RESEARCH ARTICLE

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Oxalic acid: an important organic acid to increase yield and quality in lettuce

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Abstract
In the present study, the effects of different doses of oxalic acid applications on yield and quality in lettuce cultivation were evaluated. Yedikule 5107 variety was used as plant material and four different doses of oxalic acid (0-2-4-6 mM) were applied to lettuce plants from leaves. Plant weight varied between 343.02-432.57 g/plant, plant height 28.0-30.35 cm, plant diameter 26.67-28.72 cm, leaf length 21.23-22.44 cm, root collar diameter 19.11-21.49 mm and number of leaves 47.57-55.63 per plant depending on oxalic acid doses, and the highest yield was obtained from 2 mM oxalic acid application. Total chlorophyll and total phenolic contents varied between 37.47-39.31 and 67.35-103.98 mg/100g, respectively. While the highest chlorophyll value was obtained from 2 mM oxalic acid; the highest phenolic substance value was obtained from 4 mM oxalic acid application. It was determined that L^*, a^*, b^* and C^* values varied from 47.43 to 48.76, -17.55 to -18.26, 27.89 to 28.68, and 32.95 to 34.00 depending on applications, and the highest L^* value was obtained from 2 mM oxalic acid application and the highest a^*, b^* and C^* values were observed in 2 mM and 4 mM oxalic acid applications. The antiradical values in lettuce varied between 42.36-82.64%. At the end of the study, when all these parameters were considered, it was determined that oxalic acid applications significantly and positively affect the yield and quality of lettuce.
Keywords: Oxalic acid, Lettuce, Quality, Yield

INTRODUCTION

Lettuce (*Lactuca sativa* L.) belonging to the Compositae family is one of the cool climate vegetable species (Pink and Keane, 1993). Lettuce is among the most important and consumed types of leafy vegetables in the world (Mou, 2009). According to the data for 2021, the total lettuce production in the world is 27.011.748 tons (FAO, 2023).

Technological and scientific developments not only affect individuals' social, cultural, and economic qualities but also cause significant changes in their perspectives on life and consumption habits. Especially today, one of the areas where the understanding of being a sensitive and conscious consumer is widespread is food consumption (Altunişik et al., 2003). Instead of meeting their basic nutritional needs, people are now turning to healthy foods that can reduce their discomfort and increase the length and quality of their lives (Baslam et al., 2013).

Nowadays, the attractiveness of vegetables has increased due to their beneficial nutritional value, ease of consumption as fresh, and their entry into the group of minimally processed or fresh foods, thus leading to an increase in consumer demand (Jiang et al., 2020). In addition, epidemiological studies have shown that there is a relationship between increased vegetable consumption and reduced risks of cancer, cardiovascular and chronic diseases (Hung et al., 2004; Pavia et al., 2006; Morris et al., 2006). The reasons why vegetables are beneficial to health are explained by the macro-micro nutrients and bioactive compounds they contain (Kris-Etherton et al., 2002; Soetan et al., 2010).

Lettuce is a type of vegetable that is rich in many vitamins, minerals, and nutritional content necessary for human health (Costa et al., 2015; Konatu et al., 2017; Lara et al., 2017). Lettuce is an important dietary vegetable because it contains a very low amount of calories and fat. It is also one of the types of vegetables rich in vitamins A, C, E, and antioxidants (Nicolle et al., 2004).

In the developing world, the population is constantly increasing and the need for food and food products is also increasing (Demirel et al., 2022). As the global population increases, the demand for food and the desire and efforts to achieve high yields in agricultural production has accelerated (Beacham et al., 2019). For this purpose, studies on different compounds that act as plant growth regulators have gained intensity in order to increase production and obtain quality products. One of the compounds that have been emphasized in recent years is oxalic acid.

Oxalic acid is naturally found in the structure of plants and is an organic acid that increases the resistance of plants to environmental stresses (Liang et al., 2009). It has been reported that it plays different roles for each organism in plants, fungi, and mammals (Shimada et al., 1997; Serna-Escolano et al., 2021). Oxalic acid, a final metabolite product of plants, has a variety of physiological effects, mostly enhancing the defense-related enzymes' activity and secondary metabolites such as phenolics to promote systemic tolerance against infections caused by bacteria, viruses, and fungi (Martinez-Esplá et al., 2014). The researchers suggested that more metabolic studies on oxalic acid should be done (García-Pastor et al., 2020).

