Effect of foliar application of ordinary and nano-zinc fertilizers on growth and essential oil profile of dragonhead

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Abstract

In this work, the effects of foliar zinc sprayings on some growth parameters and essential oil composition of dragonhead (*Dracocephalum moldavica* L.) were assessed. A greenhouse experiment was carried out with two sources of zinc including zinc oxide (ZnO, as ordinary fertilizer) and zinc oxide nanoparticles (ZnO-NP, as nano-fertilizer), each having four concentrations (0, 25, 50, and 100 mg/l). Results showed that sprayings with zinc had significant influence on the evaluated parameters. Treatments with ZnO and ZnO-NP significantly increased root length, shoot height, root and shoot dry weights, photosynthesis pigments contents, antioxidant enzymes activity, and essential oil percentage, and in the impact of ZnO-NP was more effective than ZnO. The highest growth parameters and pigment contents were observed at 100 mg/L ZnO-NP. Based on the results, 27 components were identified in the dragonhead essential oil in which the maximum values belonged to oxygenated monoterpenes with four main components as geranial (27.91-36.09%), geranyl acetate (18.36-25.48%), neral (19.18-21.7%), and geraniol (5.93-8.30%). The amounts of these monoterpenes under ZnO-NP (especially at 100 mg/L) were higher than ZnO treatment and control plants. The findings of this study showed that the effect of ZnO-NP in increasing the growth, the antioxidant enzymes, and bioactive ingredients of dragonhead was more than ZnO, and it may be possible to use ZnO-NP as a nano-fertilizer in the sustainable production of essential oil in medicinal plants.

Keywords: *Dracocephalum moldavica* L., essential oil, nano-fertilizer, zinc

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Introduction

The pharmaceutical herb dragonhead (*Dracocephalum moldavica* L.) is an annual plant from Lamiaceae family and Lamiales order. In traditional medicine, it is consumed in curing stomachache, liver disorders, headache, and flatulence. The secondary metabolites from this plant such as monoterpene glycosides, trypanocidal terpenoid, rosmarinic acid, and flavonoids have anti-bacterial, anti-rheumatic, anti-cancer, anti-mutagenic, antioxidant, and antiseptic properties (Mohammadi et al., 2021). Furthermore, the dragonhead essence is prevalently used in nutritional, cosmetics, and perfume industries (Nasiri, 2021).

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Zinc is one of the essential micronutrients which is basic to plant growth and development (Çakmak and Kutman, 2018). Zinc deficiency in the soil will cause zinc insufficiency in plants and thus the food chain so that about 17.3% of the human beings are in danger of Zn deficiency (Khan et al., 2022). Due to lower bioavailability of Zn in the soil, the spraying of various forms of zinc on leaves is favored over soil application methods (Hussain et al., 2018). Foliar application has been shown to reduce micro-element deficiencies, prevent toxicity symptoms, and diminish fertilizer-related contamination (Kaur et al., 2023).

In recent years, nanotechnology has gained significant impetus in agriculture, including elevating crop productivity, reducing the use of fertilizers and pesticides, and helping to conserve natural resources (Rui et al., 2016). Against conventional methods (salt fertilizers and soil application of micronutrients), spraying nano-fertilizers can feed crops gradually and in a more controlled condition, resulting in reduced toxicity symptoms in soil (Kah et al., 2018). One of the advantages of nano-fertilizers is that they are used in small amounts compared to conventional fertilizers. Nano-fertilizers are absorbed faster by the plant due to their high solubility and small size (Moradbeygi et al., 2020). These nano-scale substances provide proper situation for plant growth and have a positive effect on the production of secondary metabolites (Moradi et al., 2018). It has been reported that nano-fertilizers improve crop yield and quality via enhancing growth parameters, chlorophyll contents, photosynthesis, and stress tolerance (Ghosh and Bera, 2021).

The objective of the present experiment was to investigate and compare the effect of zinc oxide and zinc nanoparticles on growth, photosynthesis pigments contents, antioxidant enzymes activities, and secondary metabolites of the medicinal plant dragonhead under greenhouse condition.

Materials and Methods

Preparation of materials and growth conditions

The experiment was arranged following a complete randomized design with four replicates per treatments. The experimental treatments were foliar spraying at four doses of ordinary zinc oxide (0, 25, 50, and 100 mg/L) and four doses of nano-zinc oxide (0, 25, 50, and 100 mg/L).

