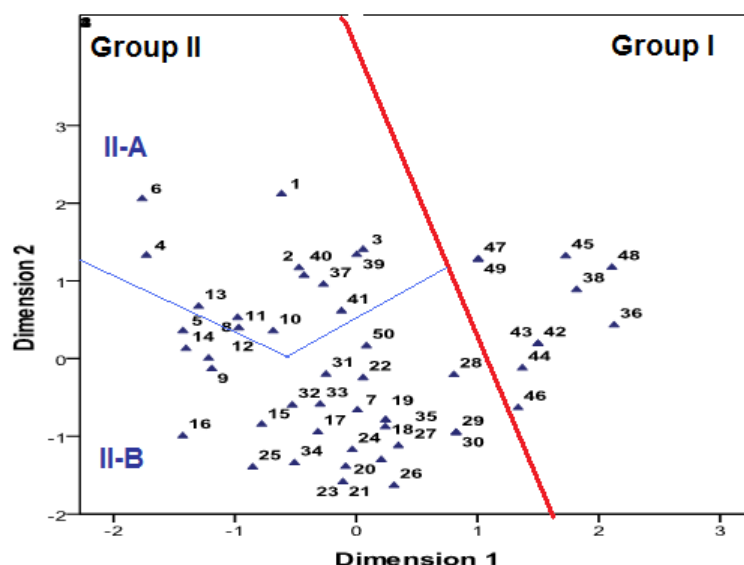


Fig. 5. Projection of matrix of the position of observation.

The results showed that the two groups of samples could be differentiated according to the presence of fibers and sclereids. Moreover, it appeared that the presence of fibers is a discriminating parameter of the group II, and served to differentiate between subgroups. Indeed, this criterion was absent the subgroup II-1.

CONCLUSION

Fifty morphological observations were analyzed from three organs of *E. spinosus* (leaves, flowers and roots). Fourteen key microscopic features were identified.

Multivariate analysis suggested the existence of two groups of samples distinguished on the basis of the presence or the lack of the sclereids. In addition, the fibers were the key feature to identify the subgroups. This finding revealed that microscopic analysis, coupled with statistical analysis, could provide a platform for plant identification, particularly in the authentication of samples of *E. spinosus* and may be extended to other medicinal plant samples (Bouzabata, 2015).

REFERENCES

- Baghdad, M., Boumediene, M., Djamel, A., Djilali, B., 2016. Ethnobotany of medicinal plants in the region Béni chougane (Mascara, Algeria), Journal of Biodiversity and Environmental Sciences, 9(1): 426-433.
- Bellakhdar, J., Claisse, R., Fleurentin, J., Younos C., 1991. Repertory of standard herbal drugs in the Moroccan pharmacopoeia, Journal of Ethnopharmacology, 35:123-143.
- Dobignard A., Chatelain C., 2011. Index synonymique de la flore d'Afrique du Nord. In: Des conservatoire et jardin botanique (ed.), volume 2, Genève. p. 258.
- Halim, A.F., Afify, M.S., Ahmed, A.F., Mira, A.S., 2011. The fact about echinopsine and first isolation of echinorine from *Echinops spinosus* L., Journal of Environmental Sciences, 40: 173-181.
- Kiyekbayeva, L.N., Akhtaeva, N.Z., Datkhayev, U.M., Omarkhan, A.B., Litvinenko, Y.A., Tynybekov, B.M., Berkenov, A.K., 2017. Pharmacognosy signs of aerial parts of medicinal plant *Echinops albicaulis* Kar et Kir., Asian. Journal of Pharmaceutical and Clinical Research, 10(6): 346-348.
- Sharma, V., Pracheta, V., 2013. Microscopic studies and preliminary pharmacognostica evaluation of *Euphorbia neriifolia* L. leaves, Indian Journal of Natural Products Research, 4(4): 348-357.
- Bouzabata A., 2015. Microscopic analysis of *Curcuma longa* using multivariate test. International Journal of Pharmacognosy, 2(4): 173-177.

World Health Organization (WHO), 1999. Monographs on Selected Medicinal Plants. Volume 1. Geneva.

