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Impact of zinc and manganese on oregano (*Origanum vulgare* L. subsp. *hirtum*) growth and essential oil composition

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Abstract

This study conducted during the 2020/2021 and 2021/2022 seasons investigated the influence of Zinc (Zn) and Magnesium (Mn), alone or combined, on oregano plants' growth, yield, and essential oil composition. Results revealed that oregano height peaked with 50 ppm Zn, while 100 ppm Zn and a combination of 50 ppm Zn with 50 ppm Mn produced the highest number of shoots and branches. Optimal fresh and dry yields occurred with 50 ppm Zn and 100 ppm Mn. The greatest essential oil percentage resulted from the combination of 100 ppm Zn and 50 ppm Mn. Zn and Mn, independently or combined, led to a significant reduction in chlorophyll "a," "b," and carotenoids, with 50 ppm Zn most affecting chlorophyll "a" and "b" and the combination of 100 ppm Zn and 50 ppm Mn predominantly impacting carotenoids. Total phenolic and flavonoid levels increased with the addition of Zn and Mn, particularly with 100 ppm Zn and 50 ppm Mn. Antioxidant activity, assessed by the DPPH assay, reaching its peak it 100 ppm Zn and 100 ppm Mn. GC-MASS analysis identified 22 compounds, with carvacrol and thymol as major constituents, carvacrol consistently surpasses thymol in concentration across treatments. Interestingly, low concentrations of Zn or Mn, when applied individually, had a more pronounced impact than their combination, particularly on carvacrol. Conversely, Mn exhibited a more noticeable effect on thymol compared to Zn. These findings suggest that incorporating Zn and Mn, especially at low concentrations, in oregano fertilization enhances both yield and quality.

Keywords:Oregano; Zn; Mn; essential oil; Carvacrol; Thymol; DPPH.

1. Introduction

Origanum vulgare L. subsp. *hirtum*, commonly referred to as Greek oregano, is a perennial plant that belongs to the Lamiaceae family. This herb finds is widely used in the food and pharmaceutical industries [1]. Greek oregano thrives in regions with ample sunlight, typically growing at altitudes of up to 1500 meters above sea level. One of its distinctive features is the presence of dense glandular trichomes, which contribute to a higher concentration of essential oils [2, 3]. In addition to its role as a popular spice and seasoning, Greek oregano is highly valued for its essential oil, primarily found in its inflorescences, leaves, and stems. The aerial parts of the plant also serve as rich sources of polyphenols, flavonoids, and triterpenoids, and they yield significant quantities of carvacrol and thymol, both well-known bioactive compounds [2, 4].

Oregano is a commonly used ingredient for its preservative and flavor-enhancing properties in a wide range of products, including foods, alcoholic beverages, cosmetics, and soaps [5, 6]. Depending on its chemical composition, oregano essential oil is

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categorized into two primary chemotypes: carvacrol and thymol. These categories are determined by the relative levels of these two phenolic compounds [3, 7]. Due to its abundance of secondary metabolites, Greek oregano is known for its antibacterial [8, 9], antifungal [10, 11], insecticidal [12], antioxidant [13] and nematicidal [14] properties.

Zinc is a vital micronutrient for plants, serving a key role in many biological processes within plant tissues. It plays essential roles in gene expression, protein synthesis, the maintenance of membrane and protein structural integrity, in addition to functions as a catalyst for numerous enzymes [15-18]. Furthermore, zinc is indispensable for vegetative growth, as it serves as a precursor to phytohormones such as auxins, which have a direct impact on cell elongation and division [19]. Zinc also plays a critical role in the process of photosynthesis and contributes to carbohydrate metabolism by stabilizing or activating proteins involved in these processes [20]. In agricultural practices, solid zinc oxide (ZnO) fertilizers are commonly employed to supply plants with the necessary zinc. The application of foliar treatments with ZnO or nano-ZnO has been demonstrated to have positive effects on growth parameters, biochemical characteristics, and ultimately, crop yield [21, 22].

Manganese (Mn) is an essential element in the biology of virtually all living organisms, serving two primary functions: as an enzyme cofactor and as a catalytic metal within biological clusters [23]. In the context of plant biology, Mn is one of the 17 essential elements critical for growth and reproduction. Although plants require Mn in relatively small quantities, its importance for growth is on par with that of essential nutrients. Manganese acts as a crucial micronutrient for plant growth and development, playing vital roles in various cellular compartments [24]. Mn is particularly indispensable for processes like photosynthesis. Mn deficiency is a common issue, often observed in sandy soils, organic soils with a pH above 6, and highly weathered tropical soils. Cool and wet conditions tend to worsen this deficiency [25]. Many crop species are susceptible to Mn deficiency in such soils, and they exhibit improved growth when Mn is added as a fertilizer. The repercussions of Mn deficiencies in these crops include reduced dry matter production, decreased yield, compromised resistance to pathogens, and increased vulnerability to drought and heat stress.

