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Effects of Different Organic Fertilizers on Plant Growth, Yield, Quality Properties and Element Contents in Spinach

Farklı Organik Gübrelerin Ispanakta Bitki Gelişimi, Verim, Kalite Özellikleri ve Element İçerikleri Üzerine Etkileri

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Abstract: This study was conducted to determine the effects of different organic fertilizers on plant growth, yield, quality properties and element contents in spinach (*Spinacia oleracea* L.). The research was carried out under open field conditions in Bolu province. In the study, Matador spinach variety was used and seven different applications were examined. The applications were as follows: 1) Control, 2) Chicken manure, 3) Turkey manure, 4) Sheep manure, 5) Cattle manure, 6) Vermicompost and 7) Chemical fertilizer. The experiment was established in randomized complete block design with three replications. According to the research findings, significant differences were found among the applications. In general, organic fertilizer applications significantly increased yield, plant height, plant wet weight, plant dry weight, dry matter content, number of marketable leaves, nitrogen, phosphorus, sulphur and selenium contents of the plant in comparison with control and chemical fertilizer. The highest yield was determined in cattle manure application. It was observed that cattle manure increased the yield by 88.08% compared to the control and increased the yield by 41.16% compared to the chemical fertilizer. In organic fertilizer applications, heavy metal contents such as arsenic, chrome, cobalt, and nickel were found to be lower than the control and, aluminum and cadmium contents were found to be lower than chemical fertilizer. As a result, it was determined that organic fertilizers examined in the study have positive effects on plant growth, yield, quality properties and element contents of spinach and organic fertilizers can be used successfully for sustainable agriculture in spinach cultivation.

Keywords: *Spinacia oleracea* L., organic fertilizers, growth, yield, quality, elements

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Öz: Bu çalışma, farklı organik gübrelerin ıspanakta (*Spinacia oleracea* L.) bitki gelişimi, verim, kalite özellikleri ve element içerikleri üzerine etkilerini belirlemek amacıyla yapılmıştır. Araştırma, Bolu ilinde açık arazi koşullarında yürütülmüştür. Çalışmada Matador ıspanak çeşidi kullanılmış ve yedi farklı uygulama incelenmiştir. Uygulamalar aşağıdaki gibidir: 1) Kontrol, 2) Tavuk gübresi, 3) Hindi gübresi, 4) Koyun gübresi, 5) Sığır gübresi, 6) Vermikompost ve 7) Kimyasal gübre. Çalışma tesadüf blokları deneme desenine göre 3 tekerrürlü olarak kurulmuştur. Araştırma bulgularına göre uygulamalar arasında önemli farklılıklar bulunmuştur. Genel olarak organik gübre uygulamaları verim, bitki boyu, bitki yaş ağırlığı, bitki kuru ağırlığı, kuru madde içeriği, pazarlanabilir yaprak sayısı, azot, fosfor, kükürt ve selenyum içeriğini kontrol ve kimyasal gübreye göre önemli ölçüde artırmıştır. En yüksek verim sığır gübresi uygulamasında tespit edilmiştir. Sığır gübresinin verimi kontrole göre %88.08, kimyasal gübreye göre ise %41.16 artırdığı gözlenmiştir. Organik gübre uygulamalarında arsenik, kobalt, krom ve nikel gibi ağır metal içerikleri kontrole göre, alüminyum ve kadmiyum içerikleri ise kimyasal gübreye göre daha düşük bulunmuştur. Sonuç olarak, çalışmada incelenen organik gübrelerin ıspanakta bitki gelişimi, verim, kalite özellikleri ve element içerikleri üzerine olumlu etkilerinin olduğu ve organik gübrelerin ıspanak yetiştiriciliğinde sürdürülebilir tarım için başarılı bir şekilde kullanılabileceği belirlenmiştir.

Anahtar Kelimeler: *Spinacia oleracea* L., organik gübreler, büyüme, verim, kalite, elementler

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INTRODUCTION

Ensuring adequate and balanced nutrition for the rapidly increasing world population is possible by increasing agricultural production and productivity. Fertilization is one of the most important factors determining yield and quality in agricultural production. Fertilizer is one of the most important inputs in agricultural production, and it alone can increase the yield close to 50%. Chemical (inorganic) fertilizers are mostly used in order to increase agricultural productivity all over the world (Karaman and Turan, 2012). Nowadays, it is known that human health and sustainability of soils are adversely affected because of excessive and unconscious chemical fertilization to obtain high yield from vegetables. On the other hand, it is reported that continuous use of chemical fertilizers causes pollution of soil and water, soil salinity, decline in organic matter content of soil, heavy metal accumulation, decrease in microbial activities of soil, nitrate accumulation, decrease in soil fertility, poor product quality and serious health problems (Savcı, 2012). For this reason, recently, organic fertilizers have gained great importance as an alternative to chemical fertilizers used in agricultural production all over the world with the new understanding focused on ensuring sustainability in agriculture due to the adverse effects of chemical fertilizers. Organic fertilizers should be preferred in order to restore and protect the deteriorated natural balance. Studies have shown that organic fertilizers are beneficial for plants, soil, environment and economy.

Organic fertilizers contain plant nutrients, organic matter and a large amount of various microorganisms. Therefore, organic fertilizers have important and positive effects on the physical, chemical and biological properties of agricultural soils (Kacar and Katkat, 2007). Organic fertilizers create a source of nutrients for plants, increase the cation exchange capacity of the soil, increase the water holding capacity of the soil, facilitate aeration and warming of the soil, help the plant nutrients in the soil to be converted into forms that can be taken by the plant, improve soil structure, give a good texture and structure to the soil, regulate soil reaction and promote plant growth (Soyergin, 2003; Mercik and Stepien, 2006; Adiloğlu and Eraslan, 2012).

Organic fertilizers consist of materials of plant and animal origin. Among the sources used as organic fertilizer, barnyard manure (farmyard manure) comes first. Barnyard manure is the oldest and most widely used organic fertilizer. Barnyard manure consists of the excrement of bovine and ovine farm animals and the bedding laid under the animals in the barns. The quality and content of barnyard manure vary depending on many factors such as the animal's breed, age, sex, nutritional status, bedding material used and storage conditions (Lampkin, 2002). The nutrient content of sheep, chicken and turkey manures is higher than that of cattle. Barnyard manure provides various plant nutrients necessary for plant growth, positively affects the physical, chemical and biological properties of the soil, accelerates the activity of microorganisms in the soil, and also acts as a source of organic matter in the soil (Watson et al., 2002; Schoenau, 2006).

