

Changes in Plant Nutrient Concentration of Olive (*Olea europaea* L.) Leaves after Different Rates of Humic Acid Application

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Abstract – This research was a one-year study to investigate how increasing rates of humic acid affect the nutrient elements [Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu)] of olive trees (*Olea europaea*) of 14 years old found in a private cultivation land located in Bozköy of Geyikli, Çanakkale (Latitude 35 S 431667, Longitude 4409876) in 2018. The humic acid used was a suspension of 15% humic and fulvic acid, which was a commercial product (Blackjak SC, pH 4-6). Different concentrations of humic acid [0 (control), 50, 100, 200, 400 mL tree⁻¹] were applied directly to soil of 20-30 cm depth under canopy projection of trees after dissolving in 10 L of water in April. A randomized block design with four replicates and five doses was adopted on 20 trees. 200 leaf samples were taken in December from annual shoot tips as reciprocal leaf couples and nutrient element analyses were carried out. The macronutrient elements N and P significantly increased upon all humic acid applications. However, the changes in the elements K, Ca, Mg, Fe, Zn, and Mn were insignificant in all applications. Humic acid at all concentrations decreased copper in leaves. As a result, addition to routine farmer fertilization, humic acid application at the rate of 50 ml tree⁻¹ was determined to be the sufficient dose for olive trees. Moreover, this mentioned dose is considered to be the most economical dose besides being the most suitable one.

Keywords – Çanakkale-Geyikli, humic acid, leaf concentration, olive, plant nutrient elements

1. Introduction

The Olive tree has been the symbol of peace. Olive production is very important for the economy of the region in Türkiye. The history of olive tree, which is one of the earliest plants cultivated, goes back to 6000 years ago [1]. Olive is one of the shrub species of *Olea* genus belonging to the Oleaceae family. It is the only species with edible fruit [2]. *Olea europaea* has been switched to cultivar *Sativa* through cutting first time in history in 4000 BC in Türkiye which is among the Mediterranean countries [3].

Mediterranean countries meet around 90% of the world's olive needs. There are about 900 million olive trees on 90 million decares on the planet. The number of trees increases day by day with the establishment of new orchards in other countries with an available climate. The most effective countries for olive production are Spain (38.6%), Italy (14.4%), Greece (9.8%), Türkiye (8.2%), and other Mediterranean countries [4]. The leading cities for olive production are İzmir, Aydın, Çanakkale, Bursa, Balıkesir, Muğla, and Gaziantep. Regionally, the Aegean region is the leading one, followed by the Mediterranean, Marmara, and Southeastern Anatolia. Apart from these regions, olive is cultivated in Blacksea region where Mediterranean climate prevails [3].

Olive cultivation has been preserved as the leading sector besides tourism in Geyikli region of Çanakkale

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province. Olive cultivation is not only a commercial activity, but also a hereditary culture ongoing for centuries. The demand for olive and olive oil is increasing in Türkiye. Chemical fertilizers have been used unconsciously to increase the yield per unit area. This study will be helpful in terms of informing the producers of the region where olive is intensely produced about the effects of humic acid application on increase of soil organic matter (OM) and yield and quality of olive plants. Humic acid, which is an OM itself, is known to enhance the efficiency of current plant nutrient elements in soil. Moreover, it increases the permeability of cell membrane and hence eases the uptake of plant nutrient elements [5]. Enhancement of durability of plants against disease and pests under stress conditions, ventilation of soil and its water retention, and development of microorganisms in soil can be achieved by humic acid applications. Thanks to humic acid, uptake of elements such as iron (Fe), zinc (Zn), phosphorus (P), nitrogen (N), and potassium (K) gets easier, accumulation of salt in soil is inhibited, and positive effects on ventilation of soil and enhancement of the structure of heavily loam soils can be observed. An increase in yield forming elements facilitates the uptake of micronutrient elements, especially Zn, which can be achieved with humic acid in many plants such as wheat [6,7]. This research aimed to investigate the effects of different rates of humic acid application on the nutrition elements of olive leaves.

2. Materials and Methods

The plant materials in this study were Ayvalık oil-type 14 years old olive trees planted in intervals of 7x7 m located in Bozköy, Geyikli region of Çanakkale Province (39° 50' 13" N, 26° 12' 4" E). Humic acid of a commercial brand (Blackjak SC, pH 4-6) was applied to the soil of olive trees. A single-year application of humic acid was carried out in 2018 to investigate the effect of humic acid on the nutrient element contents of olive leaves. Soil samples were taken in February from a depth of 0-30 cm from the crown projection of the trees representing the experimental area. Before the humic acid application, 0.5 kg tree⁻¹ potassium sulphate (K₂SO₄), 0.5 kg tree⁻¹ monoammonium phosphate (MAP) in November and 1 kg tree⁻¹ ammonium sulphate [(NH₄)₂SO₄] in April were routinely applied to all trees. The trial was conducted according to randomized block design with 4 replicates. Humic acid of rates 0 (control), 50, 100, 200, 400 mL tree⁻¹ were applied to 20-30 cm depth under the tree crown projection by diluting in 10 L of water in April.