In photosynthetic organisms, Mn plays a critical role as an essential component of the metallo-enzyme cluster in the oxygen-evolving complex within photosystem II. Despite its significance in photosynthesis, the regulation of Mn levels within plants, known as Mn homeostasis, has been insufficiently explored. Mn deficiency can become a significant nutritional issue for plants, particularly in soils with high pH and elevated oxygen levels, where Mn bioavailability can fall below the levels needed for normal plant growth [26].

Micronutrients play a crucial role in plant growth and the synthesis of active substances. The primary goal of this study was to evaluate the impact of Zn and Mn, whether applied individually or in combination, on the growth, yield, and essential oil composition of oregano plants.

2. Materials and methods

This field experiment was carried out over the consecutive seasons of 2020/2021 and 2021/2022 at the Experimental Farm of the National Research Centre in Nubaria, El-Buheira Governorate, Egypt. The objective was to investigate the influence of zinc and manganese on the growth of oregano (*Origanum vulgare* L. subsp. *hirtum*) and the composition of its essential oil. The oregano seeds, obtained from the Egyptian Ministry of Agriculture, were sown in nursery beds prepared in September for both seasons. After two months, the seedlings were transplanted into rows, spaced 60 cm apart, with a 30 cm gap between plants within hills. The soil in the field experiment had a sandy texture in both seasons, and soil and water analyses are presented in Tables 1 and 2. Each experimental unit (plot) comprised two rows, 60 cm apart, and was 3 m long.

The experiment utilized a completely randomized block design with three replicates. There were nine treatments, including a control group, and various combinations of zinc (Zn) and manganese (Mn) concentrations: 50 ppm Zn, 100 ppm Zn, 50 ppm Mn, 100 ppm Mn, 50 ppm Zn + 50 ppm Mn, 50 ppm Zn + 100 ppm Mn, 100 ppm Zn + 50 ppm Mn, and 100 ppm Zn + 100 ppm Mn. The irrigation was administered through a drip system, and standard agricultural practices were followed. Foliar spraying was conducted three times, specifically at 45, 90, and 135 days after transplanting the seedlings in both

seasons. Harvesting took place in June of each year, and data were collected for analysis.

Table (1): Physical and chemical analysisof the used experimental soil

Soil Texture	pH 1:2.5	EC dsm^{-1}	Soluble cations (meq/100 g soil)				Soluble anions (meq / l00g soil)		
			Na^{+}	K^{+}	Ca^{++}	Mg^{++}	HCO^{-3}	Cl^{-}	SO^{-4}
Sandy	7.84	0.20	0.75	0.18	0.32	0.22	0.45	0.78	0.24

Table (2): Analysis of irrigation water under study

pH 1:2.9	EC dsm^{-1}	Soluble cations meq/l				Soluble anions meq/l		
		Na^{+}	K^{+}	Ca^{++}	Mg^{++}	HCO^{-3}	Cl^{-}	SO^{-4}
7.49	0.48	2.45	0.20	1.48	0.57	1.18	2.77	0.75

2.1. Vegetative growth and yield traits

Vegetative growth parameters such as plant height (cm), the number of shoots per plant, and the number of lateral branches per plant on the main branch were documented. In terms of yield traits, the fresh weight per plant was recorded. Subsequently, the shoots were allowed to air dry under shaded conditions for one week, and the dry weight per plant was then recorded.

2.2. Extraction of essential oil

Essential oil content of each sample was determined according to AOAC [27] and expressed as mL/100 g dried sample. Essential oil was extracted according to Rafea et al.[28], using hydro-distillation method in a Clevenger-type apparatus for 3 h. The resulting oil was dried over Na_2SO_4 and stored at -20 °C in amber glass vials until analysis.

