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EFFECTS OF HUMIC ACID AND PHOSPHORUS APPLICATIONS ON NUTRIENT COMPOSITION OF LENTIL (*Lens culinaris* Medic.)

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ABSTRACT

Lentil (*Lens culinaris* Medic.) is an important food crop with high protein content. This study aimed to determine the effects of increasing doses of phosphorus and humic acid applications on P, K, Ca, Mg, Fe, Cu, Mn, Zn, protein ratio and seed yield in lentil cultivar. The study was carried out in the research and application during the 2008–2009 and 2009–2010 cropping seasons in field of Agriculture Faculty Yuzuncu Yil University, Turkey. The experiment comprised a factorial randomised complete block design replicated three times with three phosphorus treatments 0, 40 and 80 kg ha^{-1}, as triple superphosphate, and three humic acid treatments 0, 300 and 600 kg ha^{-1}. For basic fertilisation 40 kg ha^{-1} ammonium sulphate were applied for nitrogen. The nutrient contents of seed and yield were significantly increased by increasing phosphorus and humic acid levels except for Zn content nutrient. The combination between phosphorus at 80 kg P_2O_5 ha^{-1} and humic acid at 600 kg ha^{-1} gave the highest growth and production as well as minerals composition (P, K, Ca, Mg, Fe, Cu and Mn) and protein content compared to other treatments. Humic acid increased the availability of phosphors, macronutrients and micronutrients.

Keywords: nutrient content, lentil, humic acid, phosphorus.

AIMS AND BACKGROUND

The lentil, a legume crop, fixes atmospheric nitrogen in the root-nodules in a symbiotic relationship with Rhizobium bacteria. The amount of nitrogen released into the soil through the symbiotic cycle is 84 kg ha^{-1} per annum[1]. Lentil is the second leading

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grain legume crop after chickpea in Turkey. Lentil is commonly grown as a rotation crop in east and southeast of Turkey. It is an important crop because of its high protein content of seed and straw for human and animal nutrition. Phosphorus is one of the most important elements significantly affecting plant growth and metabolism[2]. Phosphorus may be a critical constraint of legumes under low nutrient environments because there is a substantial need for phosphorus in the N_2 fixation process[3]. The high requirement for phosphorus in legumes is consistent with the involvement of phosphorus in the high rates of energy transfer that must take place in the nodule. Phosphorus causes early ripening in pants, decreasing grain moisture, improving crop quality and is the most sensitive nutrient to soil pH (Ref. 4). Humic acid is produced by the chemical and biological decomposition of organic material through the help of micronutrients. It improves soil fertility and increases the availability of nutrient elements by holding them on mineral surfaces. The humic substances are mostly used to remove or decrease the negative effects of chemical fertilisers from the soil and have a major effect on plant growth, as shown by many scientists[5]. It increases cation exchange capacity and enhances soil fertility, converting the mineral elements into forms available to plants[6]. For instance, humic acid caused increases in length and dry weight of plant roots and enhanced the uptake of nitrogen, phosphorus, K^+, Ca_2^+, Cu_2^+, Mn_2^+, Zn_2^+ and Fe_3^+ (Ref. 7). In the absence of organic matter and calcareous soils, the uptake of nutrients by the plants decreases and thus some losses occur in the yield and yield components in dry land. In addition, the plants cannot benefit from the fertilisers applied. Thus, the application of organic fertilisers increased quality components also[8]. Thus, this study was conducted in order to analyse the effect of different doses of phosphorus and humic acid applications on yield and nutrient contents of seed in lentil.