EFFECTS OF FOLIAR ZINC AND HUMIC ACIDE APPLICATIONS ON THE ESSENTIAL OIL QUALITY AND SEED NUTRIENT CONTENTS OF BLACK CUMIN (*Nigella sativa* L.)

Zehra AYTAÇ¹, Nurdilek GÜLMEZOĞLU², Mine KÜRKÇÜOĞLU³

¹ Eskisehir Osmangazi University, Faculty of Agriculture, Department of Field Crops, Eskisehir, Turkey

² Eskisehir Osmangazi University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Eskisehir, Turkey

³ Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, Eskişehir

Corresponding author e-mail: zehrak@ogu.edu.tr

Abstract

Foliar zinc fertilization is important in order to increase seed zinc which is important for human nutrition. In addition, humic acid improves soil properties to increase plant yield and quality. Black cumin is one of the important medicinal plants which are also consumed as spice. A field experiment was carried out during 2016 and 2017 at the Faculty of Agriculture of Eskisehir Osmangazi University, Turkey. One *Nigella sativa* L. cultivar was used as material to study the effect of different doses of foliar zinc (0.3-0.6-0.9 %) with the incorporation of foliar humic acid (0.2 %) applications on the essential oil quality nitrogen (N) and zinc (Zn) concentrations (%) of the seeds. According to the results, significant differences were found between the foliar applications. The essential oil content was between 0.49-0.67% in both years and the highest values were obtained from 0.9% Zn and 0.2 % HA + 0.3 % Zn leaf fertilization. Both years, main compounds of the essential oil were p-Cymene and alfa-Thujene. The highest concentration of Zn in the seed was obtained from 0.9 % Zn application while there were no differences between the applications in terms of seed N concentration.

Keywords: *Nigella sativa* L., zinc, nitrogen, humic acid, essential oil components

INTRODUCTION

Black Cumin (*Nigella sativa* L.) is an annual herbaceous spice plant which is used for centuries for medicinal and culinary purposes. The seeds are used as food preservative to flavour sauces, pickles, meat dishes; sprinkled on bread and cheese (Ipore and Oyen, 1999). Black Cumin essential oil and extracts have antioxidant (Burits ve Bucar 2000), antidiabetic (Mathur et.al. 2011), antihistaminic (Kanter et.al. 2006), antihypertensive (Dehkordi and Kamkhah 2008), antiinflammatory, analgesic (Al-Ghamdi, 2001), antimicrobial (Hanafy et.al.1991) and antitumor effects (Musa, 2004).

Zinc (Zn) deficiency is affecting nearly one-third of the world population which produces yield losses in plants and causes a low accumulation of Zn in the edible parts and seeds of plants. Zinc fertilization correct Zn deficiencies of soil and is a practical method to the improvement of Zn concentration in plant parts. In addition it is important in order to increase seed zinc which is important for human nutrition (Cakmak et.al.1999; Welch and Graham, 2004).

Humic acids, a naturally occurring polymeric organic compound, is a natural resource found in soil, peat and lignites produced by the decay of organic materials in soil and it is utilized as an alternative for increasing crop production (Sharif et al., 2002). Humic acids have beneficial effects on nutrient uptake by plants and is useful for the transport and availability of micronutrients (Bohme and Thiua, 1997).

The aim of the study was to determine the effect of different foliar Zn application doses under the conditions of with and without foliar humic acid fertilization on the essential oil content, essential oil composition, nitrogen (N) and zinc (Zn) concentration (%) of Black Cumin (*Nigella sativa* L.) seeds.

MATERIAL AND METHODS

A field experiment was carried out during 2016 and 2017 at the Faculty of Agriculture of Eskisehir Osmangazi University, Turkey. One *Nigella sativa* L. cultivar (Çameli 2015) was used as material. The climatic data of both year and long term are shown at Table1.

Table 1. Mean temperature, total rainfall, relative humidity of Eskisehir (2016, 2017 and long term).

	2016	2017	Long term
Mean Temperature ($^{\circ}\text{C}$)	11.8	11.3	10.9
Total Rainfall (mm)	365.9	374.4	355.9
Relative Humidity (%)	71.5	73.0	64.5

The soil of the experimental area was slightly alkaline (pH: 8.06-8.00), loamy textured and calcareous with a low content (1.10-0.5 %) of organic matter. The phosphorus content of the soil was low (20.0-49.4 kg ha⁻¹) but the potassium content was high (2530-2996 kg ha⁻¹). There was no salinity (0.04-0.05 %) problem and the soil was low in Zn content (0.49- 0.48 %).

