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Effects of foliar zinc applications on some yield parameters and essential oil constituents of the mastic tree (*Pistacia lentiscus* var. *chia* Duham.)

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ABSTRACT

Mastic tree (*Pistacia lentiscus* var. *chia* Duham.) is an important medicinal aromatic plant which is being cultivated without any regular fertilization generally. Foliar Zn applications have great importance for many commercial plant species because of their important role in metabolic activities. In this study, the effects of foliar $ZnSO_4$ sprayings (0, 0.2, 0.4, 0.8%) applied at 50% and 100% leafing stages, on some yield parameters and essential oil composition of mastic tree were assessed. A field experiment was carried out for two consecutive years at Ege University, Turkey. Mastic resin yield was more than doubled with 0.8% Zn particularly in the second year. The highest leaf Zn content was measured as 187.8 mg l^{-1} with 0.8% zinc spraying while it was 35.4 mg l^{-1} in unsprayed trees at 100% leafing in the first year. Leaf essential oil and α-tocopherol contents were not significantly influenced by different Zn levels and leafing stages in both years. As for the major essential oil constituents, β-myrcene (62.4%), germacrene-D (14.3%), α-terpinenyl acetate (5.52%), t-caryophyllene (2.1%) and α-pinene (1.4%) were determined. Zinc sprayings increased the β-myrcene, germacrene-D and α-pinene contents particularly at 100% leafing in the first year. In mastic tree two consecutive applications of Zn seems to be necessary for reaching to desirable leaf Zn contents. Application of 0.8% Zn or more would be proper to increase the contents of most parameters assessed.

1. Introduction

Mastic tree (*Pistacia lentiscus* L.) is one of the predominant elements of the Mediterranean maqui vegetation. *P. lentiscus* var. *chia* Duham., which is a botanical variety of this species has long been grown only in the South of Chios Island for gum mastic production (Browicz, 1987). It's a dioecious plant and only male plants are being used to produce mastic resin. Due to the high essential oil and alpha-tocopherol content of leaf, shoot and resin, it has long been widely used in medicine, pharmaceutical, cosmetic and food industries as a medicinal-aromatic plant (Pachi et al., 2020).

Despite the limited investigation on the essential oil composition of *Pistacia* species, current knowledge on the essential oil composition of the aerial parts of mastic tree (*P. lentiscus*) is not clear (Aouinti et al., 2014). The southern coasts of the Aegean region of Turkey are of important potential of growing in terms of the suitability of ecological conditions.

Mastic tree has been widely grown in arid soils and there is no regular fertilization practice under growers' conditions generally (Freedman, 2011). In fact, the low amounts of micro nutrients are necessary for the growth and development of plants. One of them Zn, which is necessary for important metabolic cases such as auxin activity, triptophane biosynthesis and growth speed (Bautista-Diaz et al., 2021). Almost in every human cell Zn is found as an essential mineral. Zinc is also effective on stimulation of enzyme activity and needed for a healthy immune system and DNA synthesis (Nahida and Siddiqui, 2012). Foliar Zn treatments are quite important for fruit trees because, most agricultural soils are deficient in this element and soil applications are generally ineffective to alleviate Zn deficiency (Swietlik, 2002), therefore foliar applications are the preferred method. It was reported that foliar sprays of Zn significantly increased the Zn content of pistachio (*Pistacia vera* L.) leaves (Zhang and Brown, 1999; Soliemanzadeh and Mozafari, 2014; Norozi et al., 2019), which is a close relative of mastic tree. When applied after flowering during early leaf development in pistachio trees, foliar Zn was absorbed and transported within the tree (Zhang and Brown, 1999). The effect of foliar Zn applications on increasing the essential oil content of some aromatic plants such as; anise, basil, chamomile, lemon balm and mint was also reported by different

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researchers (Shahrajabian et al., 2022). Zn applications also increased the leaf, shoot and resin yield, besides the amount and quality of essential oil and α-tocopherol content, which is one of the basic component of vitamin E, significantly in medicinal aromatic plants such as sage and lemon balm (Geneva et al., 2010; Yadegari, 2017). α-Tocopherol (vitamin E) is naturally found in *Pistacia* leaves. Due to its pharmaceutical properties, vitamin E is widely used as a natural antioxidant (Stephens et al., 1996). Moreover, α-tocopherol is also used in cosmetics and skin-care products due to its antioxidant and anti-inflammatory effects (Silva et al., 2019). Geographical and ecological differences highly influence the Zn concentrations in leaves of wild *P. lentiscus* ecotypes (Aouinti et al., 2014). As observed in all mineral elements, Zn content of plants can be attributed to climatic conditions, soil mineral composition, preferential mineral uptake, species, age of plant and the fertilizers used (Swietlik, 2002).

Therefore, the objective of this study is to determine the effects of foliar zinc applications in different concentrations and phenological stages on some yield and biochemical quality parameters of the cultivated mastic tree (*P. lentiscus* var. *chia* Duham.).