The dragonhead seeds obtained from the Horticulture Laboratory of University of Maraghehe, Iran, were disinfected for 5 min with sodium hypochlorite 5% with a few drops of Tween 20 and then were washed several times with sterile distilled water. For germination, 30 seeds were placed on wet filter paper in each Petri dish of 8 cm in diameters.

Sandy-loamy soil (0-25 cm) was taken from the soil surface of Maragheh University Campus. The sample was mixed with sand by 4:1 ratio. The physico-chemical characteristics of the soil used in our study were as follows: 68% sand, 20% silt, 12% clay, 1.2% organic matter, 0.05% total N, 7 mg/kg available P, 35 mg/kg available K, 1.98 mg/kg available Zn, pH 7.2 and 1.28 dS/m EC. Fresh soil was air-dried and then passed through a 5-mm sieve. Uniform and well-grown seedlings (three days after seed germination) were transplanted in the selected pots (with a diameter of the opening 25 cm and height 30 cm) filled with 7 kg of the soil. Eight seeds per each pot were planted and at two-leaf stage, 4 plants were kept. The experimental dragonheads were grown in the research greenhouse of Maragheh University under a photoperiod of 14 h per day at 30 ± 2/20 ± 2 °C day/night cycle with 55-70% average relative humidity. In order to maintain soil moisture, irrigation was done every day to near field capacity using deionized water.

Ordinary zinc oxide (ZnO) and zinc oxide nanoparticle (ZnO-NP) were purchased from Pishgaman Danesh Company, Iran. The zinc nanoparticles had 99% purity as well as density of 5.606 g/cm^3 and size of 20-30 nm. For foliar treatments, four levels of zinc oxide (0, 25, 50, and 100 mg/L) and four levels of zinc oxide nanoparticles (0, 25, 50, and 100 mg/L) were prepared with the deionized water. In order to elevate the dispersion of ZnO and ZnO-NP, the suspension was ultra-sonicated for 30 min. Foliar spray was done for 3 times at an interval of 15 days, 40 days after sowing. To prevent the leaves from burning, foliar application was done at

sunset. The solutions of ZnO-NP and ZnO were freshly made in each application. For untreated plants, distilled water spray was used simultaneously with the treatments. The plant samples were harvested 15 days after the last foliar application. The dragonheads were separated into roots and shoots and washed with tap water. Then, plant height, root length, numbers of leaves per plant, and fresh weights were measured. To perform biochemical analyses, the samples were frozen in liquid nitrogen and stored at - 80 °C until laboratory experiments. The shoot and root samples were dried at room temperature in the shade for one week to reach a constant weight, and dry weights were recorded using a weighing balance.

Photosynthesis pigments assay

For determination of pigment contents, 0.5 g fresh tissue of the youngest fully expanded leaves was homogenized in 80% acetone and centrifuged at 4000 rpm for 20 min. According to Arnon's method (Arnon, 1967), the absorbance of the supernatant was recorded at 663, 645, and 470 nm for Chl a, Chl b, and carotenoids, respectively, using a UV-visible spectrophotometer (BMS, UV-2600).

Antioxidant enzyme activities assay

Leaf tissue (0.5 g) was powdered by a pre-chilled mortar and pestle, and homogenized in 5 mL ice-cold extraction buffer (50 mM potassium phosphate, pH 7.0, 4% polyvinylpyrrolidone). The extract was centrifuged at 14.000 g for 30 min at 4 °C. The supernatant was used for assays of the activities of antioxidant enzymes.

Total protein concentration in the leaf extracts was determined according to the Bradford method (Bonjoch and Tamayo, 2001) with bovine serum albumin as the standard. Catalase (CAT) activity was assayed at 240 nm by Cohen and coworkers method (Cohen et al., 1996). The hydrogen peroxide decomposition rate was monitored during 1 min at 25 °C. The activity of ascorbate peroxidase (APX) was assayed following Miyake and Asada method (1996). The reaction of ascorbic acid oxidation was started by addition of H_2O_2, and the decrease in absorbance was read at 290 nm. The activity of guaiacol peroxidase (GPX) was assayed according to Chance and Maehly (1995). The reaction of guaiacol oxidation was initiated by addition of H_2O_2, and the increase in absorbance was read at 470 nm. The enzymes activities were expressed as U/min. mg protein.

