EFFECTS OF HUMUS AND HUMIC ACID ON PLANT GROWTH AND NUTRITIONAL UPTAKE OF LETTUCE (*LACTUCA SATIVA* L.)

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Abstract. This study was carried out to determine the effects of humus and humic acid application on the morphological properties and plant nutritional contents of lettuce. Experiment was established in completely randomized design with 3 replicates including 0, 250, 500 and 1000 kg/ha humus and 0, 15 and 30 l/ha humic acid applications. The results showed that effects of humus and humic acid applications on leaf number, leaf width, leaf length, plant weight, dry matter ratio, N, P, K, Ca and Mg contents of lettuce were statistically significant. The highest mean plant weight values were obtained from the plots under 1000 kg/ha humus and 30 l/ha humic acid application at 245.68 g and 238.69 g respectively. The highest average dry matter ratio values were also obtained from the 1000 kg/ha humus and 30 l/ha humic acid applied plots as 5.93% and 5.87%, respectively. The highest average N content values were obtained in 500 and 1000 kg/ha humus applied plots at 4.28% as well as 15 l/ha and 30 l/ha gave the higher values 4.25% and 4.27% respectively. Based on the results of this study, 1000 kg/ha humus and 30 l/ha humic acid doses recommended in terms of plant growth and plant nutrient contents in lettuce.
Keywords: *Lactuca sativa L. var. crispa, biological application, plant nutrition, plant growth*

Introduction

Lettuce (*Lactuca sativa* L.) is a winter crop in the salad group, which has an important place in human nutrition due to its fibrous structure and rich mineral content (Kim et al., 2016). 94% of Turkey’s agricultural lands has been reported to be poor in terms of organic matter (Ay, 2015). In greenhouse lettuce cultivation chemical products are used excessively and in large quantities because of the cultivation of high-yielding varieties in the same place every year, providing a suitable environment for diseases and pests due to poor acclimatization of greenhouse, excessive content of nutrients in the soil and increased nutrient requirement due to the use of high-yielding varieties (Tüzel and Gül, 2008). As a result of the use of excessive inorganic fertilizers, depending on the environmental conditions, nitrogen does not decompose in the plant, causing an increase in the amount of nitrite and nitrate in the plant. Özgen et al. (2011) reported that inorganic fertilization caused three times more nitrate accumulation in lettuce and salad than organic fertilization. In addition, excessive, intense and unconscious use of chemical products in agricultural production causes the accumulation of nitrate and nitrite in the plant, as well as the accumulation of toxic and dangerous chemical substances in the environment. These pollute soil and groundwater (Li et al., 2021), threatening human health and natural life. Those practices, which cause environmental pollution, disrupt the soil structure, increase harmful chemicals in the plant and threaten human health in order to obtain high yields, have increased the demand for healthy

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agricultural production in recent years and have created a consumer pressure on the producer. In this respect, organic and good agricultural practices, which include effective and efficient use of soil, water and plant resources, protection of the environment, food safety in terms of public health, and at the end, leaving a more livable nature to future generations, have become the most important agenda of the world. In today's agricultural production, plant nutrition and fertilization are regulated, implemented and monitored not only as processes that provide high yields, but also for high quality and healthy agricultural production, protecting the environment and natural resources and observing food safety. In order to increase the quality and productivity along with sustainability in agriculture, the techniques of using biological applications instead of excessive and intensive use of chemicals have gained importance (Montalvo et al., 2020; Chianese et al., 2020). Different plant wastes, farm manure, chicken manure, garbage compost and organic industrial wastes can be used to eliminate the lack of biological source in the soil. Those materials improve the physical, chemical and biological properties of the soils and provide nutrients to the soil, thus positively affecting the yield and quality in plant production (Noroozisharaf and Kaviani, 2018; Kaya et al., 2018; Popescu and Popescu, 2018). It is stated that humic compounds constitute the most important part of coal and occur as a result of chemical change of vegetable and woody parts (Ay, 2015; Miao et al., 2018). Humic substances are a reserve for microorganisms, through elements such as C, N, S and P in their structure. Because of this feature, they enrich the microflora of the soil (Yılmaz and Alagöz, 2001; Larcher, 2003). Humic acid is one of the most important humic substances. Humic and fulvic acids are humus structures that dissolve in alkaline environment (Ay, 2015). Humic substances increase the yield in plants and sugar accumulation in fruits and vegetables by increasing the amount of organic matter and water holding capacity in the soil, improving the physical properties of the soil such as drainage and aeration and increasing the usefulness of nutrients in the soil (Kunç, 2002). The positive effect of organic fertilizers is due to the organic compounds released through the decomposition by microorganisms in the soil and the humus formed by humic and fulvic acids in their structure. Humic and fulvic acids improve soil structure by increasing aggregate formation in the soil due to their colloidal properties. It is also known that due to the ion exchange capacity of the reactive side groups of humic compounds, they interact with pesticides and herbicides to form stable structures and render them harmless to plants and groundwater (Hellal et al., 2006). It has also been reported that humic compounds provide resistance against different stress conditions in plants (Nardi et al., 2002). Furthermore, humic acid application is resulted in cadmium concentration in lettuce (Horuz et al., 2015). Humic acid also accelerate the N metabolism by increasing nitrate reductase activity (Haghighi et al., 2012).