Seeds of black cumin were sown to the research field, using a seed rate of 15 kg ha⁻¹ at the end of March both year (2016 and 2017) to 30-cm-spaced lines on a well-prepared seed bed. The experiment was arranged as a randomized complete block design with three replications. At sowing, phosphorus (P₂O₅) and nitrogen (N) were applied at a standard rate (50 kg ha⁻¹ P₂O₅, 60 kg ha⁻¹ N). The plants were irrigated once during flowering stage. The weeds were controlled by hand weeding. Harvest were performed manually at the beginning of August. After harvesting, the plants were dried until threshing.

Foliar Zn and Foliar Humic acide (HA) treatments

- 1 Control
- 2 0.3 % ZnSO₄.7H₂O
- 3 0.6 % ZnSO₄.7H₂O
- 4 0.9 % ZnSO₄.7H₂O
- 5 0.2 % humic acid
- 6 0.2 % humic acid+0.3 % ZnSO₄.7H₂O
- 7 0.2 % humic acid+0.6 % ZnSO₄.7H₂O
- 8 0.2 % humic acid+0.9 % ZnSO₄.7H₂O

Foliar Zn treatment was done 3 days after HA application. Humic acide source was HUMAS 25 cl (TKI) which contains 25 % total organic matter, 12% (H+F), 2 % Potassium oxide. Zinc source was ZnSO₄.7H₂O (23 % Zn). All spraying was done using Tween 20 as surfactant containing 0.01% (w/v) (Polyoxyethylenesorbitan monolaurate). Spraying (treatment) times were: pre-flowering stage; seed filling stage and harvest stage.

Seed N and Zn concentration: Approximately 10 g of each sample were taken for each treatment, dried at 65°C, grounded and incinerated at 550°C. The incinerated samples were dissolved in 3.3 % HCl and then filtered and diluted to a volume of 100 mL with ultra-pure distilled water. The protein content (N x 5.7) were based on the Kjeldahl method. Zinc concentration were analyzed by Atomic Absorption Spectrometer (Analytic-Jena novAA 350).

Essential oil content: Seed samples from each treatment were distilled with Neo-clevenger apparatus for about 1.0 hour and the results of essential oil content (%) were found volumetrically.

Essential Oil Components: The essential oil samples were analyzed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) using an Agilent GC-MSD system (Mass Selective Dedector-MSD). All component was analysed except Thymoquinone.

The GC-MS analysis were carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and

programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from m/z 35 to 450.

The GC analysis was carried out using an Agilent 6890N GC system. In order to obtain same elution order with GC/MS, simultaneous injection was done by using same column and inappropriate operational conditions. FID temperature was 300°C.

The components of essential oils were identified by comparison of their mass spectra with those in the Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007), MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty and Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes. Alkanes were used as reference points in the calculation of relative retention indices (RRI) (Curvers et.al., 1985). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

Statistical Analysis: All data were subjected to an analysis of variance (ANOVA) for each character. Mean differences were tested with a least significant difference (LSD) test at a $P < 0.05$ and $P < 0.01$ significance levels.

RESULTS AND DISCUSSION

The results of essential oil content (%) of black cumin seeds obtained from each experimental year were given at Table 2. The results of ANOVA showed significant ($P < 0.01$) differences of foliar Zn doses, and under the conditions of with and without foliar humic acid and only humic acid treatments in 2017 and both year (2016/2017). The first experimental year (2016) showed no significant differences. As it seen on Table 2, essential oil content changed between 0.45-0.67 % both year. The essential oil content (%) of 0.9 % foliar Zn and foliar HA + 0.3 % Zn treatments were found higher both year. Aiyafar et.al (2015) found also higher essential oil content with the increased doses of humic acid and Rezaei-Chiyaneh et.al. (2018) found enhanced essential oil percentage by foliar zinc application compared with control.