Extraction of essential oil

To extract the essential oil, 50 g of dried shoot was hydro-distilled for 3 h by a Clevenger-type apparatus. The extracted essence was dried over anhydrous sodium sulphate and kept at −20 °C until GC-MS analysis. The amount of essential oil content was recorded based on the oil volume per shoot dried sample (as % v/w, mL/100 g DW).

Analysis of essential oil

To identify the essential oil composition, GC-MS analysis was done on an Agilent 6890N gas chromatograph with a 5977 mass-selective detector (Agilent Technologies, USA). The separation was performed on an HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm diameter and 0.25 µm film thickness). Helium was used as the carrier gas with a flow rate of 1 mL/min. The system was programmed as follows: the injector temperature 250 °C, initial temperature 50 °C (for 5 min), raised to 240 °C at 3 °C/min (for 10 min), electron energy 70 eV, the transfer line temperature 270 °C, split ratio 1:30, and the acquisition scan from 50 to 240 m/z. A mixture of aliphatic hydrocarbons (C8–C40; Sigma–Aldrich, USA) was injected into the device for calculation of the retention indices of peaks. The essential oil compounds were recognized using co-injection with available authentic standards (Sigma–Aldrich, USA) and by computer matching with MS libraries (WILEY275, NIST 05, ADAMS).

Statistical Analysis

This research was conducted in the form of a completely randomized design with four replicates. Statistical analysis of data was done using SPSS software ver. 19 (Chicago, IL, USA). To compare the means, Tukey's test was used at a probability level of 5% ($p \leq 0.05$), and the data was presented as means ± standard deviation (SD).

Table 1
Effect of ZnO and NP-ZnO treatments on the growth parameters of dragonhead

Treatment	Concentration (mg/L)	Shoot height (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Control	0	47.23±3.71 e	15.70±1.93 d	1.89±0.28 b	0.91±0.11 c
ZnO	25	51.10±7.41 de	16.16±2.52 d	2.02±0.18 b	0.92±0.14 c
	50	55.56±2.16 cd	16.23±1.05 d	2.13±0.22 b	1.07±0.19 bc
	100	59.60±6.11 bc	22.00±4.58 bc	2.88±0.79 ab	1.50±0.18 b
NP-ZnO	25	59.50±1.70 bc	17.70±0.85 cd	2.22±0.18 b	1.18±0.15 bc
	50	65.33±4.01 ab	24.10±3.65 b	3.30±0.48 a	1.71±0.18 ab
	100	69.33±0.68 a	33.96±4.92 a	3.47±0.22 a	1.90±0.22 a

Values are Means ± SD. The same letter within each column indicates no significant difference among treatments using Tukey's Test at p≤0.05.

Table 2
Effect of ZnO and NP-ZnO treatments on the photosynthetic pigments and essential oil (%) of dragonhead

Treatments	Concentration (mg/L)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll a+b (mg/g FW)	Carotenoid (mg/g FW)	Essential oil (%)
Control	0	3.02±0.03 g	1.01±0.01 g	4.03±0.03 g	0.30±0.01 g	0.29±0.05 b
Zn O	25	3.32±0.03 f	1.07±0.06 f	4.39±0.05 f	0.31±0.06 f	0.29±0.05 b
	50	3.43±0.05 e	1.13±0.01 e	4.56±0.04 e	0.40±0.01 e	0.30±0.03 b
	100	3.80±0.06 c	1.23±0.03 c	5.03±0.04 c	0.53±0.03 c	0.31±0.03 b
NP-ZnO	25	3.66±0.03 d	1.21±0.02 d	4.87±0.03 d	0.41±0.02 d	0.30±0.04 b
	50	4.09±0.06 b	1.38±0.01 b	5.47±0.05 b	0.56±0.04 b	0.33±0.07 b
	100	4.27±0.03 a	1.41±0.01 a	5.68±0.03 a	0.66±0.07 a	0.53±0.07 a

Values are Means ± SD. The same letter within each column indicates no significant difference among treatments using Tukey's Test at p≤0.05.