From each tree used in the trial, 200 leaves were obtained according to [8]. Sampling of couple of leaves from the annual shoots was carried out from all four sides of the trees. Samples leaves were dried at 65 °C for 48 hours, and after that, they were grinded. The dried leaves were extracted with dry decomposition method and from the annual shoots and elements were detected with ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer, Perkin Elmer OPTIMA-5300 DV) device [9].

Variance analysis was carried out on the data according to randomized block design model [10]. Significance analysis was carried out according to *F* test using JMP statistical package program, and means were compared by Duncan multiple comparison test ($\alpha < 0.05$).

3. Results and Discussion

3.1. Soil Analysis Results

The analysis results of the soil of the experimental area are given in Table 1. The soil texture has been classified as “sandy clay loam” according to the physical analysis data. The soil has the characteristics of general soil texture of Aegean Region [11]. Olive trees can grow in a wide pH range; therefore, the soil of the region has a suitable pH range [12]. There is no saltiness problem when the soil of the trial area’s total salt limit values

was evaluated according to [13]. The lime content was intermediate according to [14]. The soil was insufficient in terms of OM according to [15]. Extractable P concentration was low according to [14]. According to [16], K concentration was sufficient, Ca was sufficient and Mg element was low.

The extractable Fe of the soil was sufficient according to [17]. Extractable Cu content was detected to be sufficient according to [18]. Extractable Mn was sufficient and extractable Zn was low according to [16].

Table 1. Chemical and physical analysis values of the soil of the study area

Soil Texture			EC (%)	CaCO ₃ (%)	OM (%)	pH
Sand (%)	Silt (%)	Clay (%)				
64	7	29	0.06	8.05	0.69	7.51
Sandy Clay Loam			Non-saline	Calcareous	Very low	Slightly-Alkaline
Macro Element Content						
N (g kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)		Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	
0.30	5.62	180.71		3637.53	59.87	
Low	Low	Sufficient		Sufficient	Low	
Micro Element Content						
Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)		Cu (mg kg ⁻¹)
7.12		8.49		0.12		1.35
Sufficient		Sufficient		Low		Sufficient

3.2. Effect of Humic Acid on N Concentration

Compared to the control trial (13.52 g kg⁻¹) the N concentration in olive leaves was detected to be 17.83, 17.8, 18.33 and 17.68 g kg⁻¹ with increasing rates of humic acid (Figure 1). All the humic acid applications significantly increased the leaf N concentrations compared to the control ($P < 0.001$), and these increases were as follows: 31.87%, 31.95%, 35.57%, and 30.76%, respectively (Figure 1).

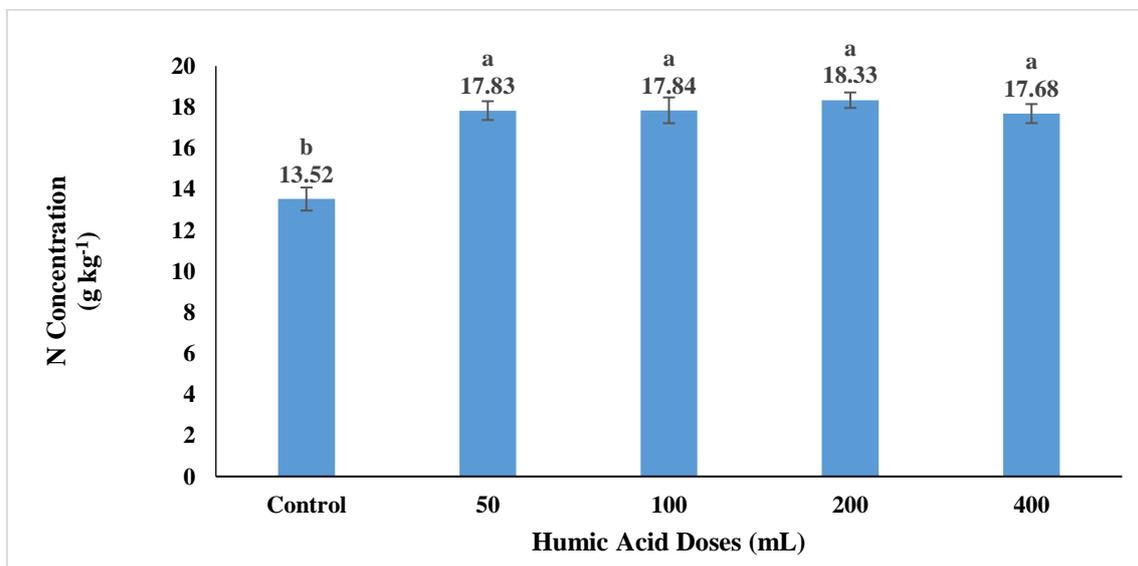


Figure 1. The effect of humic acid applied to the soil at increasing rates on leaf N concentration