Alternative organic matter sources should be used in the light of scientific data in agricultural lands where organic matter reserves are rapidly decreasing and problems are occurring in terms of the availability of nutrients. These practices such as humus-rich compost application (Solaiman et al., 2019) ensure the sustainability of soil fertility by keeping the risk of environmental pollution at a minimum level, as well as obtaining maximum productivity and quality in plant production. Studies on the interactive relationships between organic matter application and plant nutrients are still insufficient, and up-to-date scientific data are needed on the basis of different soil and product groups, especially in terms of plant growth and product quality. In this paper we

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experimented effects of different humus and humic acid doses on plant growth and mineral nutrient content of lettuce.

Materials and methods

The study was carried out in Eastern Blacksea region of Turkey (Bulancak-Giresun (Latitude: 40.874° (North), Longitude: 38.266° (East), Altitude: 421 m) in unheated plastic house. The soil properties of experimental area as follow; clay loam texture, water holding capacity 59%, $CaCO_3$ 0.48%, pH 6.88, organic matter content 4%, and without saline problem. Plant material was *Lactuca sativa* var. *crispa* cv. Olenka. Before the seedlings were planted in the experimental area, 150 kg/ha N, 100 kg/ha P and 150 kg/ha K fertilizers were applied to the all plots (Eşiyok, 2012). The experiment was established completely randomized plot design with 3 replicates. Seedlings were planted as 30 cm x 20 cm between and within the rows (plot size 2.4 m^2). Four doses (0, 250, 500, and 1000 kg/ha) of leonardite sourced Delta Super Humus (Delta Agricultural Chemicals Industry and Trade Co. Inc., Turkey) and 3 doses (0, 15 and 30 l/ha) of humic acid Delta Humate 12 (Delta Agricultural Chemicals Industry and Trade Co. Inc., Turkey) were applied to the soil before planting. Plants were harvested at 60 days after transplanting. The yellowed and carious outer leaves were removed after harvest and completely excluded from the analysis. Leaf number per plant (Ln), leaf length (Ll) and width (Lw), plant weight (Pw) and dry matter ratio (Dmr) (Dmr = (Fw/Dw)*100, where Fw: fresh weight, Dw: dry weight) properties of the lettuce were measured. The leaves were dried in the oven at 72 °C till constant weight was ensured. Plant nutrient content of lettuce leaf were measured using ICP-OES (Vista-Pro Axial, Agilent Tech., Mulgrave, AUSTRALIA) (Kacar, 2014). Prior to the measurement, 0.2 g of dry leaf sample was digested in a closed microwave digestion system (Marsexpress Cem Corp., Matthews, NC, USA) in the presence of 5 ml of concentrated nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2). Deionized water (H_2O) was used to top-up the volume of the samples to 20 ml. The analytical data was compared to the certified values of a standard reference material (SRM 1573a Tomato Leaf, National Institute of Standards and Technology, Gaithersburg, MD, USA). The variance analysis were applied to data in JMP v.10.0 (SAS inc. USA) statistical program. Means were compared using Tukey HSD post-hoc test (Tukey Honest Significant Difference).