Vermicompost is the most popular among organic fertilizers in recent years due to its positive effects on plant growth, yield and soil properties. Additionally, vermicompost is intensely applied as a reliable, economical, and sustainable method in the processing and evaluation of solid organic wastes, which has become a major environmental problem (Shetinina et al., 2019). Vermicompost, which is obtained by composting organic wastes during digestion by earthworms, is an organic product with high economic value (Garg and Gupta, 2009). Many organic wastes (plant wastes, animal manure, food wastes, urban solid waste, waste paper, sawdust, etc.) can be used in vermicompost production (Karmakar et al., 2012). Vermicompost is a well stabilized, finely divided peat-like material produced through a non-thermophilic process involving the biodegradation and stabilization of organic materials by interactions between earthworms and microorganisms. Vermicompost has very high porosity, aeration, drainage, water holding capacity and microbial activity, and a low C:N ratio (Garg and Gupta, 2009; Kumar and Topal, 2015). Vermicompost is very rich in macro and micronutrients, beneficial soil microorganisms, various enzymes, vitamins, humic acid, organic matter, and growth hormones (Özkan et al., 2016).

As in the whole world, chemical fertilizers are mostly used in fertilization to increase the yield in vegetable growing in Turkey. To provide a sustainable agriculture system that protects human health, environment, and natural resources in the long term in vegetable growing where the use of chemical fertilizers is excessive, it is necessary to expand the use of organic fertilizers instead of chemical fertilizers. The use of organic fertilizers in vegetable growing is an agricultural activity that has become widespread in Turkey with the increase in environmental awareness and the adoption of high-quality product consumption in recent years.

Spinach (*Spinacia oleracea* L.) belongs to the Chenopodiaceae family and is an annual winter vegetable produced by seed. It is known that the homeland of spinach is Western Asia (South Turkestan, Caucasus and Nepal), Iran and China (Kallo and Bergh, 1993). It has been cultivated and consumed fondly for many years in the world. Spinach whose fresh leaves are consumed is among the species with high commercial importance. It is one of the most produced and consumed winter vegetables in Turkey and it has high economic value. Spinach is a vegetable that can be grown in all regions of Turkey, limited only in the Eastern Black Sea Region which receives heavy rainfall, and is produced in large quantities. It is grown in late summer and winter in hot regions of the country, and in winter and spring in cold regions of the country. The vegetation period of spinach is short, and it can be grown in 2-2.5 months. In Turkey, spinach was grown in an area of 150.788 da with a production of 218.355 tons in 2021 (TÜİK, 2022).

Spinach, which has an important place in human nutrition, is a significant source of vitamins and minerals. Yield and quality of spinach increase in soils rich in organic matter. Since spinach is grown in a short vegetation period, fertilization is quite important in spinach cultivation in order to obtain high yield and quality from the unit area. As with other vegetables, spinach is usually grown using inorganic fertilizers. On the other hand, fertilization in spinach must be performed carefully because it is a leafy vegetable. Excessive and unconsciously used chemical nitrogen fertilizers increase the nitrate accumulation in the plant, which is harmful to human health. Spinach is one of the vegetables with the highest nitrate accumulation (Santamaria, 2006). Therefore, the use of organic fertilizers in spinach cultivation should be expanded.

It is very important to examine the effects of organic fertilizers on soil and plant productivity to increase yield and quality, improve the physical and chemical structure of the soil and prevent environmental pollution in spinach cultivation. Such studies are necessary to encourage and increase the use of organic fertilizers in vegetable cultivation. By using organic fertilizers, it is aimed to ensure sustainability in agriculture, improve agricultural soils and increase productivity with less cost per unit area.

The objective of this study was to investigate the effects of different organic fertilizers on plant growth, yield, quality properties and element contents of spinach grown under open field conditions in Bolu province. In addition to, organic fertilizers were compared with inorganic fertilizer.

MATERIAL AND METHOD

The research was carried out under open field conditions in Yeniakçakavak village of central district of Bolu province in Turkey (latitude 40°45'N, longitude 31°40'E, altitude 708 m) during the autumn growing season of 2017.

Material

Matador spinach variety (*Spinacia oleracea* L.) which is widely grown in Turkey was used as plant material in the study. Before the experiment was established, soil samples were taken from different parts of the field and some physical and chemical properties of the soil were determined by soil analysis. The soil used in the experiment was clayey, pH value of 7.3, organic matter content of 4.81%, lime ratio of 15.30% and EC value of 0.82 dS m^{-1}. The nitrogen, phosphorus and potassium contents of the soil were 0.18%, 4.50 mg kg^{-1} and 257.79 mg kg^{-1}, respectively. Some physical and chemical properties of organic fertilizers (chicken, turkey, sheep, cattle and vermicompost) used in the experiment are shown in Table 1. It was determined that organic fertilizers were rich in organic matter, nitrogen, phosphorus and potassium. The pH value of organic fertilizers varied between 6.8 and 8.1. In general, it was detected that the salt contents of organic fertilizers were high.

Table 1. Some physical and chemical properties of organic fertilizers used in the study.
Çizelge 1. Çalışmada kullanılan organik gübrelerin bazı fiziksel ve kimyasal özellikleri.

Examined properties	Determined Values				
	Chicken	Turkey	Sheep	Cattle	Vermicompost
pH	7.6	7.7	7.9	8.1	6.8
EC (dS m^{-1})	9.06	7.13	3.85	4.17	4.39
Organic matter (%)	59.33	55.24	53.48	50.17	31.28
Nitrogen (N) (%)	2.67	2.43	1.57	1.12	1.89
Phosphorus (P) (%)	2.70	2.42	1.41	1.02	1.78
Potassium (K) (%)	3.05	2.72	1.69	1.25	1.98

Method

Establishment of the Experiment, Seed Sowing and Cultivation of Plants

In the study, seven different applications were examined. The applications were as follows: 1) Control, 2) Chicken manure, 3) Turkey manure, 4) Sheep manure, 5) Cattle manure, 6) Vermicompost and 7) Chemical fertilizer. The experiment was established in randomized complete block design with three replications. There were 7 plots in each replication and there were 21 plots (7 X 3 = 21 plots) in total. The experiment field was made ready for sowing in September and 1.60 m^2 plots (1.45 m X 1.1 m) were prepared.