N is an important element for olives. N is required the most at the flowering stage and the need continues till the hardening of the seed. Fertilizer applications make N reach a sufficient level and this will affect shoot development, fruit amount and yield parameters [8]. The results of our study were obtained from other studies, too. Sözüdoğru et al. [19] researched the effects of humic acid applied to the soil on the nutrient element concentrations in beans. As a result, the concentrations of N and P were found to increase. Eryüce [8] reported the N concentration of olive leaves in the Ayvalık region to be 1.10-1.87%, which shows that our results are compatible with the literature. The study by Reuter and Robinson [20] reported the optimum leaf N

concentration in olive trees to be between 1.5-2%. Ali [21] reported the same value which is also 1.54%. Figure 1 showed that the leaf N concentration of the control plants remained below the optimum limit values, but with the addition of humic acid, it was within the range of the optimum values according to [20,21].

3.3. Effect of Humic Acid on Leaf P Concentration

When compared to the control (0.90 g kg^{-1}), the P concentration in olive leaves was detected to be 1.40, 1.46, 1.27, and 1.24 g kg^{-1} upon increasing rates of humic acid applications, respectively. All the humic acid applications increased the leaf P concentrations significantly ($P < 0.001$), and the increases were as follows: 56.17%, 62.18%, 41.6%, and 37.49%, respectively (Figure 2). The study by Reuter and Robinson [20] reported the optimum leaf P concentration in olive trees to be between 0.1- 0.3%. Figure 2 showed that the leaf P concentration of the control plants remained below the optimum limit values, but with the addition of humic acid, it was within the range of the optimum values according to [20].

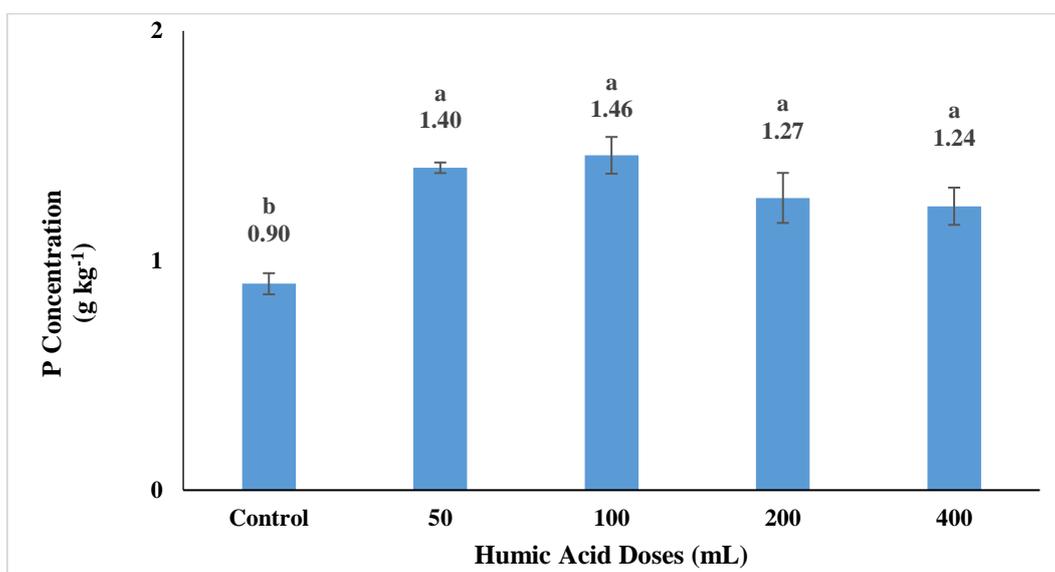


Figure 2. The effect of humic acid applied to the soil at increasing rates on leaf P concentration

Phosphorus plays an important role in formation of flowers, fruit setting and hence in decreasing the periodicity effect in olive plants [22]. Although the level of P in soil was not sufficient (Table 1), the concentration of P significantly increased with the application of humic acid, even in the first year of this study (Figure 2).

Similar results to the results of our study were detected by other researchers. Wang et al. [23] reported that when humic acid was applied to the soil together with P, P fixation was inhibited, and available P concentration was increased and also product yield increased together with the increased uptake of P by plant. Moreno et al. [5] reported that OM in soil formed compounds with Ca in soil and thus increased available P in soil, which increased plant nutrient content. In another study, Adak [24] investigated the effect of different ratios of vermicompost on nutrient elements of tomato and pepper. They reported that increasing rates of vermicompost positively affected the leaf P concentrations of pepper plants.