2. Materials and methods

2.1. Experimental site

This study was conducted in the research and production area of Ege University, Faculty of Agriculture, Department of Horticulture, Turkey (38°27'25.09"N; 27°13'20.59"E; 31 m asl) in 2020 and 2021 growing seasons. Research area has typical Mediterranean climatic conditions where the most of precipitation often falls during the winter months. Monthly total precipitation and mean temperature data of the experimental area during the growth period are shown in Table 1. All data were obtained from the Directorate of the Turkish State Meteorological Service in İzmir, Turkey.

Some physicochemical properties of the soil are shown in Table 2. The soil of experimental plots is in clay-loam texture (41% sand, 19% clay and 40% silt), slightly alkaline reaction, moderate lime content and moderate in organic matter (1.96% and 1.77% respectively, for two depths). The total content of water-soluble salt in the soil was lower than hazardous levels in terms of salinity (<0.015%) in two depths. The soil comprises sufficient total N, available K^{++}, Mg^{++}, moderately available P and very high amount of Ca^{++} and is sufficient in terms of available Fe^{++}, Cu^{++}, Zn^{++} and Mn^{++} at both depths (Estefan et al., 2013).

2.2. Plant material

In the experiment, 16 years old male mastic trees (*P. lentiscus* var. *chia* Duham.) were used as plant material. The mean height of the trees is 3 m and canopy width is 5 m respectively. Planting density is 5 * 2,5 m (80 tree ha^{-1}). Trees were grown under rainfed conditions with no chemical fertilization.

2.3. Experimental design and treatments

The field experiment was established in a randomized block design with three replications. Foliar Zn was applied in 2020 and 2021 growth periods in two different phenological stages when 50% (1st stage: 15 May and 28 May respectively) and 100% (2nd stage: 20 June and 22 June respectively) leafing occurred in the current seasons' shoots (Zhang and Brown, 1999). Trees were sprayed to full foliage wetting with Zn in the form of aqueous solution of 0 (control), 0.2%, 0.4% and 0.8% $ZnSO_47\ H_2O$ (23% Zn, GÜBRETAŞ CO./TURKEY) combined with 1% urea (46% N) and 0.5% DRESH (40% > esterified vegetable fatty acids, KOH) as surfactant, bya battery pressure sprayer, 10 and 12 liters per tree in two consecutive years. Amount of Zn solution per tree was calculated as the mean of three trees.

2.4. Soil analysis

Soil samples were collected from three different points representing the research area at the depths of 0–30 and 30–60 cm in March before the 1st year's Zn aplications.

Soil sand, clay and silt contents were determined by the hydrometric method, pH and total water-soluble salt were determined in a paste of soil, saturated with water, with a pH meter with a glass electrode and a conductometer, lime contents were determined with Scheibler calcimeter, and the organic matter content was determined by wet digestion with $K_2Cr_2O_7$ and H_2SO_4. With regard to plant nutrient elements in the soil, total nitrogen was determined by the modified Kjeldahl method, available phosphorus was determined colorimetrically after extraction with sodium bicarbonate. Available K^+, Ca^{++}, Mg^{++} contents were determined by ICP-OES after extraction with 1 N NH_4OAc. Fe, Mn, Zn, and Cu contents were determined by ICP-OES, after extraction with 0.05 M DTPA+TEA (Estefan et al., 2013).

2.5. Plant analysis

All chemical and biochemical analyses were conducted in leaves collected from 1 and 2-year old twigs from four different directions of trees on the 21st day (Zhang and Brown, 1999) following zinc applications.

Leaf samples were washed first with tap and then distilled water and dried in a drying oven (ECOCELL® MMM Medcenter Einrichtungen GmbH, GERMANY) at 65 ± 5 °C until the stable weight was reached. After the final weight was determined samples were ground to a fine powder. The leaf samples were reduced to ash at 550 °C and dissolved in 3 N HCl, and the zinc content of the extract was determined by ICP-OES (*Thermo İcap 6000 SERIES*) (Cheng et al., 2012).

To predict the resin yield, ten wounds on each tree were made by using a special apparatus, in constant length (4–5 cm), width (4–5 mm) and depth (3–4 mm) on the scaffold limbs of trees at the four different directions in mid-June when the resin flow accelerated, in two consecutive years (Sawidis et al., 2000). Mean yield of tree as g was determined in mid-September when the resin flow finished, dry resin was harvested and weighted.

The essential oil ratios of the plants were determined by a volumetric method as ml·100 g^{-1}. Leaf samples were dried at 35 °C after the harvest, then 30 g of dry material was subjected to hydrodistillation in a Neo-Clevenger apparatus for 3 h with 300 ml of deionized water for essential oil extraction. And the samples were stored at 4 °C until the gas chromatography (GC) analysis (Wichtl, 1971; Gardeli et al., 2008). A parallel determination of the dry matter content of a representative sample of the plant material (drying at 105 °C up to constancy) was determined as 7.59% (Barra et al., 2007).

α-tocopherol was separated isocratically according to AOCS Ce 8–89

Table 1
Monthly total precipitation and mean temperature data of 2020 and 2021.