No fertilizer was added to the control application. Organic fertilizers were applied as 1.5 tons da^{-1}. Organic fertilizers were mixed into the soil before sowing at 2.4 kg per plot. In chemical fertilizer application, ammonium sulphate, triple superphosphate and potassium sulphate fertilizers were applied at 12 kg N da^{-1}, 10 kg P_2O_5 da^{-1} and 12 kg K_2O da^{-1}. All of phosphorus and potassium fertilizers and half of the nitrogen fertilizer were applied before sowing, while the other half of the nitrogen fertilizer was applied two weeks after sowing.

Seed sowing was done on 27.09.2017. Seeds were sown in 3 rows at 30 cm intervals in the plots. A space of 1 m was left among the plots to prevent the mixing of fertilizers. After the emergence, when the plants reached the 3-4 leaf stage, thinning was done to be intra-row spacing 10-15 cm. All necessary cultural practices were carried out regularly during cultivation period (Vural et al., 2000). No plant protection product was used during the experiment. During the experiment, the temperature and relative humidity values in the experiment field were recorded using a temperature and humidity recorder (Onset HOBO UX100-003 Data Logger, USA) (Figure 1). The plants were harvested 67 days after sowing on 03.12.2017. For the necessary measurements and analyses, the harvested plants were brought to the laboratory of Department of Horticulture, Faculty of Agriculture, Bolu Abant Izzet Baysal University.

Measurements and Analyses Made in the Plants

In the study, 25 properties related to plant growth, yield, quality and 24 elements were examined. Plant height (cm), plant diameter (cm), root length (cm), leaf blade length (cm), leaf blade width (cm) and leaf stalk length (cm) were determined by measuring with a ruler. The root collar diameter (mm), leaf blade thickness (mm) and leaf stalk thickness (mm) were measured with a digital caliper. Plant fresh weight (g) and root fresh weight (g) were detected by weighing with a precision balance. Plant dry weight (g) and root dry weight (g) were determined by weighing with a precision balance the samples after drying in an oven at 65 °C until they reach a constant weight. The number of marketable leaves (number $plant^{-1}$) was determined by counting the marketable leaves. In order to determine the yield (g m^{-2}), all of the plants in each plot were harvested by cutting their roots and the total weight was detected over m^2 by weighing with a precision balance. The chlorophyll content of the leaves (spad) was determined with a chlorophyll meter (Apogee Chlorophyll Concentration Meter, MC-100). The dry matter content (%) was determined by using the procedures of AOAC (1990). The pH values of the samples were measured using a digital pH meter (Thermo Scientific, Orion Star A111). Total soluble solid content (%) was measured with a hand-held refractometer (ATC-1, Atago, Japan). The colour properties of the leaves (L*, a*, b*, C* and h°) were detected using a colorimeter (3NH NR60CP). The nitrate and phosphorus contents of the samples (mg kg^{-1}) were determined using UV-visible spectrophotometer (UV-1800, Shimadzu, Japan). The nitrogen and sulphur contents of the samples (%) were detected using Elemental Analyzer CHNS-O (Thermo Scientific, Flash 2000). To determine element (aluminum, arsenic, barium, boron, cadmium, calcium, chrome, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, potassium, selenium, sodium, thallium, tinnen and zinc) contents of the samples (mg kg^{-1}), dried samples were firstly ground by using a grinder (MC23200, Siemens, Germany) and then prepared for analysis according to the microwave digestion method. Element contents of the samples were detected using inductively coupled plasma-mass spectrometry ICP-MS X Series (Thermo Scientific, UK).

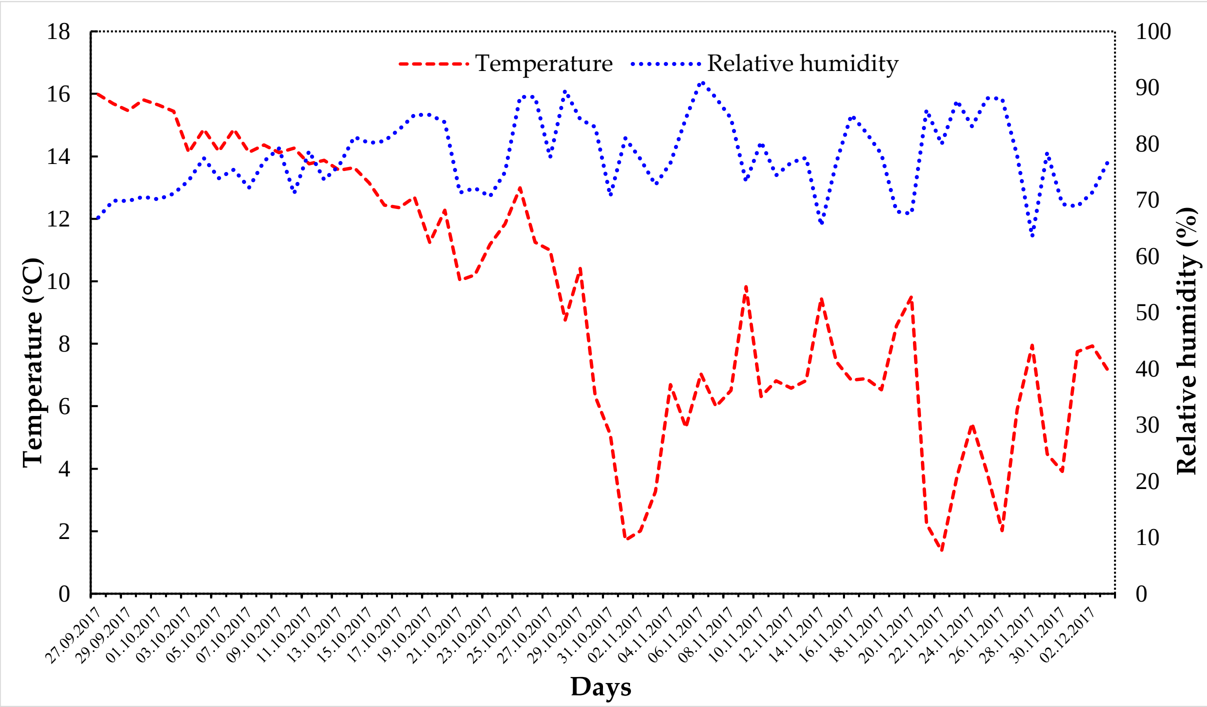

Figure 1. Temperature and relative humidity values measured in the experiment area during the experiment.