3.4. Effect of Humic Acid on Leaf K Concentration

Compared to the control (11.49 g kg^{-1}) the K concentrations of olive leaves were 12.26, 12.49, 12.38, and 11.39 g kg^{-1} upon increasing doses of humic acid (Figure 3). When the changes were analyzed, 50, 100, and 200 mL tree^{-1} humic acid applications increased leaf K concentrations by 6.7%, 8.62%, and 7.73% compared to the control; however, the highest humic acid dose of 400 mL tree^{-1} caused a negative change of 0.94%, but

this change was not statistically significant (Figure 3). As seen in Table 1, the sufficient level of K in trial soil might have been the reason for a non-significant change in leaf K levels.

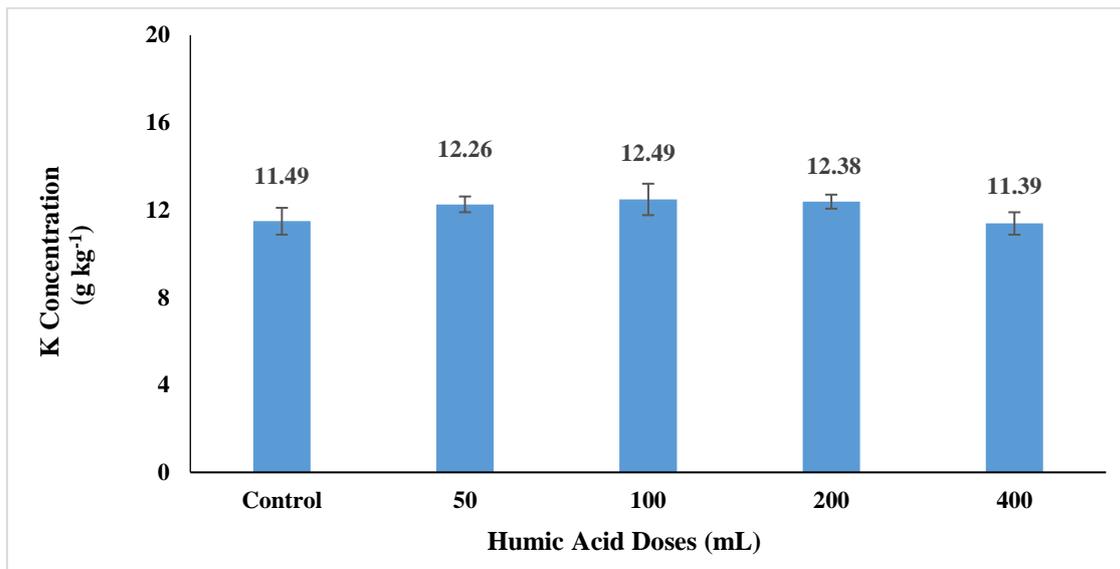


Figure 3. The effect of humic acid applied to the soil at increasing rates on leaf K concentration

Potassium is a very important nutrient element in olive cultivation. Fruit length, number of fruits, and fruit meat-seed ratio are directly related to the K nutrition. Moreover, deficiency of K increases the sensitivity of the plant to heat changes and negatively affects the product quality [25].

Similar results were reported by other researchers. Müftüoğlu and Alak [26] investigated the effects of humic acid applied to the soil in increasing doses on plant length, shoot diameter, and total K in the root and shoot of Helen-type maize plant. As the applied humic acid concentration increased, the K amount uptake by plant increased but the increase was not statistically significant. Turan et al. [27] investigated the humic acid applied on leaves on maize development and plant nutrient element content under salty conditions. The results showed an increase in plant K yet were non-significant. In another study, Çelik et al. [28] applied humic acid on the leaves under salty and limy conditions and investigated the uptake of nutrient elements by the plant. Results revealed an increase in K uptake, but it was again statistically non-significant.

Doran et al. [29], in their study with Halhalı-type olive, reported that the olive leaf K concentration was between 1.2-1.5%. The study of Reuter and Robinson [20] reported that the optimum K concentrations in olive trees were > 0.8%. Ali [21] reported a similar value as 0.87%. The leaf K concentrations in our study were between 1.13-1.24% which were similar with the findings of [20,21].

3.5. Effect of Humic Acid on Leaf Ca Concentration

Compared to the control (19.60 g kg⁻¹), the Ca concentrations in olive leaves were 19.79, 19.13, 19.34, and 18.13 g kg⁻¹ upon increasing rates of humic acid doses (Figure 4). The Ca concentration increased by 0.93% upon 50 mL tree⁻¹ application. On the other hand, 100, 200 and 400 mL tree⁻¹ humic acid applications decreased the Ca concentration of leaves, and the decreases were 2.4%, 1.32% and 7.5%, respectively, and they were not statistically significant (Figure 4). The trial soil Ca content was sufficient as seen in Table 1 which might have resulted in no change in the Ca concentrations of leaves. Similar results were obtained by other researchers. Sözüdoğru et al. [19] applied different doses of humic acid to the soil of bean plants and investigated the nutrient elements and reported that humic acid was not effective in the uptake of Ca.