	Year	March	April	May	June	July	August	September
Temperature (°C)	2020	13.5	16.4	21.6	25.1	29.7	29.3	26.9
	2021	11.1	16.7	22.9	25.4	30.6	29.9	25.1
Precipitation (mm)	2020	83.0	56.1	55.2	24.9	1.4	0.4	0.5
	2021	98.0	25.4	0.6	31.4	1.3	0.0	0.2

Table 2
Soil physical and chemical properties of the research area.

Depth (cm)	pH	EC (μS cm^{-1})	%						mg kg^{-1}							
			Organic matter	Lime	Sand	Clay	Silt	Total N	P	K	Mg	Ca	Fe	Cu	Zn	Mn
0–30	7.8	980	1.96	8.47	41	19	40	0.10	12	1089	281	12780	6.2	4.2	1.8	18
					Clay-loamy											
30–60	7.7	930	1.77	10.49	41	19	40	0.09	14	843	247	4059	5.4	3.8	1.7	17

methodology from mastic tree leaves (American Oil Chemists' Society, 1997). α-tocopherol content was determined by HPLC-FLD dedector (Agilent FLD System) (Ruggeri et al., 1984; Gapor et al., 1986) with a sample loop of 20 μl α-tocopherol was separated Lichrosorb Si 60 silica based column (5.0 μm x 250 mm × 4.0 mm; particle size x length x internal diameter). Hexane/2-propanol (99.5/0.5:v/v) was used as mobile phase, flow rate was 0.8 ml/min, which corresponded with a pump pressure of about 100 bar, with fluorescence detection (excitation at 290 nm, emission at 330 nm). Identification of the α-tocopherol was accomplished with pure standards as reference compounds (Sigma). All of the solvents and reagents in analytical grade were used.

Essential oil composition was determined by GC-MSD (HP 6890 Series GC System, MSD 5973 detector). Compounds were separated on a 60 m × 0.32 mm, 0.25 μm film thickness, INNOWAX (Hewlett Packard No: 19091N1–16) capillary column, with helium as a carrier gas at a constant flow rate of 1 ml/min and split ratio was 15:1. The oven initial temperature was 60 °C, 4 °C/min 210 °C (hold time: 3 min), 20°C/min 230 °C (hold time: 5 min) total run time was 46.50 min and the injector and detector temperatures were 250 °C. Constituents of the essential oil were identified by comparing the mass spectra of the products with data in the Wiley7N.L mass-spectra library.

2.6. Statistical analysis

The data obtained from the experiment were subjected to analysis of variance (ANOVA) by IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, N.Y., USA), according to a simple factorial randomized complete block design, for each year separately. Following the ANOVA, Tukey's Range Test ($\alpha = 0.05$) was performed where necessary.

3. Results

There was no statistically significant interaction between level and stage of Zn applications, however foliar Zn applications significantly ($p \leq 0.001$) increased the leaf Zn contents in parallel with increasing levels of Zn sprayings at both stages of leafing in the 1st year. Higher leaf Zn contents were particularly observed at the 2nd stage of leafing and the spraying of 0.8% $ZnSO_4$ gave the highest leaf content (187.80 mg l^{-1}) of Zn. In the 2nd year, similar to the 1st year of the experiment, the interaction between spraying levels and stages, was not significant, the highest figure was obtained with 0.4% $ZnSO_4$ at the 2nd stage of leafing as at the 1st stage. Moreover, the application of 0.8% Zn resulted in a slight decrease in leaf Zn contents as opposed to the 1st year. However, Zn contents of leaves tended to decrease in the 2nd year, particularly at the highest concentration was evident (Table 3).

The effect of Zn treatments on resin yield was found to be statistically not significant in both years, but resin yields remarkably increased in parallel with the increments in Zn concentration. Mastic resin yield figures were also more than doubled in the 2nd year (Table 3). Despite the non-significant interaction found between foliar Zn levels and leafing stages, essential oil contents of leaves were higher at the 100% leafing in both years. Oil content raised up to 0.15 ml 100 g^{-1} with 0.4% and 0.8% $ZnSO_4$ in the 2nd year. Means of oil contents at different stages were found to be significant ($p \leq 0.001$) only in the 2nd year and the higher amount of oil was measured at the 2nd leafing stage (Table 3).

As for the α-tocopherol content of leaves, interaction between foliar Zn levels and leafing stages was found to be not significant in both years. Inconsistent figures were observed with regard to increasing levels of Zn in two consecutive years. However, α-tocopherol contents remarkably increased compared to control with 0.4% Zn spraying in the 1st and with 0.8% Zn spraying in the 2nd year at 50% leafing stages. As the leafing advanced, dramatic decreases in α-tocopherol contents in both years were observed.

Zn applications affected the α-tocopherol contents significantly ($p \leq 0.05$) only in the 2nd year and 0.8% Zn caused an increase of over 60% in α-tocopherol content compared to control. The effect of leafing stages was found to be significant ($p \leq 0.01$), and the highest α-tocopherol content was observed at 50% leafing stage in both years (Table 3).