In studies on pre-harvest oxalic acid applications in vegetables and fruits, it was determined that applications positively affected antioxidant activity, total anthocyanin, total phenolic content (Martinez-Esplá et al., 2014), fruit weight, fruit firmness, total sugar content, and color parameters (Serna-Escolano et al., 2021). It has been revealed that the post-harvest applications increase the storage life of the products and prevent quality loss (Yücel, 2005), and also increase the resistance of the fruits to post-harvest browning, ripening, and chilling damage (Yoruk, 2002; Zheng et al., 2007a, 2007b; Huang et al., 2013a, 2013b). However, when the literature is evaluated, it becomes clear that more research is needed on the effects of preharvest oxalic acid applications on vegetable production and quality characteristics. For this purpose, our study focused on the effects of oxalic acid applications on the yield and quality of lettuce.

MATERIALS AND METHODS

The study was performed under open field conditions at Isparta (Aliköy), Türkiye (37° 48′ N and 30° 38′ E, altitude 1020 m). Lettuce seedlings (cv. Yedikule) were purchased from a commercial seedling production company (Anamas Tohum Ltd. Şti., Antalya, Türkiye) and prior to planting, diammonium phosphate was applied at a rate of 200 kg/ha. Seedlings were planted on 03 September 2019 in rows 40 cm apart with an intra-row spacing of 25 cm. 17 days after planting seedlings zinc sulfate was applied at a rate of 20 kg/ha and during the growth period, ammonium sulfate was applied to 3 times (10th day and 20th day after planting) at a rate of 100 kg/ha. The soil's characteristics of the experimental area were analyzed by ISLAB Soil and Plant Analysis Laboratory and the results are presented in Table 1.

Table 1. Characteristics of the experimental area's soil

Parameters	Values	Description
EC (dS/m)	0.12	Saltless
pH	8.38	Alkali
Lime (%)	5.75	Medium
Organic Matter (%)	1.81	Low
N (ppm)	497.00	Low
P (ppm)	7.00	Low
K (ppm)	718.21	Very high
Ca (ppm)	4 900.00	High
Mg (ppm)	436.04	Medium
Fe (ppm)	1.91	Low
Cu (ppm)	1.34	Medium
Mn (ppm)	12.57	Medium
Zn (ppm)	0.31	Low

Plants were exposed to 0, 2, 4, and 6 mM oxalic acid (Merck) solutions prepared with deionized water containing 0.5% Tween 20 (Merck) as a surfactant. Foliar oxalic acid applications were made three times at 10-day intervals from seedling planting. A randomized plot design with three replications was used, and each replication consisted of 60 plants.

The temperature and relative humidity during the experimental period varied from 9.8 to 20°C and 50.1-71.6%, respectively. Table 2 shows the climatic variables for the months of September to November during the experimental period.

After plants were harvested (19.11.2019), ten plants of each replication of the experimental plot were randomly taken and the following measurements were recorded: plant weight (g per plant), plant diameter (cm), plant height (cm), root collar diameter (mm), number of leaves, dry matter (%). For determining leaf width (cm), leaf length (cm), and total soluble solid (TSS) content (brix)

Table 2. Meteorological data

	Average Temperature (°C)	Total Precipitation (mm=kg/m²)	Average Relative Humidity (%)	Average Soil Temperature (°C) 10 cm	Average Soil Temperature (°C) 20 cm
September	20	26.5	50.1	21.4	23.1
October	15.7	9.9	59.1	16.3	18.4
November	9.8	28.6	71.6	9.8	12.1

(refractometer), the 2nd and 3rd leaves of 10 lettuce plants of each replication were used.

Also, L^* (Lightness), a^* (Red/Green Value), b^* (Blue/Yellow Value), C^* (Chroma), h^* (Hue), and chlorophyll SPAD values of leaves were determined by measuring in 3 different positions of the 2nd and 3rd leaves of 10 plants of each replication with a Minolta colorimeter (CR-400) and a portable chlorophyll meter (SPAD-502).

Ascorbic acid content

The content of ascorbic acid in lettuce leaves was determined with the method described by Cemeroğlu (2013). For this purpose, samples were taken from lettuce leaves and homogenized thoroughly with the help of a blender with the same amount of 2% oxalic acid. A certain amount of the mixture was weighed and made up of oxalic acid to 100 ml and then filtered. 10 ml of the filtrate was taken and titrated with 2.6 dichlorophenolindophenol solution. Results are expressed as mg of ascorbic acid/100 g of fresh weight.