The results of Zinc concentration (%) of black cumin seeds were given at Table 2. The results of ANOVA showed significant ($P < 0.01$) differences for foliar Zn doses, and under the conditions of with and without foliar humic acid and only humic acid treatments in 2016, 2017 and both years (2016/2017). Seed zinc concentration changed between 64.5-124.7% both year. The Zn concentration (%) of foliar 0.9 % Zn treatment were the highest both years.

Takruri (1998) studied the nutritional value of some samples of black cumin seeds and found similar amounts compared to the results of the control samples (Table 2).

Table 2. Effects of foliar Zn doses, foliar humic acid and foliar HA+Zn combinations on the seed essential oil content and seed zinc concentration of black cumin.

<i>Treatments</i>	Essential oil (%)			Seed zinc concentration (%)		
	2016	2017	2016/2017	2016	2017	2016/2017
<i>Control</i>	0.45	0.48cd	0.47cd	67.0c	62.0c	64.5d
<i>Zn0.3</i>	0.45	0.45d	0.45d	110.4b	105.4b	107.9bc
<i>Zn0.6</i>	0.57	0.62abc	0.60abc	116.5ab	111.5b	114.0b
<i>Zn0.9</i>	0.65	0.69a	0.67a	127.2a	122.2a	124.7a
<i>0.2 % HA</i>	0.50	0.52bcd	0.51bcd	69.7c	64.7c	67.2d
<i>0.2 % HA+Zn0.3</i>	0.60	0.64ab	0.62ab	107.0b	102.0b	104.5c
<i>0.2 % HA+Zn0.6</i>	0.53	0.52bcd	0.53bcd	112.4b	107.0b	109.7bc
<i>0.2 % HA+Zn0.9</i>	0.50	0.48cd	0.49bcd	111.7b	106.7b	109.2bc
Mean	0.53	0.55		102.7	97.69	
<i>F test</i>						
<i>Treatment (T)</i>	n.s	**	**	**	**	**
<i>Year (Y)</i>	-	-	n.s.	-	-	**
<i>T x Y</i>	-	-	**	-	-	n.s

The results of N concentration (%) of black cumin seeds were given at Table 3. The results of ANOVA showed no differences for foliar Zn doses, foliar humic acid and foliar HA+Zn combinations in 2016, 2017 and both years (2016/2017).

Table 3. Effects of foliar Zn doses, foliar humic acid and foliar HA+Zn combinations on the seed N concentration (%) of black cumin.

<i>Treatments</i>	N concentration (%)		
	2016	2017	2016/2017
<i>Control</i>	3.53	3.43	3.48
<i>Zn0.3</i>	3.59	3.46	3.52
<i>Zn0.6</i>	3.72	3.48	3.60
<i>Zn0.9</i>	3.71	3.48	3.59
<i>0.2 % HA</i>	3.77	3.66	3.71
<i>0.2 % HA+Zn0.3</i>	3.81	3.51	3.66
<i>0.2 % HA+Zn0.6</i>	3.91	3.61	3.76
<i>0.2 % HA+Zn0.9</i>	3.47	3.13	3.30
Mean	0.53	0.55	
<i>F test</i>			
<i>Treatment (T)</i>	n.s	n.s	n.s
<i>Year (Y)</i>	n.s	n.s	n.s.
<i>T x Y</i>	n.s	n.s	n.s

The effect of foliar Zn doses, foliar humic acid and foliar HA+Zn combinations on the main essential oil composition were shown at Table 4. The main essential oil components of black cumin were *p*-Cymene (51.9 to 62.0 %) and α -Thujene (11.3 to 19.2 %). The treatments caused an increase on *p*-Cymene rates whereas they show decreases on α -Thujene rates both years.

Table 4. Effects of foliar Zn doses, foliar humic acid and foliar HA+Zn combinations on the essential oil composition* (%) of black cumin.

		2016								IM
RRI	Compounds	Control	HA	Zn0.3	Zn0.6	Zn0.9	HA+Zn0.3	HA+Zn0.6	HA+Zn0.9	
1035	α -Thujene	14.8	13.4	14.1	12.5	13.4	14.5	11.3	14.2	MS
1280	<i>p</i> -Cymene	55.9	60.8	61.1	62.0	60.7	59.7	59.4	58.9	t _R , MS
		2017								IM
1035	α -Thujene	18.4	16.4	16.7	17.4	17.6	16.0	15.1	19.2	MS
1280	<i>p</i> -Cymene	51.9	53.8	50.8	52.1	52.7	55.4	56.1	52.2	t _R , MS

RRI : (Relative retention indices) calculated against n-alkanes, %: calculated from FID (Flame ionization detection) data; IM, identification method: t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data)

*All component were analysed except Thymoquinone.