Results

Effect of humus and humic acid on morphological features

Humus, humic acid and humus x humic acid interactions were also found to be significant ($P < 0.05$) with regard to those parameters such as leaf number, leaf length, leaf width, and plant weight, dry matter ratio (*Table 1*). The highest leaf number value was obtained in the plot that the highest humus and humic acid doses were applied (27.0) while the lowest value (13.7) was measured in control plot (no humus and humic acid applied). The average values showed that the highest leaf number values were obtained from 1000 kg/ha humus and 30 l/ha humic acid application as 23.2 and 21.0 respectively. This study showed that the number of leaves in lettuce also increased with the increase in humus and humic acid doses. Similarly the highest leaf length (28.20 cm) and leaf width (22.14 cm) values were obtained in 1000 kg/ha humus and

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30 l/ha humic acid applied plots. As increasing doses of humus and humic acid increased significantly the leaf length and leaf width mean values. Plant weight values changed between 106.40 g and 321.97 g while dry matter ratio values between 5.19 g and 6.60 g. The highest mean plant weight values were obtained from the plots 1000 kg/ha humus and 30 l/ha humic acid applied plots 245.68 g and 238.69 g respectively. Dry matter content values changed between 5.17% and 6.60%. Similar to the above parameters the highest dry matter content value was also obtained from the 1000 kg/ha humus + 30 l/ha humic acid applied plot as 6.60%. The highest average dry matter ratio values were also obtained from the 1000 kg/ha humus and 30 l/ha humic acid applied plots as 5.93% and 5.87% respectively.

Table 1. *The effects of humus and humic acid applications on leaf number, leaf length, leaf width, plant weight and dry matter ratio of lettuce*

Humus (kg/ha)	Humic acid (l/ha)	Ln	Ll (cm)	Lw (cm)	Pw (g)	Dmr (%)
0		15.6 C	18.57 C	15.41 C	130.68 D	5.23 C
250		18.0 B	21.53 B	17.42 B	166.92 C	5.33 C
500		19.1 B	22.38 B	18.24 B	200.87 B	5.66 B
1000		23.2 A	25.16 A	19.75 A	245.68 A	5.93 A
	0	17.2 C	20.17 C	16.29 C	124.34 C	5.37 B
	15	18.8 B	22.11 B	17.72 B	195.23 B	5.38 B
	30	21.0 A	23.44 A	19.11 A	238.69 A	5.87 A
0	0	13.7 d	16.89 f	14.02 f	106.40 g	5.19 d
0	15	15.6 cd	18.78 ef	16.26 def	125.70 f	5.17 d
0	30	17.5 bcd	20.04 de	15.97 ef	160.50 e	5.34 cd
250	0	15.7 cd	19.31 de	15.46 ef	110.80 g	5.29 cd
250	15	19.0 bc	22.77 bc	18.48 bcd	181.00 d	5.22 d
250	30	19.2 bc	22.51 bc	18.33 bcd	208.97 c	5.49 cd
500	0	17.8 bcd	21.12 cd	16.98 cde	126.90 f	5.46 cd
500	15	19.3 bc	22.98 bc	17.75 b-e	212.40 c	5.46 cd
500	30	20.2 b	23.02 bc	19.99 ab	263.30 b	6.06 b
1000	0	21.5 b	23.36 bc	18.71 bc	153.27 e	5.53 cd
1000	15	21.1 b	23.90 b	18.39 bcd	261.80 b	5.67 bc
1000	30	27.0 a	28.20 a	22.14 a	321.97 a	6.60 a

Ln: leaf number per plant, Ll: leaf length, Lw: leaf width, Pw: plant weight, Dmr: dry matter ratio The differences between mean values indicated by different letters are significant according to the Tukey HSD test ($P<0.05$)

Effects of humus and humic acid on leaf nutrient contents

Results related to plant nutrient content of the lettuce were given in *Table 2*. Humus and humic acid application significantly ($P < 0.5$) increased leaf N content. The highest average N content values were obtained in 500 and 1000 kg/ha humus applied plots as 4.28% as well as 15 l/ha and 30 l/ha gave the higher values 4.25% and 4.27% respectively. The highest average N content values were obtained in 500 and 1000 kg/ha humus applied plots as 4.28%, similarly 15 l/ha and 30 l/ha humic acid applications gave also high values 4.25% and 4.27% respectively. Humus and humic acid interaction was found to be significant in respect to the leaf P, K, Ca and Mg

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contents. The highest P and Ca values were obtained from the 500 kg/ha humus and 30 l/ha humic acid applied plots while 1000 kg/ha humus and 30 l/ha humic acid applied plots gave the highest K content. In respect to the Mg content the values were changed according to the humus and humic acid dose interaction. Based on the statistical processing the highest Mg content value (0.29%) obtained from the 250 kg/ha humus + 0 humic acid and 1000 kg/ha humus + 30 l/ha humic acid treatments which differed only from the 0 humus + 0 humic acid treatment. With increasing humic acid does, the average P, K, Ca and Mg values increased as well as N. Similarly, while increasing humus doses increased the mean P and K contents, the mean Ca and Mg content values were not statistically significant.