Total phenolic content

Fresh leaf samples (5 g) were homogenized in 95% ethanol (20 mL) for 2 minutes. Then, the obtained homogeneous mixture was boiled for 10 minutes and centrifuged at 8000 rpm. After centrifugation, 20 ml of 80% ethanol was added to the samples filtered through filter paper, and boiled for 10 minutes. After this process, the mixture was made up to 100 ml with 80% ethanol. Then, using the Folin-Ciocalteu reagent, total phenolic content analysis was carried out according to Coseteng and Lee (1987) and the results were given as mg/100g.

Antiradical activity

The antiradical activities of lettuce leaves were determined according to the 2,2-diphenyl-1-picrylhydrazil (DPPH) method described by Dorman et al. (2003). First, the samples were mashed, filtered, and then centrifuged. Then, 450 µL of Tris-HCl buffer (50 mM, pH 7.4) was added to the samples in 50 µl. Finally, 1.00 mL of DPPH (0.10 mM, in methanol) solution was added to the mixture, and the samples were kept in the dark for 30 minutes. The absorbance reading values of the samples were obtained at a wavelength of 517 nm in the spectrophotometer, and the results were expressed as % Inhibition (DPPH).

Statistical analysis

Using the Minitab (17) Inc. Package program, data were analyzed in one-way analysis of variance (ANOVA). Significant means were compared with Tukey's multiple range test. The analysis was performed in triplicate.

RESULTS AND DISCUSSION

The effects of oxalic acid applications on plant weight, root collar diameter, plant diameter, plant height, leaf width, leaf length, and the number of leaves per plant in lettuce are given in Table 3. According to Table 3, the effects of oxalic acid applications on leaf width were found to be insignificant, whereas the effects on plant weight, plant diameter, plant height, root collar diameter, leaf length, and the number of leaves per plant were significant ($P<0.05$).

Wang et al. (2009), in their study on jujube, determined that oxalic acid application increased the RuBisCO activase enzyme. Moreover, García-Pastor et al. (2020) reported that with the increase of this enzyme, an increase in the rate of photosynthesis occurs, and thus vegetative growth is encouraged. They also suggested that more metabolic research on this subject should be done. Anwar et al. (2018) reported that the increase in leaf area showed an increase in water ingress to plant tissues, which played a positive role in water uptake or transport of oxalic acid to vegetative organs, thus supporting vegetative growth. These reports explain the reason for the increase in plant weight between 8.1%

Table 3. Effects of applications on plant weight, plant height, plant diameter, root collar diameter, leaf width, leaf length, and the number of leaves per plant of lettuce.

Applications	Plant weight (g/plant)	Plant height (cm)	Plant diameter (cm)	Root collar diameter (mm)	Leaf width (cm)	Leaf length (cm)	Number of leaves per plant
Control	343.02 c*	28.00 c*	27.23 b*	19.11 b*	12.07 ns	21.54 b*	47.57 c*
2 mM	432.57 a	30.35 a	28.72 a	21.49 a	12.60	22.44 a	55.63 a
4 mM	376.75 b	29.07 b	26.67 b	19.60 b	12.11	21.73 b	52.20 b
6 mM	371.10 b	28.18 c	26.70 b	19.84 b	12.02	21.23 b	54.33 a

*: Means with different letter differ significantly ($P<0.05$), ns = not significant.

(6 mM oxalic acid application) and 26.1% (2 mM oxalic acid application) when compared to the control group in our study. As a matter of fact, in studies conducted by different researchers, it was determined that oxalic acid applications increased the yield by 19% in rockets, 34% in pomegranate, 30% in cherries, 13% in pears, and 21% in apricots (Uludağ, 2021; García-Pastor et al., 2020; Martínez-Esplá et al., 2014; Budak and Şan, 2017).

It was determined that the oxalic acid applications increased the plant height by 0.6% (6 mM oxalic acid application), 3.8% (4 mM oxalic acid application), and 8.3% (2 mM oxalic acid application) rates compared to the control application. Supporting our results, different researchers found that oxalic acid applications increased fruit size in pears (Budak and Şan, 2017), grapes (Kök and Bal, 2019), and apricot (Kurucu, 2019) by 2.1-6.6%, 2.6%, and 4-7%, respectively. Moreover, it was also reported that it increased plant height by 41% in tomatoes (Pérez-Labrada et al., 2019) and 6.96% in the rocket (Uludağ, 2021).