Table 3
Effect of ZnO and NP-ZnO treatments on the antioxidant enzymes activity of dragonhead

Treatment	Concentration (mg/L)	Total soluble protein	CAT (U/min. mg prot.)	APX (U/min. mg prot.)	GPX (U/min. mg prot.)
Control	0	08.24±0.21 g	15.15±0.04 g	0.53±0.03 g	13.24±0.11 g
ZnO	25	10.06±0.10 f	17.20±0.05 f	0.73±0.02 f	16.78±0.09 f
	50	10.86±0.05 e	18.71±0.03 e	0.83±0.02 e	17.12±0.01 e
	100	14.32±0.02 c	21.63±0.05 c	1.79±0.04 c	20.32±0.04 c
NP-ZnO	25	12.55±0.02 d	20.33±0.09 d	1.00±0.01 d	19.51±0.04 d
	50	15.90±0.08 b	23.94±0.04 b	2.87±0.08 b	22.81±0.03 b
	100	17.79±0.13 a	26.19±0.06 a	4.31±0.04 a	24.62±0.04 a

Values are Means ± SD. The same letter within each column indicates no significant difference among treatments using Tukey's Test at p≤0.05.

Results

Results showed that both ZnO and ZnO-NPs, treatments at different concentrations affected growth traits in dragonhead (Table 1). By increasing levels of ZnO and ZnO-NPs, the growth parameters increased. In plants sprayed with ZnO, this increase was significant (p≤0.05) for shoot height at 50 and 100 mg/L and for root length and root dry weight at 100 mg/L, compared to control (Table 1). ZnO-NPs treatment significantly (p≤0.05) increased shoot height at all applied levels, and root length and shoot and root dry weights at 50 and 100 mg/L, when compared to control plants. Also, the improving effect of ZnO-NPs on growth traits at each experimental level was greater than that of ZnO at the respective level. The minimum and maximum amounts of all growth traits were observed in control plants and those treated with 100 mg/L ZnO-NPs, respectively (Table 1). Spraying the plants with 100 mg/L ZnO-NPs improved shoot height by 46%, root length by 116%, shoot dry weight by 83%, and root dry weight by 108% over the control.

Table 4
Effect of ZnO and NP-ZnO treatments on the essential oil components of dragonhead