Şekil 1. Deneme süresince deneme alanında ölçülen sıcaklık ve nispi nem değerleri.

Statistical Analysis

All chemical analyses were performed with three replications. Measurements for morphological properties carried out with ten replications. Data obtained in the study were subjected to analysis of variance (ANOVA) using SPSS statistical software (Version 23.0). The means showing statistical significance were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Effects of different fertilizer applications on plant height, plant diameter, plant fresh weight, plant dry weight and yield in spinach are given in Table 2. The difference among the applications in terms of plant fresh weight, plant dry weight and yield was significant at the $P < 0.05$ level, while the difference among the applications in terms of plant height and plant diameter was significant at the $P < 0.01$ level. Among the applications, the highest plant height (11.18 cm) was determined in turkey and cattle manure applications, followed by chicken, vermicompost, sheep and chemical fertilizer applications which there was no statistically significant difference among them. On the other hand, the lowest plant height were detected in the control application with 9.66 cm. The highest value in terms of plant diameter was determined in cattle manure (18.27 cm), and it was closely followed by turkey, chicken, chemical, sheep and vermicompost applications which they were statistically in the same group with cattle manure. However, the lowest value in terms of plant diameter was observed in the control (15.82 cm). Plant fresh weight varied from 8.88 to 11.07 g. Turkey, cattle and chicken manure applications which there was no statistically significant difference among them possessed the highest values in terms of plant fresh weight. They were followed by sheep, vermicompost and chemical fertilizer applications. Conversely, the lowest plant fresh weight were observed in the control. When the effect of different fertilizer applications on plant dry weight in spinach was examined, the highest value was obtained from vermicompost application (1.00 g), and it was followed by chicken and turkey manure applications. On the other hand, the lowest values in terms of plant dry weight were determined in chemical, sheep, cattle and control applications. The highest yield was obtained from cattle and sheep manure applications (444.54 and 425.09 g m^{-2}, respectively). Cattle and sheep manure were followed by vermicompost, chicken, turkey and chemical fertilizer applications which they were not statistically different. Conversely, the lowest yield was recorded in the control application (236.36 g m^{-2}). In the present study, all organic fertilizer applications increased plant height, plant diameter, plant fresh weight and yield compared with the control. When compared to the control, cattle manure application increased plant height by 15.73%, plant diameter by 15.48% and yield by 88.08%. Furthermore, cattle manure application increased yield by 41.16% compared to the chemical fertilizer. It was determined that turkey manure application increased plant fresh weight by 24.66% and plant height by 15.73% compared to the control. Vermicompost application increased plant dry weight by 20.48% compared with the control. In addition, the higher values in terms of plant height, plant fresh weight, plant dry weight and yield were obtained from all organic fertilizer applications in comparison with chemical fertilizer (Table 2).

Table 2. Effects of different fertilizer applications on plant height, plant diameter, plant fresh weight, plant dry weight and yield in spinach.
Çizelge 2. Farklı gübre uygulamalarının ıspanakta bitki boyu, bitki eni, bitki yaş ağırlığı, bitki kuru ağırlığı ve verim üzerine etkileri.

Fertilizers	Plant height (cm)	Plant diameter (cm)	Plant fresh weight (g)	Plant dry weight (g)	Yield (g m^{-2})
Control	9.66b**	15.82b**	8.88b*	0.83b*	236.36b*
Chicken	10.78a	17.69a	10.78a	0.95ab	367.55ab
Turkey	11.18a	17.74a	11.07a	0.86ab	365.50ab
Sheep	10.38ab	17.39a	10.35ab	0.81b	425.09a
Cattle	11.18a	18.27a	10.84a	0.82b	444.54a
Vermicompost	10.70a	17.21a	9.92ab	1.00a	388.11ab
Chemical	10.34ab	17.50a	9.87ab	0.79b	314.93ab

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at $P < 0.05$, **: Significant at $P < 0.01$.

Similar to our results, Çıtak et al. (2011) investigated effects of barnyard manure and vermicompost on plant growth in spinach and reported that barnyard manure and vermicompost significantly increased plant height and yield compared to control and the highest plant height and yield were determined in barnyard manure. In another study, it was determined that plant height and yield in farmyard manure were significantly higher than control and chemical fertilizer in spinach (Shaheen et al., 2017). In the study conducted by Mufwanzala and Dikinya (2010) in spinach, it was determined that chicken manure significantly increased plant dry weight as compared with the control. Altuntas et al. (2018) reported that plant height and yield in vermicompost applications was found to be significantly higher than control and chemical fertilizer in spinach. Xu and Mou (2016) stated that vermicompost applications increased plant fresh weight and plant dry weight compared to control in spinach. Likewise, Ansari (2008), Özkan and Müftüoğlu (2016) and Durak et al. (2017) reported that vermicompost applications increased yield compared to the control in spinach and lettuce. In the study carried out by Tavalı et al. (2013) in summer squash, it was detected that chicken manure and vermicompost significantly increased yield as compared with the control. Our results were found to be compatible with the findings of previous researchers. Citak and Sonmez (2010) investigated the effects of chemical fertilizer and organic fertilizers (chicken manure, barnyard manure and blood meal) on plant growth in spinach and found that the highest plant height and yield were determined in chemical fertilizer, which was not compatible with our results.

As seen in Table 3, there was significant difference ($P < 0.05$) in terms of root dry weight among the applications. On the other hand, no statistically significant difference was found among the applications in terms of root length, root collar diameter and root fresh weight. Root length, root collar diameter and root fresh weight depending on the applications in the study ranged from 10.16 to 11.69 cm, 3.06 to 3.80 mm, and 2.75 to 3.68 g, respectively. Among the applications, the highest root dry weight (0.45 g) was determined in turkey manure application, followed by vermicompost, chemical, chicken and sheep manure applications which there was no statistically significant difference among them. The lowest values in terms of root dry weight were detected in control and cattle manure (0.29 and 0.32 g, respectively) applications (Table 3).