Table 3
Effects of foliar Zn applications on some yield parameters and leaf α-tocopherol content of mastic tree.

Leafing stage	Zn treatment (%)	Leaf Zn content (mg l^{-1})		Resin yield (g)		Oil yield (ml 100 g^{-1})		α-tocopherol (%)	
		2020	2021	2020	2021	2020	2021	2020	2021
50%	0	18.53 ± 3.00^{e}	18.58 ± 4.57	0.573 ± 0.07	1.271 ± 0.60	0.05 ± 0.00	0.08 ± 0.02	4.83 ± 1.05	2.04 ± 0.97
	0.2	32.72 ± 3.24^{e}	36.25 ± 2.88	0.641 ± 0.27	1.494 ± 0.86	0.05 ± 0.02	0.09 ± 0.02	5.81 ± 0.14	1.92 ± 0.21
	0.4	56.29 ± 0.76^{de}	74.60 ± 2.45	0.773 ± 0.29	1.664 ± 0.39	0.05 ± 0.00	0.10 ± 0.03	6.88 ± 0.82	2.04 ± 0.20
	0.8	97.24 ± 11.31^{c}	68.23 ± 2.27	0.804 ± 0.27	2.258 ± 0.48	0.06 ± 0.02	0.08 ± 0.02	5.17 ± 0.45	4.71 ± 0.20
100%	0	35.41 ± 7.16^{e}	31.98 ± 0.61			0.07 ± 0.02	0.12 ± 0.03	3.96 ± 0.70	1.86 ± 0.21
	0.2	61.18 ± 11.18^{d}	65.04 ± 3.22			0.08 ± 0.06	0.12 ± 0.01	4.56 ± 0.17	1.03 ± 0.22
	0.4	132.40 ± 13.45^{b}	126.49 ± 3.78			0.12 ± 0.01	0.15 ± 0.03	3.73 ± 0,0.69	1.50 ± 1.22
	0.8	187.80 ± 2.55^{a}	120.50 ± 1.84			0.10 ± 0.02	0.15 ± 0.02	4.23 ± 0.27	1.62 ± 0.26
Zn mean	0	26.97 ± 7.66^{D}	25.28 ± 3.45^{B}			0.06 ± 0.02	0.10 ± 0.03	4.39 ± 0.82	1.95 ± 0.63^{AB}
	0.2	46.95 ± 9.89 ^{C}	50.64 ± 3.14^{AB}			0.06 ± 0.01	0.11 ± 0.02	5.18 ± 1.07	1.47 ± 0.50^{B}
	0.4	94.35 ± 10.21^{B}	100.55 ± 3.40 ^{A}			0.08 ± 0.05	0.12 ± 0.03	5.31 ± 1.09	1.77 ± 0.33^{B}
	0.8	142.52 ± 12.20 ^{A}	94.37 ± 2.34 ^{A}			0.08 ± 0.03	0.12 ± 0.03	4.70 ± 0.94	3.16 ± 0.83 ^{A}
Stage mean	50%	51.19 ± 3.56^{B}	49.41 ± 2.81^{B}			0.05 ± 0.01	0.09 ± 0.03^{B}	5.67 ± 1.15 ^{A}	2.68 ± 0.94 ^{A}
	100%	104.20 ± 6.02 ^{A}	86.00 ± 4.42 ^{A}			0.09 ± 0.03	0.14 ± 0.02 ^{A}	4.12 ± 0.34^{B}	1.50 ± 0.34^{B}
Significance	Zn X Stage	* **	n.s			n.s	n.s	n.s	n.s
	Treatment	* **	* *	n.s	n.s	n.s	n.s	n.s	*
	Stage	* **	*			n.s	* **	* *	* *

The effects indicated with * , * * and * ** were statistically significant at $p \leq 0.05$, $p \leq 0,01$ and $p \leq 0001$, n.s. indicates no significance. Means with different letters at the same column differ significantly according to Tukey's Range Test ($\alpha = 0.05$).

In this study 40 constituents were detected in the essential oil of *P. lentiscus* var. *chia* leaves with β-myrcene (62.4%), germacrene-D (14.3%), α-terpinenyl acetate (5.5%), t-caryophyllene (2.1%) and α-pinene (1.4%) as the major constituents. The effects of Zn applications on the amounts of principal constituents of leaf essential oil showed remarkable changes in terms of leafing stages and Zn levels.

The predominant component β-myrcene, was significantly ($p \leq 0.05$) affected by different levels of Zn and leafing stages. In the 1st year, the highest content was measured as 77.7% with 0.4% Zn spraying at the 100% leafing stage. The highest concentration of Zn (0.8%) significantly decreased the β-myrcene contents compared to control at both leafing stages. In the 2nd year, slight increments of β-myrcene were observed at 0.4% and 0.8% Zn at 100% leafing. Zn applications and different stages significantly ($p \leq 0.001$ and $p \leq 0.01$) influenced the β-myrcene contents and the highest figures were obtained with 0.4% Zn at the second leafing stage in the 1st year. In the 2nd year, although no statistically significant effects were observed, an almost similar pattern regarding the β-myrcene content could be seen in Table 4.