EXPERIMENTAL

Sazak-91 cultivar was well adapted lentil in Van, Turkey ecological conditions[9]. The trial was established in 2008–2009 and 2009–2010 years in eastern Turkey (longitude 43°17´E´, latitude 38°33´N´, and altitude 1655 m). The experiment comprised a factorial randomised complete block design replicated three times with three phosphorus treatments P_1 (0 kg ha^{-1}), P_2 (40 kg ha^{-1}) and P_3 (80 kg ha^{-1}), as triple superphosphate, and three humic acid treatments HA_1 (0 kg ha^{-1}), HA_2 (300 kg ha^{-1}) and HA_3 (600 kg ha^{-1}) as leonardit in field. The lentil seeds were sown 5 cm deep by hand in 10 cm inter rows down each plot, with a row spacing of 20 cm. Winter lentil was seeded at 250 seeds per square meter sown on 13 October 2008 and 14 October 2009. Fertilisers phosphorus and humic acid were applied in bands three cm below rows sown to lentil. The climatic data are summarised in Table 1. In both years the soils were sampled to a depth of 0–20 cm of the soil, air-dried and sieved (two mm) for soil analyses. Some physical and chemical properties of soils are given in Table 2. The soils in the trial fields have sandy loam properties and pH was 8.7 and 8.3. Organic substance (1.89–1.79%) in both soils was quite low. The phosphorus content was moderate (4.84–5.11 ppm P_2O_5), in 0–20 cm of both years. Soil total salt was 0.019 and 0.020%. Grain was used to measure total nitrogen by the Kjeldahl method[10]. Total N was multiplied by 6.25 to obtain % crude grain protein. Macronutrients (P, K, Ca and Mg) as well as micronutrients (Fe, Cu, Mn and Zn) were determined according to the method described by Cottenie[11]. All data were subjected to statistical analysis according to procedure outlined by SAS (Ref. 12).

Table 1. Meteorological data of Van in 2008–2009 and 2009–2010 and long term (TSMS: Reports of Turkish State Meteorological Service, Ankara, Turkey, 2011)

Month	Precipitation (mm)			Mean temperature (°C)			Relative humidity (%)		
	2008–2009	2009–2010	long term	2008–2009	2009–2010	long term	2008–2009	2009–2010	long term
October	56.6	15.9	44.4	11.0	10.5	10.7	60.4	46.8	59.0
November	210	91.1	48.2	4.9	4.4	4.4	60.4	61.1	66.0
December	36.7	34.8	37.1	–1.7	1.8	–0.9	62.6	63.5	67.0
January	42.9	51.6	34.2	–3.9	0.1	–3.5	67.6	63.4	68.0
February	49.2	71.1	32.3	0.0	1.3	–3.1	69.1	65.6	68.0
March	74.8	38.3	45.9	1.8	5.7	1.0	63.2	59.0	68.0
April	47.1	46.3	55.0	6.5	8.3	7.5	57.0	62.2	61.0
May	31.9	69.8	45.6	13.1	13.2	13.0	46.3	61.2	56.0
June	27.1	41.0	17.7	17.5	19.8	18.1	47.7	43.6	50.0
July	21.2	–	5.5	21.1	24.0	22.2	43.9	34.3	45.0
Total	408.5	459.9	365.9						
Average				7.03	8.9	6.9	57.8	56.0	60.8

Table 2. Some properties of the < 2 mm fraction of the top 20 cm of soil used for each site

Soil properties	2008–2009	2009–2010
Texture	sandy loam	sandy loam
pH	8.7	8.3
Clay (%)	16.3	18.6
$CaCO_3$ (%)	15.6	17.4
P (ppm)	4.84	5.11
Total salt (%)	0.019	0.020
Organic matter (%)	1.84	1.79

RESULTS AND DISCUSSION

The data on the effects of different humic acid (HA_1 (0 kg ha^{-1}), HA_2 (300 kg ha^{-1}) and HA_3 (600 kg ha^{-1}) and phosphorus levels (P_1 (0 kg ha^{-1}), P_2 (40 kg ha^{-1}) and P_3 (80 kg ha^{-1})) on yield and nutrient contents of seed in lentil are given in Table 3. The year effects were separately evaluated since there were significantly differences between years, as found from combined analysis.