Similar to our results, Kashem et al. (2015) and Kumari et al. (2017) reported that vermicompost applications significantly increased root dry weight as compared with the control in tomato and eggplant, respectively. In another study, there was no statistically significant difference with respect to root length among control and vermicompost applications in lettuce (Adiloğlu et al., 2018), which was compatible with our findings. In the study carried out by Kovacs et al. (2016) in spinach, root wet weight in cattle manure application was found to be significantly higher than the control. It was reported that vermicompost applications significantly increased root wet weight compared to the control in spinach (Özkan et al., 2016).

Table 3. Effects of different fertilizer applications on root length, root collar diameter, root fresh weight and root dry weight in spinach.
Çizelge 3. Farklı gübre uygulamalarının ıspanakta kök uzunluğu, kök boğazı çapı, kök yaş ağırlığı ve kök kuru ağırlığı üzerine etkileri.

Fertilizers	Root length (cm)	Root collar diameter (mm)	Root fresh weight (g)	Root dry weight (g)
Control	10.32^{ns}	3.06^{ns}	2.75^{ns}	0.29b*
Chicken	11.36	3.74	3.52	0.36ab
Turkey	10.44	3.80	3.68	0.45a
Sheep	11.54	3.45	2.94	0.35ab
Cattle	10.93	3.44	2.80	0.32b
Vermicompost	11.69	3.46	2.87	0.39ab
Chemical	10.16	3.53	3.12	0.38ab

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test.
*: significant at $P < 0.05$, ns: non-significant.

The difference among the applications in terms of leaf stalk thickness and number of marketable leaves was significant at the $P < 0.05$ level, while the difference among the applications in terms of leaf blade length, leaf blade width, leaf blade thickness and leaf stalk length was significant at the $P < 0.01$ level. Leaf blade length, leaf blade width and leaf blade thickness depending on the applications ranged from 5.02 to 6.41 cm, 4.14 to 5.28 cm, and 0.87 to 1.19 mm, respectively. The highest values in terms of leaf properties mentioned above were obtained from turkey and chicken manure applications, while the lowest values were observed in the control. Leaf stalk length and number of marketable leaves varied from 3.76 to 4.82 cm and 7.81 to 8.57, respectively. Although the lowest leaf stalk length and number of marketable leaves were determined in the control application, the highest values were observed in all fertilizer applications which they were statistically in the same group. Leaf stalk thickness varied between 6.68 (control) and 7.73 (chicken manure) mm. In the study, it was determined that all organic fertilizer applications significantly increased leaf blade length, leaf blade width, leaf blade thickness, leaf stalk length, leaf stalk thickness and number of marketable leaves in comparison with control. In addition, the higher values with regard to number of marketable leaves were obtained from all organic fertilizer applications compared to chemical fertilizer. When compared to the control, turkey manure application increased leaf blade length by 27.69%.

It was found that chicken manure application increased leaf blade width by 27.54% and number of marketable leaves by 9.73% compared with the control (Table 4).

Çıtak et al. (2011) reported that barnyard manure and vermicompost significantly increased leaf stalk length, leaf stalk thickness and number of leaves compared to the control in spinach. It was detected that vermicompost applications significantly increased leaf length, leaf width and number of leaves as compared with the control in spinach (Özkan et al., 2016). Xu and Mou (2016) reported that vermicompost applications increased number of leaves compared to the control in spinach. Likewise, it was found that vermicompost applications increased leaf length, leaf width and number of leaves as compared with the control in lettuce (Özkan and Müftüoğlu, 2016; Adiloğlu et al., 2018). The results obtained in this study regarding leaf properties are consistent with the findings in the literature. Citak and Sonmez (2010) reported that the highest leaf stalk length, leaf stalk diameter and number of leaves in spinach were determined in chemical fertilizer among chicken manure, barnyard manure, blood meal and chemical fertilizer, which was not consistent with our findings.

Table 4. Effects of different fertilizer applications on leaf blade length, leaf blade width, leaf blade thickness, leaf stalk length, leaf stalk thickness and number of marketable leaves in spinach.

Çizelge 4. Farklı gübre uygulamalarının ıspanakta yaprak ayası boyu, yaprak ayası eni, yaprak ayası kalınlığı, yaprak sapı uzunluğu, yaprak sapı kalınlığı ve pazarlanabilir yaprak sayısı üzerine etkileri.

Fertilizers	Leaf blade length (cm)	Leaf blade width (cm)	Leaf blade thickness (mm)	Leaf stalk length (cm)	Leaf stalk thickness (mm)	Number of marketable leaves (number plant^{-1})
Control	5.02c**	4.14d**	0.87c**	3.76b**	6.68c*	7.81b*
Chicken	6.34a	5.28a	1.12ab	4.49a	7.73a	8.57a
Turkey	6.41a	5.21a	1.19a	4.81a	7.49ab	8.52a
Sheep	5.81b	4.64bc	1.08abc	4.46a	6.85bc	8.52a
Cattle	6.19ab	5.00ab	0.98bc	4.82a	7.16abc	8.33a
Vermicompost	5.74b	4.40cd	0.93bc	4.32a	6.83bc	8.43a
Chemical	5.91ab	4.73bc	0.95bc	4.41a	7.11abc	8.14ab

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: significant at $P < 0.05$, **: Significant at $P < 0.01$.