Positive effects of 2 mM oxalic acid application were observed on plant diameter and root collar diameter of lettuce. It provided increases of 5.4% and 12.4% compared to the control application, respectively. Similarly, Anwar et al. (2018) stated that oxalic acid has positive effects and increases the width and length of fruits in strawberries. In a study on pears, the highest values in fruit width were obtained in the application of 1 mM oxalic acid compared to the control (Budak and Şan, 2017).

Although the effects of the applications on leaf width were found to be insignificant in our study, it was observed that 2 mM oxalic acid application increased the leaf width by 4.3% compared to the control. Also, this dose increased the leaf length by 4.1% compared to the control application. Garcia-Pastor et al. (2020) and Martínez-Esplá et al. (2014) reported that oxalic acid applications had favorable effects on fruit volume in their study on pomegranates and cherries, respectively. Despite the increase in leaf width and length, the number of leaves per plant did not decrease, on the contrary, it increased. We can see that 4 mM, 6 mM, and 2 mM oxalic acid applications increased the number of leaves per plant in lettuce by 9.7%, 14.2%, and 16.9%, respectively. This can be explained by the fact that oxalic acid increases photosynthesis, water, and nutrient uptake, and encourages vegetative growth.

Significant differences were observed in the colors and chlorophyll values (Table 4).

The positive effect of oxalic acid, which was stated in different studies (Kurucu, 2019; Budak ve Şan, 2017; Huang et al., 2013a, 2013b; Martínez-Esplá et al., 2014) on color parameters were also determined in our study. It has been found that in general 2 mM and 4 mM oxalic acid applications increased the color values compared to the control. Wang et al. (2009), in their study on jujube, determined that oxalic acid prevented the chloroplast from breaking down and the reddening of the fruit peel. This situation can be thought of as a result of the reduction of ethylene production by inhibiting the synthesis of 1-aminocyclopropane-1-carboxylic acid of oxalic acid, thus reducing the respiratory rate and lowering metabolic activity (Razzaq et al., 2015). Zheng and Tian (2005), Huang et al. (2013b), and Whangchai et al. (2006) stated in their study that oxalic acid prevented browning in postharvest lychee, banana, and longan fruits. Yoruk and Marshall (2003) found that different levels of oxalic acid prevent browning on the surface of freshly cut apples. These studies support the findings of Tang et al. (2020) who have reported that oxalic acid applications delay aging and reduce the losses in color values. As a matter of fact, our findings were found to be parallel to these reports.

Chlorophyll is the pigment, which gives plants their green color, so, while chlorophyll values are compared with the a^* values, it is seen that the results are similar to each other. In our work, the chlorophyll values increased by 1.5%, 2.4%, and 4.9% in 6 mM, 4 mM, and 2 mM oxalic acid applications respectively, compared to the control application. Wang et al. (2009) and Huang et al. (2013b) reported in their studies that oxalic acid application prevents/delays chloroplast/chlorophyll degradation.

The effects of the applications on TSS were found insignificant, but a positive effect of oxalic acid was observed with increasing doses compared to the control application. Similarly, in the studies carried out on pomegranate (García-Pastor et al., 2020), apricot (Kurucu, 2019), kiwifruit (Ali et al., 2019), and rocket (Uludağ, 2021) oxalic acid applications were found to increase the amount of TSS. Martínez-Esplá et al. (2014), have reported that it is due to the fact that oxalic acid is effective in the increase of photosynthesis. A similar circumstance was also observed in the dry matter parameter. Although the effect of the oxalic acid application on the dry matter

Table 4. Effects of applications on colors, chlorophyll, TSS, and dry matter of lettuce.

Applications	L^*	a^*	b^*	C^*	H°	Chlorophyll (SPAD)	TSS (%)	Dry matter (%)
Control	47.53 c*	-17.55 b*	27.89 b*	32.95 b*	122.18ns	37.47 b*	3.33ns	5.26ns
2 mM	48.76 a	-18.26 a	28.68 a	34.00 a	122.48	39.31 a	3.34	5.29
4 mM	48.12 b	-18.10 a	28.58 a	33.83 a	122.34	38.38 ab	3.65	5.37
6 mM	47.43 c	-17.66 b	27.98 b	33.09 b	122.26	38.04 ab	3.69	5.62

*: Means with different letter differ significantly (P<0.05), ns = not significant.