Components	Empirical Formula	Molecular Weight	Category	RT	RI	RI Lit.	Control	ZnO 25	ZnO 50	ZnO 100	ZnO NPs 25	ZnO NPs 50	ZnO NPs 100
Camphene	$C_{10}H_{16}$	136.23	Bicyclic Monoterpene	3.19	947	946	0.112	0.201	0.218	0.231	0.139	0.148	0.236
Sabinene	$C_{10}H_{16}$	136.23	Bicyclic Monoterpene	19.51	970	969	0.144	1.113	0.966	0.830	0.981	0.988	1.100
1,8-Cineole	$C_{10}H_{18}O$	154.25	Bicyclic Monoterpene	22.76	1030	1026	0.530	0.534	0.659	0.655	0.693	0.747	0.511
Fenchone	$C_{10}H_{16}O$	152.23	Bicyclic Monoterpene	26.44	1084	1083	0.079	-	-	0.137	0.198	0.310	0.344
Linalool	$C_{10}H_{18}O$	154.25	Acyclic Monoterpene	27.78	1095	1101	0.144	0.234	0.241	0.234	0.322	0.304	0.375
trans-Pinocarveol	$C_{10}H_{16}O$	152.23	Bicyclic Monoterpene	28.52	1135	1138	0.297	0.270	0.214	0.252	0.412	0.305	0.417
Camphor	$C_{10}H_{16}O$	152.23	Bicyclic Monoterpene	28.66	1141	1144	0.625	0.491	0.619	0.350	0.390	0.468	0.539
trans-Chrysanthemal	$C_{10}H_{16}O$	152.23	Monocyclic Monoterpene	28.66	1153	1149	0.092	-	-	-	-	-	-
Borneol	$C_{10}H_{18}O$	154.25	Bicyclic Monoterpene	29.39	1165	1163	0.906	0.734	0.794	0.639	0.683	0.674	0.417
Menthol	$C_{10}H_{20}O$	156.27	Monocyclic Monoterpene	29.78	1167	1171	0.159	0.183	-	0.176	0.178	0.512	0.404
Myrtenol	$C_{10}H_{16}O$	152.23	Bicyclic Monoterpene	30.83	1194	1193	0.061	-	-	-	-	-	-
n-Dodecane	$C_{12}H_{26}$	170.33	Alkane	31.16	1200	1200	0.154	0.201	-	-	0.092	0.137	0.139
Nerol	$C_{10}H_{18}O$	154.25	Acyclic Monoterpene	31.82	1227	1227	0.539	0.701	0.675	0.754	0.574	0.628	0.484
Z-Citral	$C_{10}H_{16}O$	152.23	Acyclic Monoterpene	32.58	1238	1241	19.188	17.269	18.935	19.008	20.064	21.291	21.707
Geraniol	$C_{10}H_{18}O$	154.25	Acyclic Monoterpene	32.95	1252	1255	5.932	6.861	6.733	7.373	7.503	8.275	8.309
Geranial (E-Citral)	$C_{10}H_{16}O$	152.23	Acyclic Monoterpene	33.93	1267	1272	27.919	28.065	35.593	34.875	35.432	35.568	36.069
Carvacrol	$C_{10}H_{14}O$	150.22	Monocyclic Monoterpene	35.26	1298	1300	0.076	0.071	0.075	0.079	0.080	0.090	0.112
Methyl Geranate	$C_{11}H_{18}O_2$	182.26	Acyclic Monoterpene	36.55	1322	1321	0.171	0.217	0.182	0.214	0.204	0.252	0.301
Neryl Acetate	$C_{12}H_{20}O_2$	196.29	Acyclic Monoterpene	37.75	1361	1363	0.985	1.024	1.374	1.232	1.941	1.523	1.349
Geranyl Acetate	$C_{12}H_{20}O_2$	196.29	Acyclic Monoterpene	38.65	1381	1385	18.369	18.719	21.431	22.487	22.376	24.863	25.486
n-Tetradecane	$C_{14}H_{30}$	198.39	Alkane	39.13	1400	1397	0.169	0.150	0.164	0.170	0.198	0.173	0.181
beta-Caryophyllene	$C_{15}H_{24}$	204.35	Bicyclic Sesquiterpene	39.57	1417	1414	0.115	0.175	0.382	0.125	0.193	0.161	0.214
Germacrene D	$C_{15}H_{24}$	204.35	Monocyclic Sesquiterpene	45.38	1484	1475	0.126	0.590	0.637	0.654	0.319	0.141	0.190
beta-Selinene	$C_{15}H_{24}$	204.35	Bicyclic Sesquiterpene	46.32	1489	1480	0.106	0.112	0.136	0.154	0.425	0.497	0.439
Spathulenol	$C_{15}H_{24}O$	220.35	Tricyclic Sesquiterpene	47.69	1577	1571	0.113	0.164	0.198	0.285	0.118	0.161	0.201
Caryophyllene Oxide	$C_{15}H_{24}O$	220.35	Bicyclic Sesquiterpene	47.75	1582	1576	0.184	0.167	0.169	0.187	0.287	0.196	0.194
n-Hexadecane	$C_{16}H_{34}$	226.41	Alkane	47.85	1600	1600	0.087	0.102	0.144	0.148	-	0.165	0.152
Monoterpenes							76.331	76.687	88.709	89.526	92.170	96.946	98.160
Hydrocarbon Monoterpenes (HM)							0.256	1.314	1.184	1.061	1.120	1.136	1.336
Oxygenated Monoterpenes (OM)							76.075	75.373	87.525	88.465	91.050	95.810	96.824
Sesquiterpenes							0.644	1.208	1.522	1.405	1.342	1.156	1.238
Hydrocarbon Sesquiterpenes (HS)							0.347	0.887	1.155	0.933	0.937	0.799	0.843
Oxygenated Sesquiterpenes (OS)							0.297	0.331	0.367	0.472	0.405	0.357	0.395
Others							0.410	0.453	0.308	0.318	0.290	0.475	0.472
Total							77.385	78.348	90.539	91.249	93.802	98.577	99.870

As shown in Table 2, photosynthesis pigments were influenced by foliar spraying treatments. Application of ZnO and ZnO-NPs in all studied levels significantly ($p \leq 0.05$) improved the pigment contents including chlorophyll a and b, total chlorophyll, and carotenoids in comparison with control dragonheads. Improving the level of photosynthetic pigments had a similar trend with increasing the level of both ZnO and ZnO-NPs. Under each level of treatment, the plants treated with ZnO-NPs showed a higher level of the pigments than the plants treated with ZnO. The highest and lowest pigment contents were found under 100 mg/L of ZnO-NPs and 0 mg/L of ZnO NPs (or 0 mg/L ZnO), respectively (Table 2). Foliar treatment of ZnO-NPs at 100 mg/L enhanced the contents of chlorophyll a by 41%, chlorophyll b by 39%, total chlorophyll by 40%, and carotenoids by 120% over their respective controls.