Germacrene-D contents in the 1st year were significantly ($p \leq 0.01$) affected by the interaction between Zn levels and leafing stages.

The effects of increasing Zn levels were more pronounced at 100% leafing and the highest content (20.9%) was observed at 0.8% Zn application. Zn levels did not significantly affect the germacrene-D contents. However, the effect of leafing stages was significant ($p \leq 0.01$) and later application of Zn resulted in an increment of over 40% in germacrene-D content compared to 50% leafing stage. In the 2nd year, there was no statistically significant effects were observed (Table 4).

The interaction between the factors and Zn levels regarding the α-terpinenyl acetate content were not statistically significant, in both years, whereas the effect of leafing stage was significant for both the 1st ($p \leq 0.001$) and the 2nd year ($p \leq 0.05$) of the study. In both years α-terpinenyl acetate content was higher at the 50% leafing stage (Table 4).

Interaction of Zn levels and leafing stages significantly ($p \leq 0.01$) affected the amounts of t-caryophyllene in the 1st year. Despite the inconsistent figures were observed in terms of different Zn levels, 0.8% Zn gave the highest t-caryophyllene content at the 1st stage. Means of Zn levels significantly ($p \leq 0.01$) affected the content figures, 0.8% Zn gave the highest amount of t-caryophyllene. In the 2nd year non-significant interaction was found between Zn levels and leafing stages. Also, means of Zn levels and leafing stages did not significantly affect the content figures (Table 4).

α-Pinene contents were significantly ($p \leq 0.01$) affected by different Zn levels and leafing stages in the 1st year. The highest content was obtained with 0.4% Zn at 50% leafing. However, 0.8% Zn treatment decreased the amount of α-pinene at this stage. But in the 2nd stage, the amounts of α-pinene gradually increased in parallel with increasing Zn levels. Effects of mean Zn levels and leafing stages significantly ($p \leq 0.05$) affected the α-pinene contents and the highest figures were obtained with 0.4% Zn at 50% leafing. In the 2nd year, despite a non-significant interaction was observed between Zn levels and leafing stages, increasing levels of Zn tended to increase the α-pinene contents

Table 4
Effects of foliar Zn treatments on predominant constituent contents of mastic tree essential oil.

Leafing stage	Zn treatment (%)	β-myrcene (%)		Germacrene-D (%)		α-tepinenyl acetate (%)	
		2020	2021	2020	2021	2020	2021
50%	0	60.21 ± 1.99^{c}	62.43 ± 2.67	14.34 ± 1.54bc	14.36 ± 0.38	5.57 ± 0.74	3.14 ± 0.51
	0.2	70.66 ± 1.28^{ab}	56.98 ± 2.72	5.66 ± 1.08d	12.68 ± 0.62	4.56 ± 0.17	4.50 ± 0.64
	0.4	66.98 ± 3.57^{bc}	61.43 ± 1.96	13.45 ± 0.73bc	11.40 ± 171	3.75 ± 0.39	3.66 ± 0.86
	0.8	53.36 ± 1.90^{c}	55.87 ± 1.71	11.36 ± 1.96 cd	15.15 ± 1.58	5.29 ± 0.32	3.74 ± 0.19
100%	0	70.30 ± 1.18^{ab}	56.96 ± 2.63	14.88 ± 1.72a-c	14.55 ± 1.64	2.24 ± 0.81	2.85 ± 0.48
	0.2	66.76 ± 1.24^{bc}	56.07 ± 0.81	18.09 ± 0.91ab	13.29 ± 0.93	2.25 ± 0.33	2.43 ± 0.45
	0.4	77.77 ± 1.08^{a}	63.74 ± 1.27	9.77 ± 1.36 cd	11.23 ± 1.00	2.55 ± 0.42	2.25 ± 0.61
	0.8	60.80 ± 1.28^{c}	64.18 ± 1.55	20.99 ± 2.49a	11.80 ± 0.91	2.45 ± 0.43	2.69 ± 0.38
Zn mean	0	65.25 ± 1.17^{B}	59.69 ± 1.17	14.61 ± 1.77	14.45 ± 0.47	3.91 ± 0.88	2.99 ± 0.54
	0.2	68.71 ± 3.93^{AB}	56.52 ± 1.93	11.87 ± 1.38	12.98 ± 1.01	3.40 ± 0.69	3.46 ± 0.34
	0.4	72.37 ± 2.88 ^{A}	62.58 ± 2.12	11.61 ± 1.89	11.31 ± 1.20	3.15 ± 0.78	2.95 ± 0.76
	0.8	57.08 ± 1.64 ^{C}	60.03 ± 1.38	16.18 ± 2.79	13.48 ± 1.52	3.87 ± 1.31	3.22 ± 0.72
Stage mean	50%	62.80 ± 1.10^{B}	59.17 ± 2.05	11.20 ± 1.76B	13.39 ± 2.00	4.79 ± 1.17A	3.76 ± 0.78A
	100%	68.91 ± 1.97 ^{A}	60.24 ± 2.51	15.93 ± 1.99A	12.72 ± 1.92	2.37 ± 0.47B	2.56 ± 0.43B
Significance	Zn X Stage	*	n.s	* *	n.s	n.s	n.s
	Treatment	* **	n.s	n.s	n.s	n.s	n.s
	Stage	* *	n.s	* *	n.s	* **	*