The analysis of variance showed that there were significant differences ($P < 0.01$) among the applications in terms of chlorophyll content, dry matter content, pH, total soluble solid content and nitrate content in spinach. Chlorophyll content in spinach plants belonging to different applications varied between 54.71 and 68.93 spad. The highest chlorophyll content was observed in chicken manure application, followed by sheep manure application, though the lowest chlorophyll content was recorded in the control application. The maximum and minimum dry matter contents were obtained from cattle manure (5.89%) and control (4.32%) applications, respectively. In the present study, pH content of spinach plants varied between 6.53 and 6.76. The highest pH values were determined in turkey, chemical, sheep and chicken manure applications which they were statistically in the same group. On the other hand, the lowest pH was detected in the plants applied vermicompost fertilizer. The total soluble solid content varied between 1.51 and 2.28% depending on the applications. The highest values with regard to total soluble solid content were found in turkey, chemical, sheep, control and cattle manure applications, while the lowest values were observed in vermicompost and chicken manure applications. There was a wide range variation in nitrate content of spinach plants. When the nitrate content was examined, the highest values were found in chicken, vermicompost, chemical and cattle manure applications (1182.62, 1109.93, 1095.97 and 1017.29 mg kg^{-1}, respectively). Whereas, the lowest nitrate content was detected in the control application (518.71 mg kg^{-1}). It was determined that organic fertilizer applications increased chlorophyll content compared to the control. Chicken manure application increased the chlorophyll content in the plant by 25.99% compared to the control application. The higher values in terms of dry matter content were obtained from all organic fertilizer applications in the study in comparison with control and chemical fertilizer. All fertilizer applications increased the nitrate content in the plant compared to the control. However, nitrate contents of plants applied sheep, turkey and cattle manure were lower than that of chemical fertilizer. The nitrate content of the plants applied sheep manure was 77.40% lower than the plants applied chemical fertilizer (Table 5).

Baliah and Muthulakshmi (2017) and Kumarpandit et al. (2017) stated that farmyard manure and vermicompost significantly increased chlorophyll contents of spinach and okra compared to the control, which was consistent with our findings. Degwale (2016) and Mohanta et al. (2018) determined that vermicompost increased dry matter content as compared with the control in garlic and broccoli, respectively, which was agreed with our findings. Contrary to our results, it was determined that vermicompost applications increased total soluble solid content compared to the control in spinach (Peyvast et al., 2008). Tavalı et al. (2013) and Tavalı et al. (2014) reported that there was no statistically significant difference with respect to pH value and total soluble solid content among control, vermicompost and chemical fertilizer applications in cauliflower and cabbage. In the present study, the higher nitrate content detected in organic and chemical fertilizer applications compared to the control may be due to the high nitrogen content of these fertilizers. Nevertheless, the nitrate content determined in this study found to be lower than the critical values recommended for human health according to World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO). Tsai (2005) reported that the high nitrate content in the plant at increasing doses of organic fertilizers is due to increased nitrogen uptake. In the study conducted by Vigardt (2012) on spinach, higher nitrate content in vermicompost applications was found as compared with the control, which was consistent with our findings. In the studies carried out in spinach, broccoli and carrot, the highest nitrate content was determined in chemical fertilizer among vermicompost, chicken manure, barnyard manure and chemical fertilizer (Abubaker et al., 2010; Citak and Sonmez, 2010; Çıtak, 2014).

Table 5. Effects of different fertilizer applications on chlorophyll content, dry matter content, pH, total soluble solid content and nitrate content in spinach.

Çizelge 5. Farklı gübre uygulamalarının ıspanakta klorofil içeriği, kuru madde oranı, pH, suda çözünebilir kuru madde miktarı ve nitrat içeriği üzerine etkileri.

Fertilizers	Chlorophyll content (spad)	Dry matter content (%)	pH	Total soluble solid content (%)	Nitrate content (mg kg^{-1})
Control	54.71c**	4.32c**	6.63bc**	2.00a**	518.71d**
Chicken	68.93a	4.59bc	6.72ab	1.53b	1182.62a
Turkey	63.08b	4.62bc	6.76a	2.28a	875.25bc
Sheep	64.19ab	5.61ab	6.73ab	2.17a	617.77cd
Cattle	59.94b	5.89a	6.57cd	1.85ab	1017.29ab
Vermicompost	61.88b	5.03abc	6.53d	1.51b	1109.93ab
Chemical	63.22b	4.05c	6.75a	2.26a	1095.97ab

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. **: Significant at $P < 0.01$.

Significant difference ($P < 0.01$) was found among the applications with regards to h° (Hue angle) colour value. However, no statistically significant difference was found among the applications with respect to L*, a*, b* and C* (Chroma) colour values. The h° colour values of the spinach samples varied from 117.88 (vermicompost) to 122.12 (chemical fertilizer). An apparent effect of different fertilizer applications on the colour properties of spinach was not observed (Table 6).

Colour is one of the most important factors in terms of quality in spinach. Similar to our results, in the study carried out by Özen (2018) in lettuce, the effect of different organic materials (waste mushroom compost, leonardite and vermicompost) and their different doses on colour was found to be insignificant.

Table 6. Effects of different fertilizer applications on colour properties of the leaves (L*, a*, b*, C* and h°) in spinach.
Çizelge 6. Farklı gübre uygulamalarının ıspanakta yaprakların renk özellikleri (L, a*, b*, C* ve h°) üzerine etkileri.*

Fertilizers	L*	a*	b*	C*	h°
Control	37.97^{ns}	-6.66^{ns}	11.53^{ns}	13.32^{ns}	120.10ab**
Chicken	37.86	-5.98	10.71	12.27	119.13b
Turkey	37.79	-5.89	11.40	12.85	117.91b
Sheep	38.06	-5.75	9.95	11.50	120.17ab
Cattle	38.40	-6.59	11.19	13.00	120.41ab
Vermicompost	37.57	-6.22	11.63	13.19	117.88b
Chemical	37.30	-6.05	9.64	11.38	122.12a

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. **: Significant at $P < 0.01$, ns: non-significant.

Effects of different fertilizer applications on element contents in spinach are presented in Table 7. The differences among the applications in terms of boron (B), barium (Ba), calcium (Ca), chrome (Cr), copper (Cu), potassium (K), magnesium (Mg), manganese (Mn), nitrogen (N), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), sulphur (S), selenium (Se), tinnen (Sn) and zinc (Zn) contents were significant at the $P < 0.01$ level, while the differences among the applications in terms of aluminum (Al), arsenic (As), cadmium (Cd) and iron (Fe) contents were significant at the $P < 0.05$ level. On the other hand, no statistically significant difference was found among the applications in terms of cobalt (Co) content. In addition to, it was determined that the mercury (Hg) and thallium (Tl) contents of spinach plants belonging to different applications examined in the study were below the detection limits (Table 7).