Table 3. Effects of different humic acid and phosphorus levels on yield and nutrient contents of seed in lentil

Treatments	Protein (%)		P (ppm)		K (%)		Ca (ppm)		Mg (ppm)	
Humic acid levels (kg ha^{-1})	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010
0	22.45 b	23.06 c	2247.8 c	2463.1 c	2.15 c	2.32 c	1673.7 b	1793.7 b	2262.7 c	2710.5
300	23.07 b	24.65 b	2631.1 b	2761.5 b	2.37 b	2.58 b	1720.5ab	1882.0 b	2370.0 b	2721.4
600	25.32 a	26.00 a	2913.0 a	3046.1 a	2.51 a	2.97 a	1895.8 a	2055.6 a	2402.0 a	2805.7
Mean	23.61 b	24.57 a	2597.3 b	2756.9 a	2.34 b	2.62 a	1763.3 b	1910.4 a	2344.9 b	2745.8 a
P_2O_5 levels (kg ha^{-1})										
0	21.91 b	21.81 c	2202.0 c	2259.8 c	1.84 c	2.02 c	1608.4 b	1628.1 b	2178.7 c	2452.6 b
40	24.53 a	24.96 b	2696.2 b	2706.8 b	2.42 b	2.73 b	1772.6ab	2000.8 a	2259.8 b	2891.0 a
80	24.41 a	26.94 a	2893.7 a	3304.0 a	2.76 a	3.12 a	1909.1 a	2102.4 a	2596.1 a	2894.1 a
Mean	23.61 b	24.57 a	2597.3 b	2616.5 a	2.34 b	2.62 a	1763.3 b	1910.4 a	2344.8 b	2745.9 a

Treatments	Fe (ppm)		Cu (ppm)		Mn (ppm)		Zn (ppm)		Seed yield (kg ha^{-1})	
Humic acid levels (kg ha^{-1})	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010	2008–2009	2009–2010
0	106.2 b	112.2 b	13.8 b	15.2 c	22.2 1 c	23.45 c	39.52 a	41.02 a	911 c	1308 b
300	113.3 a	129.2 b	14.6 ab	16.3 b	23.61 b	25.01 b	37.03 a	38.17 b	1012 b	1536 a
600	112.1 a	154.1 a	15.4 a	17.9 a	24 53 a	26.32 a	33.25 b	36.02 c	1097 a	1625 a
Mean	110.5 b	131.8 a	14.6 b	16.4 a	23.45 b	24.92 a	36.60 b	38.40 a	1006 b	1489 a
P_2O_5 levels (kg ha^{-1})										
0	105.4 c	106.4 b	11.5 b	14.4 b	22.38 b	22.66 b	40.84 a	44.65 a	900 c	1226 c
40	110.5 b	165.4 a	15.8 a	17.1 a	23.27 b	25.68 a	38.43 a	39.74 b	989 b	1483 b
80	115.6 a	123.6 b	16.4 a	17.9 a	24.68 a	26.43 a	30.53 b	30.82 c	1135 a	1756 a
Mean	110.5 b	131.8 a	14.5 b	16.4 a	23.44 b	24.92 a	36.60 b	38.40 a	1008 b	1488 a

Values in a column with different letters are significantly different from each other (the Duncan multiple range tests, < 0.05).

The protein ratio significantly increased with increasing the humic acid applications in both years. The highest protein ratios were obtained from HA_3 with 25.32% in first year and 26.0% in second year. While the lowest protein ratio was obtained from control plots in both years, the difference between HA_1 and HA_2 applications was statistically insignificant in first year. The effects of phosphorus levels were statistically significant on protein ratio in both years. The highest protein ratio was obtained from P_2 with 24.53% in first year. The difference between P_2 and P_3 applications was statistically insignificant in this year (Table 3). In the second year, the highest protein ratio was obtained from P_3 with 26.94%. The lowest values of protein ratio were recorded in control plots in both years. Kandil[13] in a study conducted about humic acid and phosphorus doses reported that the protein ratio enhanced with increase of humic acid and phosphorus doses. Also, Togay[14], Yetim and Yalcın[15] and Unsal and Tufenkci[16] reported that humic acid increased the protein.