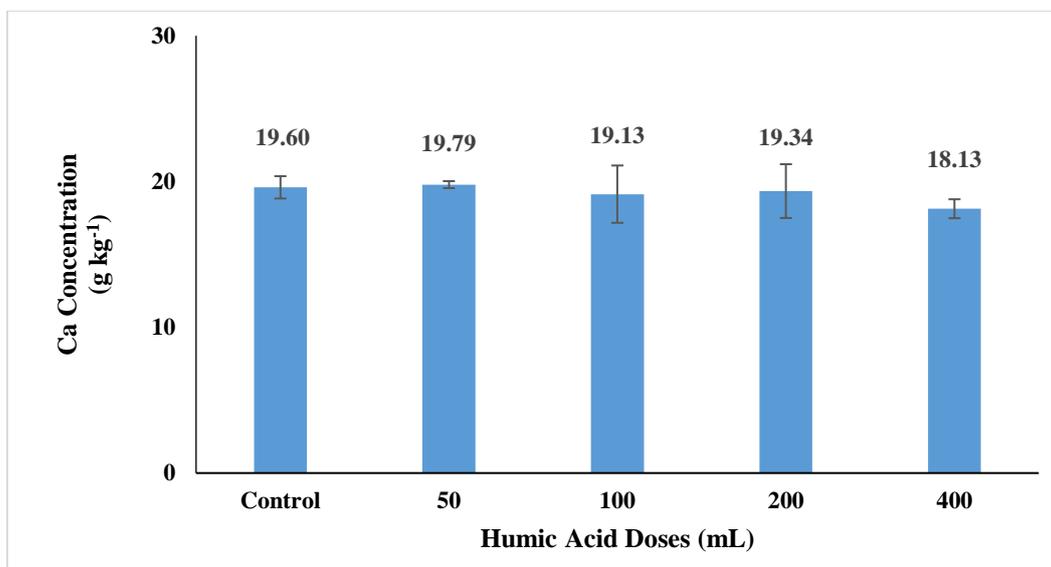


Figure 4. The effect of humic acid applied to the soil at increasing rates on leaf Ca concentration

Demir et al. [30] investigated the effect of different organic fertilizers and NPK fertilizers on nutrient element concentrations of tomato fruit and found that Ca concentration was not statistically different than the control. Eryüce [8] researched Ayvalık-type of olive and found that Ca concentration can change between 0.88-2.14%. Soyergin [31] reported that Ca concentration was between 1.26-2.94% in Gemlik-type olive. Seferoğlu [32] conducted a study on two different regions and found that leaf Ca concentrations were 1.10-1.80% in Ayvalık region, while 1.00-1.20% in Edremit region. The study of Reuter and Robinson [20] reported the optimum Ca concentrations of olive trees as >1.0 . Ali [21] reported a similar value as 1.48%. In our study, leaf Ca concentrations were found to be between 1.81-1.97%, which were compatible with results from [20,33].

3.6. Effect of Humic Acid on Leaf Mg Concentration

Compared to the control condition (1.52 g kg^{-1}), the Mg concentrations in olive leaves were 60, 1.68, 1.65, and 1.56 g kg^{-1} upon application of increasing rates of humic acid (Figure 5). The applications of 50, 100, 200 and 400 mL tree^{-1} humic acid increased the leaf Mg concentrations, and the changes were 33%, 10.86%, 8.82%, and 2.76%, respectively. However, the changes were not statistically significant (Figure 5). Similar results were reported by other researchers. Demirtaş et al. [34] used different doses of municipal waste compost for greenhouse cultivation of tomatoes. Compared to the control, they found that increase in Mg concentrations was not statistically significant. Demir et al. [30] investigated how different organic fertilizers and NPK fertilizers affected tomatoes, and again the increase in Mg concentrations was not found to be statistically significant. Eryüce [8] studied Ayvalık-type olive and reported that leaf Mg concentrations were between 0.12-0.37%. Püskülcü [35] found that leaf Mg concentrations of Memecik-type olive were changing between 0.22-0.34%. Soyergin [31] reported leaf Mg concentrations of Gemlik-type olive to be 0.12-0.37%. Seferoğlu [32] also worked on Ayvalık-type olive and reported the Mg concentrations changing between 0.15-0.31%. In our study, the Mg concentrations were between 0.15-0.16%, and inside limit values according to [20,33]. The trial soil Mg was low (Table 1). The Mg concentration tended to increase with humic acid applications, and it is expected that with humic acid applications, the effects will be more visible in the upcoming years.