In the current study, a considerable variation in terms of element contents was determined among the applications. Potassium, calcium, magnesium, phosphorus, sodium, nitrogen and sulphur contents depending on the applications varied from 20724 to 25067 mg kg^{-1}, 11455 to 15641 mg kg^{-1}, 1909 to 2507 mg kg^{-1}, 489.26 to 518.82 mg kg^{-1}, 397.61 to 770.18 mg kg^{-1}, 3.43 to 5.91% and 2.18 to 7.21%, respectively. Among the applications, the lowest values in terms of potassium, calcium, magnesium, phosphorus, nitrogen and sulphur contents were observed in the control. The highest calcium contents were detected in chemical, turkey, vermicompost, cattle and sheep manure applications which they were statistically in the same group. It was determined that the plants applied vermicompost fertilizer had the highest potassium, phosphorus, nitrogen and sulphur contents. The highest values regarding magnesium content were found in turkey, cattle, sheep, chemical and vermicompost applications which they were not statistically different. The highest sodium content was observed in cattle manure application, though the lowest sodium content was recorded in sheep manure application. It was determined that organic fertilizer applications significantly increased essential mineral contents such as potassium, calcium, magnesium, phosphorus, nitrogen and sulphur in the plant compared to the control. When compared to the control, vermicompost application increased potassium content by 20.95% and nitrogen content by 72.30%. Furthermore, vermicompost application increased the sulphur content of the plant approximately 3 times compared with the control. It was found that turkey manure application increased calcium content by 35.26% and magnesium content by 31.36% compared to the control. In addition to this, the higher values in terms of phosphorus, nitrogen and sulphur contents were obtained from all organic fertilizer applications in comparison with chemical fertilizer (Table 7).

Iron, manganese, zinc, boron, copper, barium and selenium contents depending on the applications varied from 107.78 to 170.96 mg kg^{-1}, 98.05 to 117.44 mg kg^{-1}, 74.06 to 97.06 mg kg^{-1}, 44.72 to 52.09 mg kg^{-1}, 21.11 to 29.21 mg kg^{-1}, 13.85 to 24.81 mg kg^{-1}and 0.10 to 0.16 mg kg^{-1}, respectively. The highest iron contents were obtained from turkey, chicken, chemical and cattle manure applications which they were not statistically different. Conversely, iron content in control, sheep manure and vermicompost applications was found to be the lowest. The lowest values in terms of manganese, zinc and selenium contents were observed in the control. Manganese content was the highest in chemical and cattle manure applications followed by turkey manure application. Zinc content was the highest in cattle manure application. The highest values in terms of selenium content were determined in cattle, sheep, vermicompost and turkey manure applications which they were statistically in the same group. The maximum and minimum copper contents were obtained from vermicompost and chemical fertilizer applications, respectively. When the boron content was examined, it was determined that there was no statistically significant difference among turkey, sheep, vermicompost, cattle and chemical fertilizer applications and they had the highest boron contents. However, the lowest boron content was observed in chicken manure and control applications. The highest value with regard to barium content was found in turkey manure application, while the lowest value was observed in chemical fertilizer. It was detected that, all organic fertilizer applications significantly increased iron, manganese, zinc and selenium contents in the plant compared to the control. When compared to the control, cattle manure application increased selenium content by 60.00%, zinc content by 31.06% and manganese content by 17.79%. It was observed that turkey manure application increased iron content by 58.62% compared with the control. Additionally, the higher values in terms of selenium content were obtained from all organic fertilizer applications in comparison with chemical fertilizer (Table 7).

It was determined that the plants applied chemical fertilizer had the highest aluminum content (125.55 mg kg^{-1}). Conversely, aluminum content in sheep manure application (76.02 mg kg^{-1}) was found to be considerably lower than those of other applications examined in the study. The highest arsenic content was observed in the control application (0.41 mg kg^{-1}), though the lowest arsenic content was recorded in vermicompost application (0.25 mg kg^{-1}). In the study, the maximum and minimum values for cadmium were detected in chemical fertilizer (0.53 mg kg^{-1}) and vermicompost (0.40 mg kg^{-1}) applications, respectively. The highest chrome content was found in the control application (0.49 mg kg^{-1}), whereas the lowest chrome content was observed in the fertilizer applications (chicken, turkey, sheep, cattle, vermicompost and chemical fertilizer) examined in the study. Nickel content was found to between 1.07 (chemical fertilizer) and 2.28 mg kg^{-1} (control). Lead content was in the range from 0.11 (control) to 0.75 mg kg^{-1} (chicken manure). The highest tinnen content was found in cattle manure application with 1.83 mg kg^{-1}. On the contrary, tinnen content was the lowest in sheep manure application (1.22 mg kg^{-1}). The arsenic, chrome, cobalt, and nickel contents in organic fertilizer applications were found to be lower than the control. Additionally, aluminum and cadmium contents in organic fertilizer applications were lower than that of chemical fertilizer. This is very important considering that arsenic, chrome, cobalt, nickel, aluminum and cadmium are heavy metals. On the other hand, it was determined that lead content in organic fertilizer applications was significantly higher than control and chemical fertilizer (Table 7).

It is thought that the potassium, nitrogen and phosphorus contents of organic fertilizers used in the experiment is effective in increasing potassium, nitrogen and phosphorus contents of plant in organic fertilizer applications compared to the control in this study. Similar to our results, Peyvast et al. (2008) determined that vermicompost applications increased potassium, calcium, magnesium, nitrogen, phosphorus, iron, manganese, zinc and copper contents in spinach compared to the control. Likewise, Hernandez et al. (2010), Tavalı et al. (2014) and Durak et al. (2017) reported that vermicompost applications increased potassium, calcium, nitrogen, magnesium, phosphorus, iron, zinc, copper and manganese contents in lettuce and cabbage compared to the control. Çıtak (2014) found that barnyard manure and vermicompost applications increased potassium, sulphur and iron contents in broccoli and carrot compared to control and chemical fertilizer. In the study conducted by Mufwanzala and Dikinya (2010) in spinach, it was determined that chicken manure significantly increased nitrogen and phosphorus contents in the plant compared to the control. Our results were found to be compatible with the findings of previous researchers. Contrary to our results, Çıtak et al. (2011) reported that potassium, magnesium, nitrogen, iron, and zinc contents in barnyard manure and vermicompost applications were lower than the control in spinach. Researchers also found that there were no significant differences with respect to manganese and copper contents among vermicompost, barnyard manure and control applications. In another study, Hınıslı (2014) found that the highest potassium, phosphorus, and boron contents in lettuce were determined in chemical fertilizer among vermicompost, cow manure and sheep manure. Kumarpandit et al. (2017) reported that farmyard manure significantly decreased cadmium content of spinach compared to the control.