Leafing stage	Zn treatment (%)	t-caryophyllene (%)		α-pinene (%)	
		2020	2021	2020	2021
50%	0	1.54 ± 0.35^{ab}	2.12 ± 0.25	0.92 ± 0.07^{ab}	1.45 ± 0.09
	0.2	0.60 ± 0.02^{c}	2.43 ± 0.27	1.05 ± 0.03^{ab}	1.42 ± 0.16
	0.4	1.33 ± 0.10^{abc}	2.06 ± 0.21	1.14 ± 0.14^{a}	1.68 ± 0.29
	0.8	1.98 ± 0.15^{a}	2.35 ± 0.16	0.63 ± 0.04^{bc}	1.89 ± 0.32
100%	0	1.35 ± 0.06^{abc}	2.04 ± 0.09	0.38 ± 0.03^{c}	1.24 ± 0.24
	0.2	1.40 ± 0.12^{abc}	2.13 ± 0.08	0.43 ± 0.11^{c}	0.97 ± 0.10
	0.4	0.78 ± 0.04^{bc}	1.92 ± 0.12	0.87 ± 0.31^{abc}	1.38 ± 0.09
	0.8	1.73 ± 0.15^{ab}	1.90 ± 0.11	1.07 ± 0.04^{ab}	1.46 ± 0.05
Zn mean	0	1.45 ± 0.25^{AB}	2.08 ± 0.24	0.65 ± 0.30^{B}	1.34 ± 0.17
	0.2	1.00 ± 0.04^{B}	2.28 ± 0.24	0.74 ± 0.32^{AB}	1.20 ± 0.32
	0.4	1.05 ± 0.34^{B}	1.99 ± 0.10	1.00 ± 0.36 ^{A}	1.53 ± 0.23
	0.8	1.86 ± 0.23 ^{A}	2.13 ± 0.27	0.85 ± 0.34^{AB}	1.68 ± 0.28
Stage mean	50%	1.36 ± 0.51	2.24 ± 0.19	0.93 ± 0.26 ^{A}	1.61 ± 0.29 ^{A}
	100%	1.31 ± 0.39	2.00 ± 0.18	0.69 ± 0.26^{B}	1.26 ± 0.21^{B}
Significance	Zn X Stage	* *	n.s	* *	n.s
	Treatment	* *	n.s	*	n.s
	Stage	n.s	n.s	*	*

The effects indicated with * , * * and * ** were statistically significant at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, n.s. indicates no significance. Means with different letters at the same column differ significantly according to Tukey's Range Test ($\alpha = 0.05$).

particularly in the 1st stage of leafing. Moreover, mean effect of leafing stage found to be significant ($p \leq 0.01$) and the higher figure of α-pinene content was found at the 1st stage of leafing (Table 4).

4. Discussion

In mastic tree, foliar Zn sprayings significantly influenced the leaf Zn contents in relation with the Zn concentration used and the leafing stage of the tree. The increasing levels of Zn resulted with a remarkable accumulation of Zn in the leaves, especially at 100% leafing in the 1st year. In fact, 0.8% Zn gave rise to more than 5-fold increment in leaf Zn content compared with the unsprayed trees. In the 2nd year, although the Zn content of the leaves increased with Zn applications, there was no statistically significant effect of interaction, and unlike the first year of the study, there was a quadratic effect with a peak value at 0.4% Zn in both stages (Table 3). On the other hand, Zn levels and leafing stages significantly influenced the Zn content of leaves. Overall, increasing concentration of Zn applications, especially in the second leafing stage, resulted in increments of in leaf Zn contents (Table 3). There are no published articles regarding the effects of foliar Zn application on cultivated mastic tree to best of our knowledge. However, it is a common practice in pistachio (*P. vera*) which is a decidious close relative of mastic tree (Zhang and Brown, 1999; Kızılgöz et al., 2010; Soliemanzadeh and Mozafari, 2014). Similar to our results, different articles reported that foliar $ZnSO_4$ sprayings up to 2 g l^{-1} significantly increased the leaf Zn contents in relation with the phenological stage, concentration used, application frequency and application year in pistachio (Kızılgöz et al., 2010; Soliemanzadeh and Mozafari, 2014). Effects of foliar Zn applications also seems to be in relation with the species used as formerly reported by Swietlik (2002). As a matter of fact, Zn sprayings ranged between 66 and 120 mg l^{-1} were significantly augmented the leaf Zn contents according to phenological stage and application frequency in pomegranate (*Punica granatum* L.) (Davarpanah et al., 2016) and damask rose (*Rosa damascena* var. *trigintipetala* Dieck.) (Ali et al., 2021). From this point of view, two consecutive applications of Zn seem to be necessary for reaching to desirable leaf Zn contents. Application time by using the phenological stages might be optimised because of the evergreen feature of mastic tree. Despite the non-significant effects of different levels of Zn on the mastic resin yield, the figures increased in parallel with increasing Zn applications in both years. However, the resin yield more than doubled in the 2nd harvest year compared to the 1st year of the study (Table 3). Yearly increases in thickness of the wounded branches may have caused this result. Since resin ducts are located in the phloem tissue of the vascular bundles (Sawidis et al., 2000), yearly increase in the mastic resin yield was probably affected by the enlargement of this tissue and/or slow movement and distribution of foliar Zn (Zhang and Brown, 1999).