REFERENCES

- Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publ. Corp, Carol Stream, IL.
- Aiyafar, S., Minab Poudineh, H., Forouzandeh, M., 2015. Effect of Humic Acid on Qualitative and Quantitative Characteristics and Essential Oil of Black Cumin (*Nigella sativa* L.) under Water Deficit Stress. J. Sci, 4(2), 2277-5641.
- Al-Ghamdi, M. S. 2001. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*,” Journal of Ethnopharmacology, vol. 76, no. 1, pp. 45-48.

- Bohme, M., Thi Lua H., 1997. Influence of mineral and organic treatments in the rhizosphere on the growth of tomato plants. *Acta Horticulturae* 450: 161–168.
- Burits, M., Bucar, F., 2000. Antioxidant activity of *Nigella sativa* essential oil, *Phytotherapy Research*, vol. 14, no. 5, pp. 323-328.
- Cakmak I, Kalayci M, Ekiz H, Braun HJ, Yilmaz A.,1999. Zinc deficiency as an actual problem in plant and human nutrition in Turkey: A NATO-Science for Stability Project. *Field Crops Research* 60: 175-188.
- Curvers, J., Rijks, J., Cramers, C., Knauss, K., Larson, P., 1985. Temperature pro-grammed retention indexes: calculation from isothermal data. Part 1: Theory. *Journal of High Resolution Chromatography* 8, 607–610.
- Dehkordi, F. R., Kamkhah, A. F., 2008. Antihypertensive effect of *Nigella sativa* seed extract in patients with mild hypertension, *Fundamental and Clinical Pharmacology*, vol. 22, no. 4, pp. 447-452.
- Hanafy, M. S. M., Hatem, M. E., 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin), *Journal of Ethnopharmacology*, vol. 34, no. 2, pp. 275-278.
- Hochmuth, D. H., 2008. MassFinder-4, Hochmuth Scientific Consulting, Hamburg, Germany.
- Ipor, I. B., Oyen, L. P. A., 1999. *Nigella sativa* L., Plant resources of south-east Asia, pp. 148-151.
- Kanter, M., Coskun, O., Uysal, H. 2006. The antioxidative and antihistaminic effect of *Nigella sativa* and its major constituent, thymoquinone on ethanol-induced gastric mucosal damage, *Archives of Toxicology*, vol. 80, no. 4, pp. 217-224.
- Mathur, M. L., Gaur, J., Sharma, R., Haldiya, K. R., 2011. Antidiabetic properties of a spice plant *Nigella sativa*, *Journal of Endocrinology and Metabolism*, vol. 1, no. 1, pp. 1-8,
- McLafferty, F.W., Stauffer, D.B., 1989. *The Wiley/NBS Registry of Mass Spectral Data*, J.Wiley and Sons: New York.
- Musa, D., Dilsiz, N., Gumushan, H., Ulakoglu, G., Bitiren, M., 2004. Antitumor activity of an ethanol extract of *Nigella sativa* seeds, *Biologia Bratislava*, vol. 59, no. 6, pp. 735-740.
- Rezaei-Chiyaneh, E., Rahimi, S., Rahimi, A., Hadi, H., Mahdavia, H., 2013. Response of Seed Yield and Essential oil of Black Cumin (*Nigella sativa* L.) Affected as Foliar Spraying of Nano-fertilizers.
- Sharif, M., Khattak, R. A., Sarir M. S., 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. *Communication in Soil Science and Plant Analysis* 33: 3567–3580.
- Takruri, H. R., Dameh, M. A.,1998. Study of the nutritional value of black cumin seeds (*Nigella sativa*L). *Journal of the Science of Food and Agriculture*, 76(3), 404-410.
- Welch RM, Graham RD.,2004. Breeding crops for enhanced micronutrient content. *Plant Soil* 245:205–214.