Table 2. *The effects of humus and humic acid applications on N, P, K, Ca, and Mg contents of lettuce*

Humus (kg/ha)	Humic acid (l/ha)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
0		4.15 B	0.64 B	8.61 C	1.09	0.25
250		4.18 B	0.64 B	9.24 B	1.20	0.27
500		4.28 A	0.69 A	9.44 B	1.16	0.25
1000		4.28 A	0.71 A	10.17 A	1.14	0.26
	0	4.16 B	0.61 C	8.84 C	1.08 B	0.25
	15	4.25 A	0.64 B	9.30 B	1.12 B	0.26
	30	4.27 A	0.74 A	9.96 A	1.25 A	0.27
0	0	4.11	0.58 e	8.12 d	1.00 c	0.20 b
0	15	4.20	0.67 cd	8.83 cd	1.06 bc	0.28 ab
0	30	4.15	0.66 cde	8.88 cd	1.23 abc	0.27 ab
250	0	4.10	0.59 de	9.23 bc	1.11 abc	0.29 a
250	15	4.17	0.61 de	9.32 bc	1.29 ab	0.25 ab
250	30	4.27	0.71 bc	9.18 bc	1.19 abc	0.26 ab
500	0	4.20	0.60 de	9.14 bc	1.00 c	0.22 ab
500	15	4.31	0.66 cde	9.30 bc	1.14 abc	0.27 ab
500	30	4.34	0.82 a	9.87 b	1.34 a	0.26 ab
1000	0	4.23	0.66 cde	8.85 cd	1.23 abc	0.28 ab
1000	15	4.30	0.67 cd	9.74 b	0.97 c	0.22 ab
1000	30	4.31	0.79 ab	11.91 a	1.23 abc	0.29 a

N: Nitrogen, P: Phosphorus, K: Potassium, Ca: Calcium, Mg: Magnesium. The differences between mean values indicated by different letters are significant according to the Tukey HSD test ($P<0.05$)

Discussion

Application of humic substance such as humus and humic acid contributed plant growth in lettuce. Bozkurt et al. (2004) and Tüfenkçi et al. (2006) reported that humic acid application had been increased the leaf number in lettuce. In addition, Çivit (2010) reported that organic matter sourced leonardit application also increased the lettuce leaf number. Aksu (2017) reported that humic acid application increased the leaf length and width as in our study. Application of plant wastes such as hazel nut husk and tea waste composts also contributed positively to plant growth in lettuce (Çağlar, 2014). The researcher reported that leaf length and leaf width values increased significantly by application of those plant waste compost. Furthermore Horuz et al. (2015), Mirdad

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(2016) and Rodrigues et al. (2018) reported that humic acid application increased dry matter content of lettuce. Other than humus and humic acid organic fertilizer such as Ko-Humax also increased plant dry matter in lettuce (Demir et al., 2003). Humic substances such as humus and humic acid contribute to plant growth positively by stimulating the root growth (Canellas et al., 2002; Busato et al., 2010; Zandonadi et al., 2010; Martinez-Balmori et al., 2014; Maji et al., 2017). Humic acid also increased plant biomass by stimulating cell respiration and increasing photosynthetic activity rate in the plant (Fan et al., 2014; Olivares et al., 2015). In addition, humus together with shading contributes seedling emergence in lettuce (Pallaoro et al., 2020). Humus-rich compost application to the soil increase nutrient uptake by stimulating mycorrhizal colonization and ensure sustainability of soil fertility (Solaiman et al., 2019). On the other hand, Roosta et al. (2017) reported that containing humic substance nano-fertile fertilizer might be effective positively on plant nutrient uptake in also hydroponic system.

Conclusion

Today, organic and sustainable agricultural practices continue to increase. In order to realize quality productions with environment-friendly production techniques, farmers have been using many different techniques and applications to complete the deficient organic matter in the soil. The addition of different organic sourced matters such as plant wastes or humus and humic acids to the soil is the most practical of these applications. It is known that there has been a significant increase in the use of organic materials such as humus and humic acid in recent years. Application of organic materials such as humus and humic acid to the soil increases the availability of macro and micro nutrients. Furthermore, due to the regulation of the soil structure, it is thought that it will significantly increase plant growth in lettuce production, and prevent the use of excessive fertilizers. In this study, it was determined that with the increase of humus and humic acid doses applied to the soil, plant growth and the amount of plant nutrients in the leaves increased. The highest humus and humic acid doses gave the highest values in both plant growth parameters and plant nutrient content values. In addition to the use of biological applications such as humus and humic acid applied in this study, it is also recommended to research the effect of different plant waste composts.

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