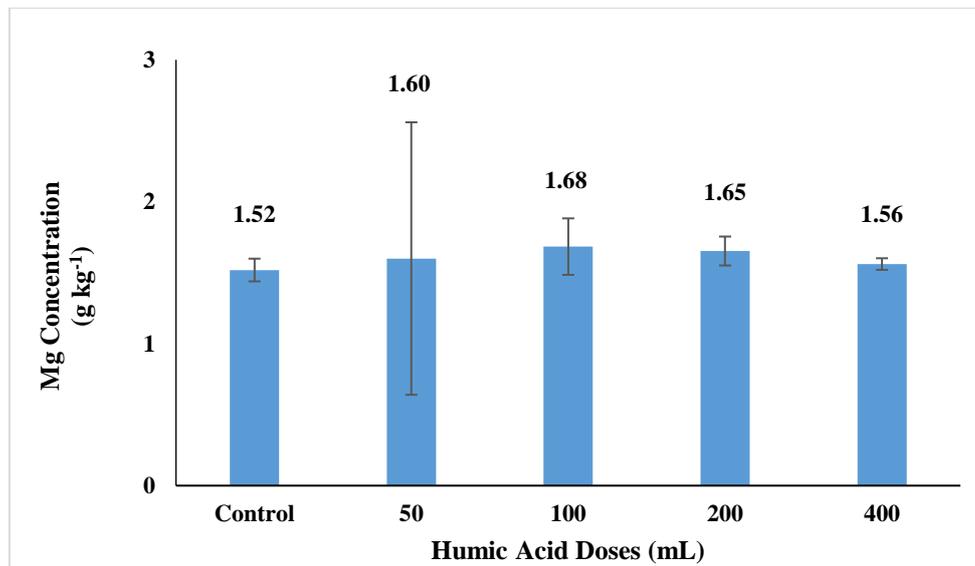


Figure 5. The effect of humic acid applied to the soil at increasing rates on leaf Mg concentration

3.7. Effect of Humic Acid on Leaf Fe Concentration

When compared to the control (111.85 mg kg⁻¹) the Fe concentrations in the leaves of olive trees were 103.87, 103.85, 103.25, and 94.55 mg kg⁻¹ upon increasing doses of humic acid applications (Figure 6). In the order of the applied humic acid (50, 100, 200, and 400 mL tree⁻¹) the decrease of Fe concentrations were as follows: 7.13%, 7.15%, 7.68%, and 15.46%, and they were not statistically significant (Figure 6).

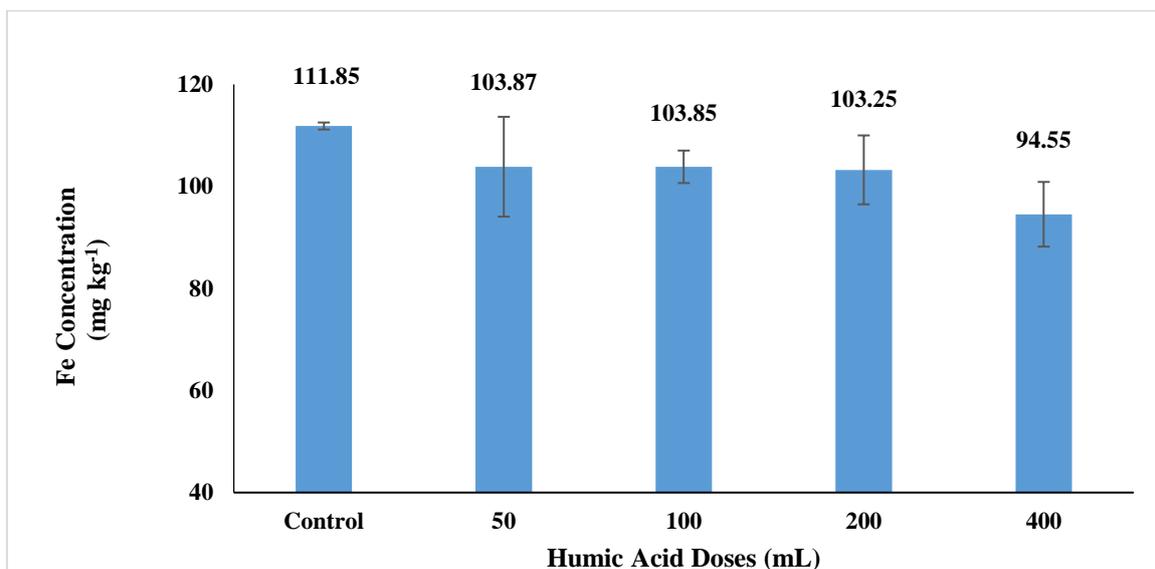


Figure 6. The effect of humic acid applied to the soil at increasing rates on leaf Fe concentration

Demir et al. [30] could not report a change in Fe concentrations upon applications of different organic fertilizers and NPK fertilizers. Humic acid of low pH (pH 4-6) applied to the soil was expected to increase the Fe availability, however, there was no significant change. On the contrary, there was a decrease in Fe concentration as seen in Figure 6. Seferoğlu [32] found the Fe concentrations in Ayvalık-type olive in Ayvalık region to be between 55-115 mg kg⁻¹, and between 60-140 mg kg⁻¹ in Edremit region. Soyergin et al. [36] reported the Fe concentration of Gemlik-type olive to be 88-280 mg kg⁻¹. In our study, the Fe concentrations were between 94.55-111.85 mg kg⁻¹, and they were compatible with the literatures. The detected Fe concentrations were inside the limit values according to [20,33].