The effects of phosphorus and humic acid applications were statistically significant for phosphorus and potassium contents of seed in both years. The maximum phosphorus and potassium contents were obtained from HA_3 and P_3 applications, whereas the lowest phosphorus and potassium contents were obtained from HA_1 and P_1 (control plots) in both years (Table 3). Togay[14] reported that fertilisation with phosphorus increased phosphorus and potassium content of lentil seeds. Kandil[13] who pointed that application of humic acid at 10% with 80 kg P_2O_5 ha^{-1} significantly increased all growth and yield parameters. Maximum P and K concentration were obtained at 10% humic acid with 80 kg P_2O_5 ha^{-1} in seeds.

Applications of humid acid and phosphorus doses were found to be statistically significant in the calcium contents in both years. The highest calcium contents were obtained from HA_3 and P_3 applications, whereas the lowest calcium contents were obtained from control plots. The difference between HA_1 and HA_2 applications and the difference between P_2 and P_3 applications were statistically insignificant in second year. Ca contents of root and stem were increased by increasing humic acid application[17].

While the effects of humic acid applications were statistically insignificant on magnesium contents in second year, the effects of humic acid applications were statistically significant on magnesium contents in first year. The highest value was obtained from HA_3 application, and the lowest – from HA_1 application in first year. The effects of phosphorus applications were statistically significant on magnesium contents in both years. While the highest values were obtained from P_3 applications with 2596.1 ppm in first year and 2894.1 ppm in second year, the lowest values were obtained from control plots in both years. The difference between P_2 and P_3 applications was statistically insignificant in second year. Cimrin[18] pointed that the combined effects of humic acid and phosphorus application was significantly increased Mg content of shoot of pepper seedling.

The effects of humic acid and phosphorus applications were statistically significant for iron contents in both years. The maximum value was obtained from HA_2 application with 113.2 ppm. The difference between HA_2 and HA_3 application was statistically insignificant in first year. In the second year, maximum value was obtained from HA_3 application with 154.1 ppm. The lowest values were obtained from control plots both two years. But the difference between HA_1 and HA_2 application was statistically insignificant in second year. The highest values were obtained from P_3 application in first year and P_2 application in second year. The lowest values were obtained from control plots. The difference between P_1 and P_3 application was statistically insignificant in second year. Kandil[13] who announced that foliar application of humic acid at 10% with 80 kg P_2O_5 ha^{-1} significantly increased all growth and yield parameters. Maximum Fe concentration was obtained at 10% humic acid with 80 kg P_2O_5 ha^{-1} in seeds.

The highest values of copper contents of seed in lentil were obtained from HA_3 and P_3 applications in both years. The difference between P_2 and P_3 application was statistically insignificant in both years. The lowest values were obtained from control plots both humic acid and phosphorus applications. Kandil[13] in a study conducted about humic acid and phosphorus doses reported that copper contents of seed enhanced with increase of humic acid and phosphorus doses. Also, Cimrin[18] reported that the combined effects of both humic acid and phosphorus application were significantly increased Cu content of shoot.

Applications of humid acid and phosphorus doses were found to be statistically significant in the manganese contents in both years. The highest values were obtained from HA_3 and P_3 applications in both years. While the difference between P_2 and P_3 application was statistically insignificant in first year, the difference between P_1 and P_2 application was statistically insignificant in second year. The lowest values were obtained from control plots. Kandil[13] who pointed that foliar application of humic acid at 10% with 80 kg P_2O_5 ha^{-1} significantly increased all growth and yield parameters. Maximum Mn concentration was obtained at 10% humic acid with 80 kg P_2O_5 ha^{-1} in pea seeds.