Table 5. Effects of applications on ascorbic acid, total phenolic and antiradical activity of lettuce.

Applications	Ascorbic acid (mg/100g)	Total Phenolic (mg/100g)	%Inhibition
Control	11.15^{ns}	72.81 b*	70.74 b*
2 mM	11.25	67.35 c	42.36 c
4 mM	11.51	103.98 a	82.64 a
6 mM	11.57	72.58 b	80.28 a

*: Means with different letter differ significantly (P<0.05), ns = not significant.

was found to be insignificant, an increase of 0.5%, 2%, and 6.8% was observed in 2 mM, 4 mM, and 6 mM oxalic acid applications, respectively. Anwar et al. (2018), in their study on strawberries, suggest that with the improvement of vegetative growth, the uptake of N, P, and K nutrients increases, and as the transportation of these nutrients to the leaves becomes easier the amount of dry matter increases. Also, Pérez-Labrada et al. (2019) found that the amount of dry matter in the leaves of the tomato plant increased and stated that this may be due to the increase in plant height.

Table 5 shows the findings related to ascorbic acid, total phenolic content, and antiradical activity of lettuce. The applications had significant effects (P<0.05) on total phenolic content and antiradical activity of lettuce.

When the table is examined, it is seen that the effects of the applications on vitamin C in lettuce are insignificant. However, a slight increase is observed depending on the doses. A similar result was found by Zhu et al. (2016), who reported that the pre-harvest application of oxalic acid increased the ascorbic acid content of kiwifruit at harvest.

In the study, it was determined that 4 mM oxalic acid application increased the total phenolic substance content by 42.8% when compared to the control application. In their studies, different researchers reported that pre-harvest oxalic acid applications increased the total phenolic content of artichoke (Martínez-Esplá et al., 2017), coriander (El-Zaeddi et al., 2017), and pomegranate (García-Pastor et al., 2020). In addition, it has been reported that the total amount of phenolic substance is preserved in studies where the oxalic acid application was applied after harvest. As a matter of fact, Koyuncu et al. (2019) stated in their storage study on pomegranate that oxalic acid application protects and increases the total phenolic content. In addition, Martínez-Esplá et al. (2017) reported that oxalic acid application increased the amount of phenolic substances by 30-50% in their study on artichokes. Also, oxalic acid applications increased the antiradical activity up to 17% (4 mM oxalic acid application) as compared to the control application. In other studies that support our results, postharvest oxalic acid application on tomato (Kant et al., 2013), peach (Zheng et al., 2007a), plum (Wu et al., 2011), mango (Zheng and Tian 2005; Zheng et al., 2007b, 2012a, 2012b), jujube (Wang et al., 2009), pomegranate (Sayyari et al., 2010), cherry (Valero et al., 2011) and banana (Huang et al., 2013a) fruits increase the antioxidant potential. Dinçay (2021), in his study on the freshly cut rocket, reported that oxalic acid application increased antioxidant activity. In addition, in different studies, it has been determined that oxalic acid applications are effective in the regulation of enzymes such as LOX, SOD, and APX in lychee and mango fruits (Zheng and Tian 2005; Zheng et al., 2007b). Wang et al. (2018) reported that oxalic acid application increased the APX enzyme in their study on melon.

CONCLUSION

In recent years, the use of plant growth regulators, which is one of the cultural practices, has become widespread, as well as the studies on different compounds that can be alternatives to plant growth regulators. In this study, the effects of oxalic acid, which can be an alternative and one of the compounds that have been emphasized recently, on the yield and quality characteristics of lettuce were investigated. As a result of the study, when all the data are evaluated together, it has been seen that oxalic acid applications in lettuce cultivation provided positive contributions to the yield and quality values. Also, it was determined that especially 2 mM and 4 mM oxalic acid doses could play an active role in increasing yield and biochemical properties, respectively.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

According to the authors, there is no potential, actual, or perceived conflict of interest with this research article.

Author contribution

The authors each contributed equally to the current work. The authors have read the final manuscript and given their approval. Every author verify that the text and tables are unique and have never been published previously.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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