Interaction between Zn levels and leafing stages was found to be not significant in terms of essential oil content of leaves. However, oil contents were remarkably higher at 100% leafing in both years, and leaf oil content significantly affected by leafing stage in the 2nd year (Table 3). Bampouli et al. (2014), reported that leaf oil contents of cultivated mastic tree leaves collected in February were detected as 0.06–0.03% in fresh and dried leaves respectively. Although the leaf oil contents of our study for the first year were slightly higher than those formerly reported. In the second year of the study, leaf oil contents were nearly doubled. In former studies conducted on the wild ecotypes of *P. lentiscus* from different locations of the Mediterranean basin, leaf oil contents ranged between 0.14% and 0.45% (Boelens and Jimenez, 1991; Duru et al., 2003; Aouinti et al., 2014). Furthermore, the effect of harvest time on leaf oil content of *P. lentiscus* was also reported and the highest oil content was obtained in May under Moroccan conditions (Aouinti et al., 2014). For this reason, climatic and soil conditions together with harvesting time could be predictive on the essential oil contents of this study. Two successive applications of Zn seemed to be useful to increase the oil contents of leaves as formerly discussed in leaf Zn contents. In our study, although not statistically significant, two successive applications of Zn seem to be useful to increase the leaf oil content, similar to the results of Moghimipour et al. (2017), who reported that 1.5 g l^{-1} $ZnSO_4$ spraying nearly doubled the essential oil content of holly basil (*Ocimum sanctum* L.) leaves in two successive cuts.

Former studies showed that α-tocopherol is the most abundant form of tocopherol in plant leaves (Ouchikh et al., 2011). In mastic tree, the effects of Zn sprayings at different leafing stages on α-tocopherol content of leaves showed non-significant interactions in two consecutive years. α-tocopherol contents were generally inconsistent at different leafing stages, although measured contents were much higher in the 1st year at two different stages than the 2nd year. These differences probably derived from different yearly climatic conditions. The highest content (6.88%) was obtained with 0.4% Zn at 50% leafing in the 1st year. Moreover, effects of leafing stages were found as significant and the highest α-tocopherol contents were observed at 50% leafing particularly in the 1st year (Table 3). For this reason, time of Zn spraying seems to be important for the highest content of this constituent. The highest concentrations of α-tocopherol ranged between 0.69 and 42.68 mg 100 g^{-1}on dry weight basis were measured in the leaves of 62 edible trophical plants (Ching and Mohamed, 2001). In this study, the α-tocopherol contents of leaves were changed between 1.03% and 6.88% with Zn applications. Similarly, maximum α-tocopherol content was determined as 0.005% on dry weight basis in cultivated mastic tree leaves without any application (Kıvçak and Akay, 2005). Therefore, mastic tree leaves might be considered as a potential source of α-tocopherol.

Formers studies showed that β-myrcene, which is a monoterpene hydrocarbon (Nahida et al., 2012), was one of the main components of leaf essential oil of cultivated mastic tree (Kıvçak et al., 2004; Bampouli et al., 2014). In this study, β-myrcene was determined as the major compound. Zn sprayings at different stages significantly affected the amount of this constituent in the1st year. Spraying of 0.2% Zn at the 1st and 0.4% Zn at the 2nd stage of leafing gave the highest figures (70.6% and 77.7% respectively) of β-myrcene compared to unsprayed trees. However, 0.8% Zn decreased the amount of this compound at both stages considerably (Table 4). In this study, β-myrcene amounts have seemed quite higher than the amounts that were given in former studies. As a matter of fact, the amounts of this constituent were calculated as 13.9% and 19.5% (Kıvçak et al., 2004; Bampouli et al., 2014) in leaf oil of cultivated mastic trees from different locations.