Zinc contents of seed in lentil gave the significant negative correlation with humic acid and phosphorus applications. While the highest zinc contents were obtained from HA_1 and P_1 applications (control plots), the lowest zinc contents – from HA_3 and P_3 applications in both years. The difference between HA_1 and HA_2 applications and P_1 and P_2 application was statistically insignificant in first year. Cakmak[19] reported that the combination of high pH, $CaCO_3$ and low organic matter (< 2%), together with low annual precipitation, ranging from 280 to 400 mm were the major factors for Zn deficiency in Central Anatolia. In this study, in addition to low organic matter content, high pH and $CaCO_3$ contents in the soil. A numerous studies indicated that Zn deficiency in plants might cause high concentration of phosphorus that are potentially toxic[20,21]. On the other hand, Erdal et al.[22] reported that zinc fertilisation led to decrease in grain P concentrations and related parameters.

The seed yield in both cropping seasons significantly increased with increasing the humic acid and phosphorus fertilisation and the highest yields were obtained at the highest humic acid and phosphorus application rate (HA_3 and P_3 applications).

The lowest seed yields were obtained from control plots. All phosphorus levels increased seed yield. Kandil[13] pointed that the highest growth parameters were obtained from 80 kg P_2O_5 ha^{-1}. Getachew[23] found that application of phosphate fertiliser at the rates of 10, 20 and 30 kg P_2O_5 ha^{-1} increased mean grain yields by 36, 67 and 57%, respectively compared to the control.

CONCLUSIONS

Phosphorus and humic acid applications caused increases in all seed contents except for Zn content. In the final course of the study, it was concluded that, in the soils of this region, which have poor phosphorus content and are highly alkaline, maximum P, K, Ca, Mg, Fe, Cu, Mn concentration and protein percentage were obtained at 600 kg ha^{-1} humic acid and 80 kg ha^{-1} phosphorus fertilisation (HA_3 and P_3) in lentil seeds. It would bring good results and thus could be beneficial in order to have adequate lentil farming in humic acid phosphorus fertilisation.

REFERENCES

1. S. SEHIRALI: Grain Legumes. University of Ankara, Pub. Number: 314, Ankara, Turkey, 1988.
2. M. TESFAYE, J. LIU, D. L. ALLAN, C. P VANCE: Genomic and Genetic Control of Phosphate Stress in Legumes. Plant Physiol. **144**, 594 (2007).
3. G. E. TSVETKOVA, G. I. GEORGIEV: Changes in Phosphate Fractions Extracted from Different Organs of Phosphorus Starved Nitrogen Fixing Pea Plants. J Plant Nutr, **30**, 2129 (2007).
4. M. J MALAKOOTI: Sustainable Agriculture and Yield Increment by Optimum Fertilizer Utilization in Iran. 2nd ed. Agricultural Extension Publications, Iran, 2000.
5. E. A. GHABBOUR, G. DAVIES: Humic Substances: Structures, Models and Functions. Royal Society of Chemistry, England, 2001.
6. F. J. STEVENSON: Humus Chemistry: Genesis, Composition, Reactions. 2nd ed. John Wiley and Sons, Inc., New York. 1994.
7. B. J. EYHERAGUIBEL, P. SILVESTRE, P. MORARD: Effects of Humic Substances Derived from Organic Waste Enhancement on the Growth and Mineral Nutrition of Maize, Bioresource Technol, **99**, 4206 (2008).
8. Y. TOGAY, M. OZALP: Effects of Yield and Quality Components of Traditional Fertilization and Different Organic Manure Sources on Tir Wheat (*Triticum aestivum* L. var. Leucospermum (Körn.) Farw.). Oxid Commun, **38** (4), 1947 (2015).
9. M. ERMAN: Effect of Different Levels of Nitrogen and Rhizobium Inoculation on Yield and Yield Components in Some Winter Lentil (*Lens culinaris* Medik.) Cultivars under Van Ecological Conditions. Ph.D. Thesis, Yuzuncu Yil University, Van, Turkey, 1998.
10. J. M. BREMNER: Methods of Soil Analysis, Part: 2. American Society of Agronomy Inc., Publisher Medison, Wiconsin, USA, 1965.
11. A. COTTENIE, M. VERLOO, L. KIEKENS, G. VELGH, R. CAMERLYNK: Effect of Cobalt Supplement on Rosemary (*Rosmarinus officinals* L.) a Herb Yield, Essential Oil Content and Its Composition. Chemical Analysis of Plants and Soils, State Univ. Ghent, Belgium, **63**, 44 (1982).
12. SAS: Statistical Analysis Software, Version. 6–12. SAS. Ins.Cary. N.C. USA, 1998.
13. H. KANDIL: Response of Pea Plants (*Pisum sativum* L.) to Phosphorus Levels and Humic Acid Levels. In: Proc. of the International Conference of Agricultural Engineering. AgEng 2014, Zurich 6–10 July, Abstract No P0136, 2014.