Based on Table 2, the essential oil percent was significantly elevated by application of ZnO-NPs at 100 mg/L (82.7% compared to control) while other levels of ZnO-NP and ZnO did not have a significant effect on the percentage of essential oil.

The data presented in Table 3 illustrated that both treatments including ZnO and ZnO-NPs affected the total soluble protein and antioxidant enzymes so that with increasing levels of ZnO and ZnO-NPs, the soluble protein content and the activities of CAT, APX, and GPX were elevated. The maximum and minimum amount of leaf soluble protein and leaf enzymes activities were observed at 100 mg/L of ZnO-NPs and control plants, respectively (Table

3). The treatment of ZnO-NPs at 100 mg/L elevated the content of soluble protein by 115%, CAT activity by 73%, APX activity by 713%, and GPX activity by 86% over their respective controls (Table 3).

Twenty-seven (27) essential oil components were identified in different concentrations of ZnO and ZnO-NPs (Table 4). The major constituents of *D. moldavica* oil were found as geranial or E-citral (27.91-36.09%), geranyl acetate (18.36-25.48%), Z-Citral or neral (19.18-21.7%), and geraniol (5.93-8.30%) (Table 4). Treatments of the study caused an increase in the levels of the essential oil compounds. In the meantime, the effect of zinc oxide nanoparticles on the level of compounds was more than ordinary zinc oxide. The highest amount of these essential oil compounds was seen under nanoparticle treatment at 100 mg/L. Foliar application of ZnO-NPs at 100 mg/L elevated the amounts of geranial by 29.1%, geranyl acetate by 38.7%, Z-Citral by 13.1%, and geraniol by 39.9% in comparison with control plants. In this study, the level of oxygen-containing monoterpenes (76.07-96.82%) was the highest among essential oil compounds of *D. moldavica* under different levels of the treatments. The highest levels of oxygenated monoterpenes were observed in ZnO-NPs treatment at 100 mg/L, so that spraying *D. moldavica* plants with 100 mg/L ZnO-NPs increased the amounts of oxygenated monoterpenes by 28.6% in comparison to control plants (Table 4).

Discussion

Foliar application of micronutrients such as zinc is one of the easiest and fastest ways to eliminate or reduce the deficiency of these nutrients in plants. It is shown that spraying zinc is a highly efficient and practical method to improve the absorption and accumulation of this element, rather than soil application (Xu et al., 2021). In this way, zinc was rapidly translocated via phloem to different organs of the plant (Gupta et al., 2016). Zinc plays an important role in protein synthesis, indole acetic acid synthesis, cell elongation, regulating membrane stability and function, and enzyme activities (Umair Hassan et al., 2020). In our study, foliar spraying of ZnO and ZnO-NPs enhanced growth attributes in terms of plant height, root length, and shoot and root dry matter in dragonhead, and in this regard, the effectiveness of ZnO-NPs was higher than ordinary ZnO. Awan et al. (2021) reported more efficiency of ZnO-NPs for enhancing shoot and root lengths and leaf numbers compared to the macro size Zn salt in *Brassica oleracea*. Also, Rossi et al. (2019)showed a more positive role of ZnO-NPs on coffee growth and physiology than conventional Zn salts and argued that this positive impact may be due to increased ability of nanoparticle to penetrate the leaf. They stated that dissolution of zinc nanoparticles in water is relatively slow, so that about 2% of zinc from nanoparticles is dissolved in 1 day. However, after attaching Zn-NPs to leaf surfaces, zinc might be continuously released, providing a long-term source of Zn. The leaf cuticle permeability to water and lipophilic substances increases by mobility and solubility of these molecules, and in this regard, ZnO-NPs which are having more lipophilicity and mobility than non-NPs (Prasad et al., 2022), can penetrate via the lipophilic cuticle of leaf, and distribute via phloem in different parts of plants. The lower water solubility of ZnO-NPs can prevent fast falling off from leaf surface in compare to other supplements. The bioavailability of nanoparticles due to their small size and large surface area can also be higher than that of ZnO, resulting in a higher absorption of Zn to leaf.