3.8. Effect of Humic Acid on Leaf Mn Concentration

Compared to the control (45.44 mg kg⁻¹) the Mn concentrations of olive leaves upon increasing rates of humic acid applications were found to be 51.60, 52.43, 52.53, and 50.29 mg kg⁻¹ (Figure 7). All the applications increased the Mn concentrations compared to the control at 13.55%, 15.37%, 15.6%, and 10.68% in the order of humic acid concentrations respectively, however, the results were not statistically significant (Figure 7).

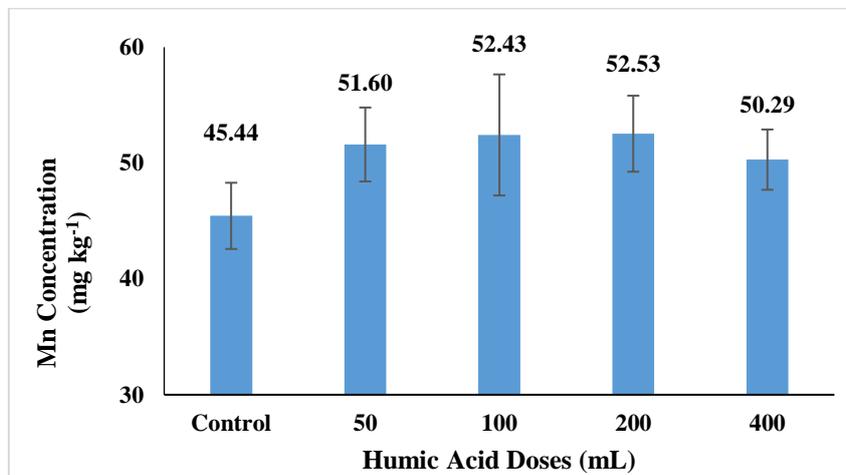


Figure 7. The effect of humic acid applied to the soil at increasing rates on leaf Mn concentration

Similar results were observed by different researchers. Demir et al. [30] investigated the effects of different organic materials and NPK fertilizers on tomatoes and found that their effect on Mn concentration was not significant. Demirtaş et al. [37] investigated the effects of plant-based liquid organic fertilization of tomatoes and found that its effect on Mn concentration was not statistically significant. Şahin [38] found the Mn concentrations in leaves of olives of organic cultivation to be 49.75-61.33 mg kg⁻¹. In our study, the Mn concentrations were found to be 45.44-52.53 mg kg⁻¹ which are inside the limit values according to [20,21,33].

3.9. Effect of Humic Acid on Leaf Zn Concentration

Compared to the control (15.79 mg kg⁻¹) the results of applying increasing doses of humic acid were 16.36, 18.01, 17.24, and 15.32 mg kg⁻¹ Zn in leaves of olive (Figure 8). Compared to the control, 50, 100, and 200 mL tree⁻¹ humic acid increased the Zn concentration in leaves by 3.6%, 14.05%, and 9.18%, respectively; however, 400 mL tree⁻¹ humic acid decreased Zn as 2.97%. these changes were not found to be statistically significant (Figure 8).

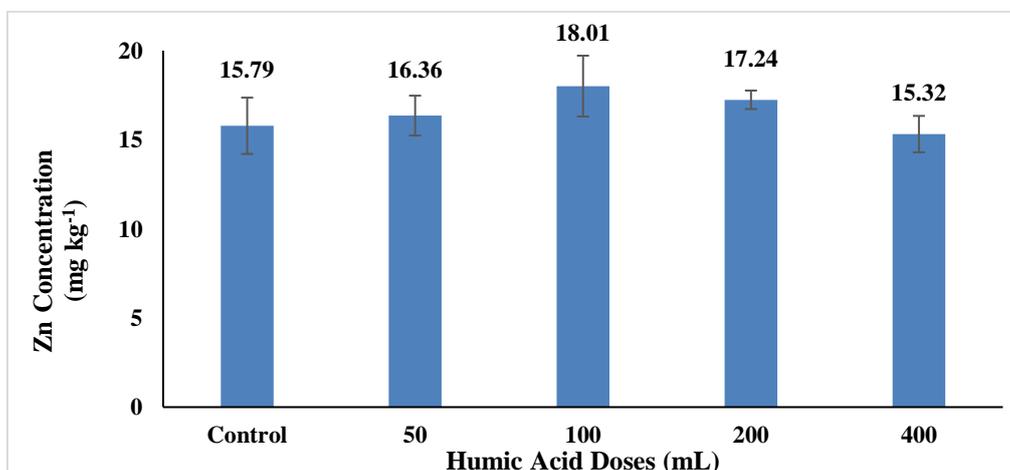


Figure 8. The effect of humic acid applied to the soil at increasing rates on leaf Zn concentration

Similar results were obtained by other researchers. Demir et al. [30] found that different organic materials and NPK fertilizers did not significantly affect the Zn concentration tomato compared to the control condition. Demirtaş et al. [37] investigated the effects of plant-based liquid fertilizer on plant nutrient elements in the tomato plant. Similarly, they did not find a statistically significant change in Zn concentrations. Tokay and Yaşar [39] reported that Zn levels significantly play an important role in olive fruit development.