Table 7. Effects of different fertilizer applications on element contents in spinach.
Çizelge 7. Farklı gübre uygulamalarının ıspanakta element içerikleri üzerine etkileri.

Fertilizers	**Al (mg kg^{-1})**	**As (mg kg^{-1})**	**B (mg kg^{-1})**	**Ba (mg kg^{-1})**	**Ca (mg kg^{-1})**	**Cd (mg kg^{-1})**
Control	116.14abc*	0.41a*	44.83b**	19.94b**	11455c**	0.43b*
Chicken	105.30abc	0.35abc	44.72b	19.95b	12763bc	0.47ab
Turkey	117.86ab	0.37ab	52.09a	24.81a	15494a	0.46ab
Sheep	76.02c	0.33abc	51.44a	17.84b	13908ab	0.45b
Cattle	99.78abc	0.36ab	49.92a	19.17b	14342ab	0.46b
Vermicompost	81.99bc	0.25c	50.47a	19.95b	14627a	0.40b
Chemical	125.55a	0.29bc	47.73ab	13.85c	15641a	0.53a
Fertilizers	**Co (mg kg^{-1})**	**Cr (mg kg^{-1})**	**Cu (mg kg^{-1})**	**Fe (mg kg^{-1})**	**Hg (mg kg^{-1})**	**K (mg kg^{-1})**
Control	0.39^{ns}	0.49a**	24.63b**	107.78b*	nd	20724d**
Chicken	0.27	0.37b	23.25bc	157.56ab	nd	22584c
Turkey	0.38	0.39b	24.06b	170.96a	nd	23594bc
Sheep	0.25	0.30b	24.47b	109.51b	nd	23477bc
Cattle	0.33	0.38b	24.80b	125.32ab	nd	23905abc
Vermicompost	0.26	0.32b	29.21a	113.77b	nd	25067a
Chemical	0.32	0.36b	21.11c	141.32ab	nd	24864ab
Fertilizers	**Mg (mg kg^{-1})**	**Mn (mg kg^{-1})**	**N (%)**	**Na (mg kg^{-1})**	**Ni (mg kg^{-1})**	**P (mg kg^{-1})**
Control	1909c**	98.05c**	3.43g**	597.26abc**	2.28a**	489.26f**
Chicken	2135b	102.78bc	5.80b	575.11abc	1.98a	499.61d
Turkey	2507a	114.79ab	5.35c	521.87bc	2.01a	505.27c
Sheep	2400a	105.27abc	4.94e	397.61c	1.61ab	514.88b
Cattle	2445a	115.49a	5.03d	770.18a	1.65ab	496.91de
Vermicompost	2351a	106.47abc	5.91a	650.92ab	1.70ab	518.82a
Chemical	2388a	117.44a	4.75f	543.10bc	1.07b	493.06ef
Fertilizers	**Pb (mg kg^{-1})**	**S (%)**	**Se (mg kg^{-1})**	**Sn (mg kg^{-1})**	**Tl (mg kg^{-1})**	**Zn (mg kg^{-1})**
Control	0.11b**	2.18g**	0.10c**	1.32cd**	nd	74.06c**
Chicken	0.75a	6.25b	0.12bc	1.40c	nd	82.92bc
Turkey	0.16b	5.48c	0.14ab	1.59b	nd	77.03bc
Sheep	0.56a	3.64e	0.15a	1.22d	nd	75.89bc
Cattle	0.50a	5.05d	0.16a	1.83a	nd	97.06a
Vermicompost	0.54a	7.21a	0.14ab	1.54b	nd	76.30bc
Chemical	0.13b	2.29f	0.11bc	1.41c	nd	84.63b

Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test. *: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ns: non-significant, nd: not detected.

CONCLUSION

Nowadays, the use of organic fertilizers in vegetable cultivation has gained great importance in terms of both human and environmental health and sustainability in agriculture. In this study, the effects of different organic fertilizer applications on plant growth, yield, quality properties and element contents of spinach grown under open field conditions in Bolu province were investigated. According to findings obtained from the present study, the applications evaluated in the study significantly affected the plant growth, yield, quality properties and element contents in spinach. The results also indicated that organic fertilizer applications generally increased yield, plant height, plant wet weight, plant dry weight, dry matter content, number of marketable leaves, nitrogen, phosphorus, sulphur and selenium contents of the plant in comparison with control and chemical fertilizer. The highest yield was determined in cattle manure application. An apparent effect of different fertilizer applications on the colour properties of spinach was not observed. In organic fertilizer applications, heavy metal contents such as arsenic, chrome, cobalt, and nickel were found to be lower than the control. Additionally, the contents of heavy metals such as aluminum and cadmium in organic fertilizer applications were lower than that of chemical fertilizer. Consequently, all organic fertilizer applications in terms of yield, plant height, plant wet weight, dry matter content, number of marketable leaves, nitrogen, phosphorus, sulphur and selenium contents; chicken, turkey and sheep manures in terms of chlorophyll content; chicken, turkey and cattle manures in terms of leaf blade length, leaf blade width and leaf stalk thickness; turkey and sheep manures in terms of total soluble solid and nitrate contents; vermicompost in terms of potassium content; turkey, vermicompost, cattle and sheep manures in terms of calcium, magnesium and manganese contents; turkey, chicken and cattle manures in terms of iron content; cattle and chicken manures in terms of zinc content were found to be the most successful organic fertilizer applications. It was concluded that organic fertilizers examined in the study have positive effects on plant growth, yield, quality properties and element contents of spinach and organic fertilizers can be used successfully for sustainable agriculture in spinach cultivation. The findings of this study will be beneficial in terms of reducing the use of chemical fertilizers in agricultural production, contributing to the widespread use of organic fertilizers, and obtaining healthier and higher quality agricultural products in Turkey.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

Beyhan Kibar: Design of the study, statistical analysis, evaluation of the study, writing of the manuscript
Özgül Yaman Türkkan: Carrying out of the experiment, performing of the field and laboratory studies,

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