Among the treatments used in this research, 100 mg/L ZnO-NPs was found optimum for foliar application as it produced the highest increase in the growth and dry biomass of dragonheads over all other treatments. In wheat plants and under foliar spray treatment with ZnO-NPs, increasing Zn-NPs concentration increased the growth, and maximum increase in growth attributes was achieved in the wheats treated with 100 mg/L ZnO-NPs (Adrees et al., 2021). Also, Rizwan et al. (2019) showed that foliar spray of 100 mg/L ZnO-NPs increased shoot dry biomass by 65%, root dry biomass by 86%, shoot length by 34%, and number of leaves per plant by 72% in maize plants compared to the control under cadmium stress. The apparent increase in the growth of the plants exposed to nano-fertilizers can be attributed to their homogeneous distribution in different parts of the plant (Awan et al., 2021). Furthermore, Li et

al. (2018) argued that the slow release of ZnO-NPs in tomato and soybean plants prevents the toxicity of large amounts of zinc in plants by preventing its sudden uptake.

According to Table 2, maximum chlorophyll a and b, total chlorophyll, and carotenoid contents were observed in plants treated with 100 mg/L ZnO-NPs. Similar results were found in maize plants so that foliar application of 100 mg/L ZnO-NPs elevated the chlorophyll a and b concentrations by 64% and 67%, respectively (Rizwan et al., 2019). Foliar application of ZnO-NPs increased the amounts of light harvesting pigments, i.e., chlorophyll a, b, and carotenoids in sugarcane seedlings (Elsheery et al., 2020). A High carotenoid content in leaves of sugarcane plants treated with ZnO-NPs elevated the non-photochemical quenching (NPQ) of PSII (Elsheery et al., 2020).

Xanthophylls (as oxygen containing carotenoids) are involved in quenching of excess energy on PSII through the xanthophyll cycle, which is important to prevent photo-damage to the photosystems and maintain the photosynthesis rate (Derks et al., 2015). Zn affects the concentration of micro- or macro-nutrients involved in chlorophyll biosynthesis (such as Fe and Mn), which are part of chlorophyll structure such as N and Mg (Kumar et al., 2021). Amino levulinic acid dehydratase (an enzyme in the chlorophyll biosynthesis pathway) requires Mg^{2+} and Zn^{2+} for its activity (Zhu et al., 2023), so its activity is enhanced by Zn treatment. It could be hypothesized that an increase in the growth and photosynthesis pigment contents in dragonheads might be a result of physiological responses caused by zinc sufficiency under Zn fertilization.

The findings showed that a concentration of 100 mg/L ZnO-NP significantly increased the essential oil percentage in plant tissues.The increase in the content of essential oil under nano-zinc application have been observed by other researchers such as Nekoukhou et al. (2022) in dragonhead and Fallahi et al. (2016) in basil. Faizan et al. (2018) reported that zinc as a cofactor by increasing the activity of Rubisco enzyme and carbon metabolism promotes the synthesis of essential oils, especially monoterpenes. In other words, optimal supply of zinc allocates more assimilates (such as glucose) to the production of essential oils by improving the photosynthetic capacity (Nekoukhou et al., 2022; Saleh et al., 2018).

Nanoparticles cause calcium and reactive oxygen species signaling at the cell surface along with complex physiological changes at the organism level (Abdal Dayem et al., 2017). The results from Table 3 showed that by increasing levels of ZnO and ZnO-NPs, activities of CAT, APX, and GPX significantly increased. Application of nano-zinc and salt-zinc as priming treatment and foliar spray treatment significantly increased CAT and POD activities compared to control in corn plants (Naseer et al., 2023). Hernandez-Fuentes et al. (2023) reported that foliar application of zinc nanoparticles promoted greater CAT, APX, and POD activities, compared to the control in pear plants. Application of ZnO-NPs by 50-200 mg/L significantly boosted CAT, APX, and GPX activities under arsenic stress in both seedlings and plants before the reproductive stage in *Vigna mungo* (Banerjee et al., 2023). Increased antioxidant enzyme activities reflect the generation of ROS and then oxidative damage caused by the zinc treatment in dragonhead plants. The antioxidant enzymes of CAT, APX, and GPX play an important role in plant defense mechanisms against oxidative stress via converting H_2O_2 to water in plant cell organelles. Although zinc is an important element for many metabolic processes in the plant cell, its high concentration disrupts photosynthesis and creates oxidative stress. In this study, the effect of ZnO-NPs in each of the treatment levels on antioxidant enzymes activities was greater than the effect of ZnO at the same level. In alfalfa, the activities of CAT, APX, and POD (peroxidase) were noticeably higher in nano-ZnO treated plants than in bulk-ZnO under non-stress and salt-stress conditions (Hassan et al., 2023). CAT and APX activities increased by 88-106%, with 100 mg/L of in comparing to bulk Zn and control in tomato seedlings (Azim et al., 2022). The results of our experiment along with the results of previous studies confirm that the increase in the amount of antioxidant enzymes can be the plant's defense response to the increase in free radicals caused by the increase in zinc levels in the plant, although the dragonheads were able to compensate this