Şahin [38] conducted a study on organic olive cultivation and found that the Zn concentrations as 16.78-132.81 mg kg⁻¹. Our study reported the Zn concentrations as 15.32-18.01 mg kg⁻¹ which are inside the limit values according to [20,33].

3.10. Effect of Humic Acid on Leaf Cu Concentration

Compared to the control condition (37.16 mg kg⁻¹) the Cu concentrations in olive leaves were 14.69, 17.50, 22.05, and 13.39 mg kg⁻¹ upon increasing doses of humic acid applications (Figure 9). The Cu contents significantly decreased in the left upon applications of all the humic acid doses, and these changes were as follows compared to the control: 60.48%, 52.92%, 40.67%, and 63.98% (Figure 9). Sözüdoğru et al. [19] investigated the effects of humic acid on nutrient elements of plants and found that humic acid did not affect Cu uptake, which was in contrast to our study. Şahin [38] reported the Cu contents to be 17.40-25.54 mg kg⁻¹ in the study conducted on organic cultivation olive gardens. The results of our study revealed that leaf Cu values were between 15.39-37.16 mg kg⁻¹ being inside the limit value according to [20,21,33].

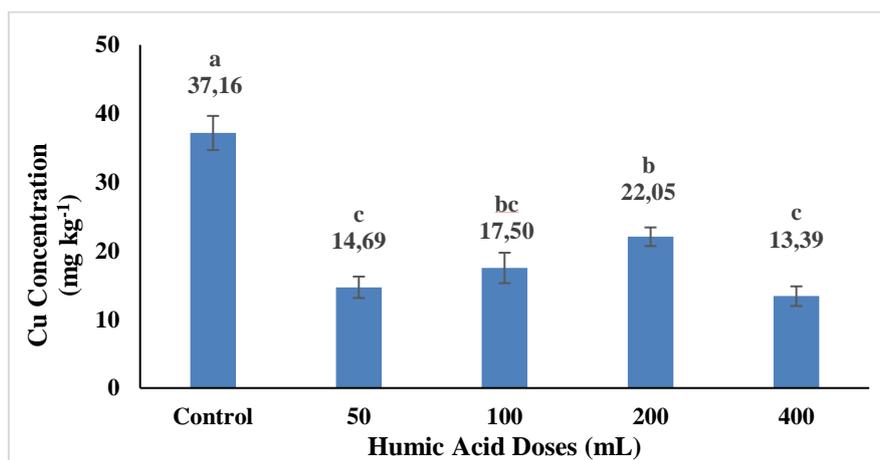


Figure 9. The effect of humic acid applied to the soil at increasing rates on leaf Cu concentration

4. Conclusion

This experiment was carried out on Ayvalık oil-type olive trees of age 14 in Bozköy located in Geyikli region of the city Çanakkale in field conditions for the year according to randomized block design. Increasing rates of humic acid increased the N and P nutrient elements, and decreased Cu concentration, while the concentrations of Ca, K, Mg, Fe, Mn, and Zn did not change. The concentrations of all the nutrient elements stayed inside the suggested limit values. Humic acid application is an important contributor to the increase of OM in soil. Although a decrease in Cu values and an increase in Fe values were observed, both elements were inside the acceptable leaf limit values. However, these micronutrient elements should be applied to the soil or leaves in case these values decrease over time. In this study, olive leaf N and P concentrations in routine farmer fertilization remain below the limit value, and with the addition of humic acid, they are within the optimum limit values. The findings of the current study show that humic acid application at the rate of 50 ml tree⁻¹ is sufficient for olive tree nutrition. Moreover, this dose is economically proper for the farmers. Since the olive

tree is perennial with a deep root structure, continuous humic acid applications are thought to be positive in terms of macro and micronutrient elements for the plant. The deficiency of N and P concentrations in the soil of Türkiye can be eliminated by the addition of humic acid. Our study is a two-year study, and the results will be shared to inform the producers of the region.

Author Contributions

Both authors devised the main conceptual ideas and developed the theoretical framework. The second author conceived and designed the analysis and wrote the article. The second author also performed the experiment and necessary analyses under the supervision of the first author. All authors read and approved the final version of the paper. This paper is derived from the first author's master's thesis supervised by the first author.

Conflicts of Interest

All the authors declare no conflict of interest.

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