14. Y. TOGAY, TOGAY N., Y. DOGAN: Research on the Effect of Phosphorus and Molybdenum Applications on the Yield and Yield Parameters in Lentil (*Lens culinaris* Medic.). African Journal of Biotechnology, **7** (9), 1256 (2008).
15. S. YETIM, R. S. YALCIN: Effect of Different Levels of Nitrogen and Humic Acid on the Yield, Nitrogen Uptake and Protein Content of Bean Plant (*Phaseolus vulgaris*). In: Proc. of the 4th National Plant Nutrition and Fertilizer Congress, 8–10 October, Konya, Turkey, 2008, 417–427.
16. H. UNSAL, S. TUFENKCI: The Effect of Humic Acid and Zinc Applications on Yield and N–P–K Contents of Chickpea in Alkaline Soils. In: Proc. of the 5th National Plant Nutrition and Fertilizer Congress, 15–17 September, Izmir, Turkey, 2010, 334–340.
17. S. SOZUDOGRU, C. KUTUK, R. YALCIN, S. USTA: The Effects of Humic Acid on the Growth of Beans and Its Nutrients Uptake. Agriculture Faculty of Ankara, University Publications, Publication No 1452, 1996.
18. M. K. CIMRIN, O. TURKMEN, M. TURAN, B. TUNCER: Phosphorus and Humic Acid Application Alleviate Salinity Stress of Pepper Seedling. African Journal of Biotechnology, **9** (36), 5845 (2010).
19. I. CAKMAK, A. YILMAZ, M. KALAYCII, B. TORUN, B. ENOGLU, H. J. BRAUN: Zinc Deficiency as a Critical Problem in Wheat Production in Central Anatolia. Plant Soil, **180** (1), 165 (1996).
20. N. W. CHRISTENSEN, T. L. JACKSON: Potential for Phosphorus Toxicity in Zinc-stress Corn and Potato. Soil Sci Soc Am J, **45**, 904 (1981).
21. J. F. LONERAGAN, D. L. GRUNES, R. M. WELCH, F. A. ADUAYI, A. TENGH, V. A. LAZAR, E. E. CARY: Phosphorus Accumulation and Toxicity in Leaves in Relation to Zinc Supply. Soil Sci Soc Am J, **46**, 435 (1982).
22. I. ERDAL, M. KAYA, Z. KUCUKYUMUK: Effects of Zinc and Nitrogen Fertilizations on Grain Yield and Some Parameters Effecting Zinc Bioavailability in Lentil Seeds. Agricultural Research Communication Center, Legume Research, **37** (1), 55 (2014).
23. A. GETACHEW: Phosphate Fertilizer and Weed Control Effects on Growth and Yield of Field Pea on Nitisols of Highland Ethiopia. SINET Ethiop J Sci, **32** (2), 1 (2009).

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