negative effect by increasing the antioxidant enzymes, and thus improving their growth and biomass accumulation.

In the present study, the dominant compounds of the essential oils in the dragonhead plant, in the control and treated plants with different concentrations of zinc oxide and zinc nanoparticles, were oxygenated monoterpenes, and among them four dominant substances were geranial, geranyl acetate, Z-citral, and geraniol, respectively. Although their relative levels were different in the treatments. An increase in the levels of oxygenated monoterpenes, including geraniol, geranial, geranyl acetate, and neral, indicates an improvement in the quality of the synthesized essential oil in dragonhead, as most of the desirable physical, chemical, and biological properties of essential oils are attributed to these compounds (Brahmi et al., 2017). Review of the literature on chemical compositions of dragonhead suggests that the largest number of the compounds belongs to geranial + neral (Z-citral) + geranyl acetate chemotype (Aćimović et al., 2022). Some other researchers have reported the major compounds of dragonhead as follows: geranyl acetate + geranial + geraniol (Amini et al., 2020; Pouresmaeil et al., 2022). The chemotype of the essential oil extracted from *D. moldavica* harvested in Urmia, Iran included geranial, neral, geraniol, and geranyl acetate (Ehsani et al., 2017). The variation in essential oil composition can be caused by the factors such as regional climate, plant species and variety, distillation conditions, and maturation stage (Aminzare et al., 2015).

The results of Table 4 showed that the application of foliar zinc (zinc oxide and zinc nanoparticles) at different concentrations was effective on the composition of dragonhead's essential oil. Monoterpenes such as geranyl acetate, geranial, geraniol and neral are synthesized via MEP (methylerythritol phosphate) pathway (Lukas et al., 2015). The key enzyme of MEP pathway (2-C-methyl-D-erytrithol 2, 4-cyclodiphosphate synthase) requires zinc as a cofactor for its activity (Steinbacher et al., 2003). It can be said that the optimal supply of zinc can increase the level of monoterpenes. Hassanpouraghdam et al. (2011) reported that methylchavicol as the main ingredient of essential oil of *Ocimum basilicum* increased with zinc sulfate treatment.

In our study, positive effects of ZnO-NP in increasing oxygenated monoterpenes were more pronounced in comparison with the same level of ZnO. In a previous work, the levels of the monoterpenes (neral, geraniol, and geranial) significantly increased with ZnO-NP (160 mg/L) compared to the equivalent level of ZnS or control (Nekoukhou et al., 2022). Nanoparticles may cause a slight increase in the production of reactive oxygen species (ROS) in cells, which can cause the changes in secondary metabolites and activate the antioxidant system (Marslin et al., 2017). Therefore, the use of nano-fertilizers as an elicitor, by changing the chemical composition of the essential oil, can be useful for increasing the production of the desired secondary metabolites.

The results of this research showed that foliar application of ordinary ZnO and ZnO-NPs at different levels had a significantly positive effect on growth, photosynthetic pigments, antioxidant enzymes, and the quantity and quality of dragonhead essential oil. The highest amount of growth indicators, contents of chlorophyll a, b, and carotenoids, antioxidant enzymes activities as well as the percentage of essential oils were observed in plants sprayed with 100 mg/L of ZnO-NP. The investigation of the main compositions of dragonhead essential oil revealed that oxygenated monoterpenes constitute an important percentage of the secondary compounds in the essential oil. The four compounds of geranial, geranyl acetate, Z-citral, and geraniol contain the highest levels of essential oil. Foliar application of zinc nanoparticles at 100 mg/L produced the highest amount of the above-mentioned essences. Therefore, foliar application of zinc, especially zinc nanoparticles, plays an important role in increasing the biomass and essential oil of the valuable dragonhead plant.

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