As a sesquiterpene, germacrene-D was found to be the second major constituent. In the 1st year, different Zn levels and leafing stages significantly affected the constituent figures. Despite the inconsistent figures were found at the 50% leafing stage, increasing levels of Zn tended to increase germacrene-D contents up to 20.9% with 0.8% Zn at 100% leafing. The mean effect of leafing stages significantly affected the constituent amounts and it was higher at the 2nd stage. In the 2nd year, the effects of Zn levels and leafing stages did not significantly influence the amounts of this constituent. The effects of mean Zn levels and stages were also non-significant respectively (Table 4). In former studies, germacrene-D contents were reported as 20.1% (Kıvçak et al., 2004) and 24.7% (Bampouli et al., 2014) which are higher than the figures obtained from unsprayed trees in two consecutive years in this study. These differences are probably the result of the diverse climatic and soil conditions where the trees were grown, as well as the differences in sampling time of leaves as formerly reported (Duru et al., 2003; Kıvçak et al., 2004; Aouinti et al., 2014). From this point of view, two successive applications of $ZnSO_4$ at 50% and 100% leafing at 0.4% level seemed to be proper for the highest levels of two major constituents. Leafing stages might be optimized by using the development of different organs such as flower clusters due to coincidence of active blooming with leafing in mastic tree as formerly reported (Barra et al., 2007; Gardeli et al., 2008).

Contents of α-terpinenyl acetate were not affected by different Zn levels and leafing stages significantly in both years. Inconsistent figures were obtained with Zn applications. Effects of mean Zn levels were also non-significant in two consecutive years. Means of leafing stages significantly affected the constituent figures in the 1st year. Although at the 100% leafing, contents of this constituent decreased in both years (Table 4).

t-Caryophyllene contents were significantly affected by different Zn levels and leafing stages in the 1st year. Applications of 0.8% Zn gave the highest values of this constituent (1.9% and 1.7%) at both stages. Means of Zn levels were found to be significant in the 1st year and the highest level of Zn (0.8%) gave the highest amount of this constituent. Leafing stages did not significantly affect the constituent values in the 1st year. In the 2nd year, a non-significant interaction found between Zn levels and stages. But the contents of t-caryophyllene were higher than the 1st year. The effect of Zn levels and leafing stages was not found statistically significant (Table 4).

Different Zn levels and leafing stages significantly affected the α-pinene contents in the 1st year. The highest figure (1.1%) was observed with 0.4% Zn at 50% leafing and 0.8% Zn decreased the amount of this constituent. At the 2nd stage, increasing Zn levels gradually increased the amounts of α-pinene and the highest figure was found as 1.0% with 0.8% Zn while the control figure was 0.3%. Effects of mean Zn levels and stages were significantly affected the α-pinene figures, highest values were obtained with 0.4% Zn at the 1st stage. Despite the remarkably higher values were determined in the 2nd year, Zn levels and leafing stages did not significantly influence the α-pinene contents. Although Zn sprayings at 0.4% and 0.8% gradually increased the α-pinene content according to control trees particularly at 50% leafing. Mean Zn levels did not significantly affect the α-pinene content, but 0.4% and 0.8% Zn were markedly increased the figures compared to unsprayed trees. Leafing stages significantly affected the α-pinene content and 50% leafing resulted in a higher value for this compound (Table 4). In the limited studies on mastic essential oil, three compounds were detected as the major essential oil constituents, namely α-terpinenyl acetate, t-caryophyllene and α-pinene. They belong to different groups of essential oils such as oxygenated monoterpene, sesquiterpene and monoterpene hydrocarbon, respectively. The contents of these compounds were ranged between 0% and 4.8% (α-terpinenyl acetate), 0.2–10.8% (t-caryophyllene) and 0–0.5% (α-pinene) in fresh weight basis, respectively from the samples from Turkey and Greece (Kıvçak et al., 2004; Bampouli et al., 2015; Pasias et al., 2021). In this study, the highest contents of these three compounds were measured as 5.5%, 2.1% and 1.4%, respectively in the leaves of unsprayed trees, which is not quite different from those reported in former studies. The differences at the amounts of mentioned compounds reported in former studies and this study were probably derived from the location differences and sampling times. In fact, sampling time was significantly effected the content of these compounds and the highest values were obtained at flowering (May) and fruiting (August) periods as opposed to pre-flowering (February) in wild mastic tree (Gardeli et al., 2008). Similarly, at 50% leafing stage in two consecutive years (Table 4), which was coincided with the third and fourth week of May in this study, these three compounds had higher values. Therefore, leafing stage can be considered as a proper indicator for planning Zn sprayings in cultivated mastic tree. Concentrations of Zn at 0.4–0.8% or more can be used, but re-optimization of Zn levels is needed.

5. Conclusions

In cultivated mastic tree, foliar Zn applications showed promising results on leaf Zn contents together with some yield parameters assessed. The effects of Zn sprayings were highly affected by the concentration used and phenelogical stage of the tree. Year differences were also found effective on some yield and essential oil compound values. Mastic resin, which is the most important product of the mastic tree, remarkably increased with increasing Zn levels in both years. Leaf Zn contents were also influenced by the Zn concentrations applied. The highest level of Zn gave rise to more than five fold increament in leaf Zn content depending on application stage and year. Zn applications have shown effects on the amounts of essential oil constituents as well. Consecutive applications at possibly much higher concentrations of Zn seems to be more efficient compared to a single application to reach the desirable leaf Zn contents. Proper stages for spraying might be re-optimized by following the development of flower clusters which coincided with annual shoot elongation and leafing of tree.

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