2.3. Essential oils analysis by Gas Chromatography Mass spectrometry

The GC-MS analysis of the oregano essential oil samples was carried out using TRACE GC Ultra Gas Chromatographs (THERMO Scientific™ Corporate, USA), coupled with a single quadrupole mass spectrometer (Thermo Scientific ISQ™ EC) at the Department of Medicinal and Aromatic Plants Research, National Research Centre, Egypt. The GC-MS system was equipped with a TR-5 MS column (30 m × 0.32 mm i.d., 0.25 µm film thickness) and helium as carrier gas at a flow rate of 1.0 mL/min. Oven temperature was held at 60°C for 1 min; then raised by 4.0°C/min to 240°C and held for 1 min, while the temperature of injector and ion source were held at 210°C. Essential oil samples were dissolved in hexane (1:10 v/v) and 1 µL was injected with a split ratio of 1:10. Mass spectra were recorded by electron ionization (EI) at 70 eV and scan range of m/z from 40 to 450.

The separated compounds were identified using two different methods: (a) KI, Kovats indices in reference to n-alkanes (C9-C22) by using AMDIS software (www.amdis.net), and (b) based on the mass spectra of the National Institute of Standards and Technology (NIST) and Wiley spectral library collection.

2.4. Photosynthetic pigments

To determine pigments, 0.5 g of leaf samples underwent extraction with methanol using a magnetic stirrer at 700 rpm for 15 minutes. Following the separation of the supernatant, the extraction process was repeated. Chlorophyll a, chlorophyll b, and total carotenoids were quantified using a UV-Vis spectrophotometer (UVS-2800, Labomed Inc., USA). The absorbance readings were taken at 470, 645, and 666 nm using a glass cuvette. The concentrations of chlorophylls and carotenoids were calculated based on the method described by Costache et al. [29].

2.5. Total phenolic content

Total phenolics of dried leaves were determined according toYousif et al. [30] using Folin-Ciocalteu technique. Briefly, 100 µL of the extract was put in a test tube, and distilled water were used to adjust the volume to 3.5 mL. For oxidation, 250 µL of Folin-

Ciocalteau reagent was then added. A 20% aqueous sodium carbonate (Na_2CO_3) solution containing 1.25 mL was added to the mixture to neutralizeit after five minutes. The absorbance was measured at 725 nm against the solvent blank after an incubation period of 40 minutes. Using a calibration curve made using gallic acid, the total phenolic content was calculated and represented as milligrammes of gallic acid equivalent (mg GAE) per gramme of the sample.

2.6. Determination of total flavonoid content:

The total flavonoid content of the dried leaves was ascertained using an aluminum chloride ($AlCl_3$) colorimetric assay, in accordance with the methodology reported by Yousif et al. [30]. A simple approach involved mixing 100 µL of the extract with 300 µL of 5% sodium nitrite ($NaNO_2$). Following a 6-minute duration, 300 µL of a 10% AlCl3 solution was added, and the volume was then adjusted with distilled water to 2.5 mL. After seven more minutes, 1.5 mL of 1 M NaOH was added, and the mixture was centrifuged for ten minutes at 5000 g. At 510 nm, the supernatant's absorbance was calculated in relation to the solvent blank. Using a calibration curve made using catechin, the total flavonoid content was calculated and represented as milligrams of catechin equivalent.

2.7. Radical DPPH scavenging activity

The extracts' ability to scavenge free radicals was evaluated through the application of the stable DPPH* (2,2-diphenyl-1-picrylhydrazyl) technique, as described by Mehaya et al. [31]. 3.0 mL was the overall reaction volume, and 200 µM was the final concentration of DPPH*. Following a 60-minute dark incubation period, the absorbance at 517 nm was measured in comparison to a pure methanol blank. The DPPH free radical's % inhibition was computed using the formula:

$$\boldsymbol{Inhibition}\ (\%) = \frac{\boldsymbol{A_{control} - A_{sample}}}{\boldsymbol{A_{control}}} \times \mathbf{100}$$

Where: Acontrol is the absorbance of the control reaction (containing all reagents except the test compound), Asample is the absorbance with the test compound.

A standard curve was generated using Trolox, and the results were expressed as milligrams of Trolox equivalents (TE) per gram of the sample. If the measured DPPH value exceeded the linear range of the standard curve, additional dilution was performed.

2.8. Statistical analysis

The data obtained underwent statistical analysis utilizing the "F" Test, as described by Snedecor and Cochran [32]. Subsequently, the means were compared using a least significant difference (L.S.D.) test, following the methodology outlined by Gomez and Gomez [33]. The entire statistical analysis was conducted using the Statistix 8.1 program.

3. Results

Analysis of variance showed highly significant effects of Zn and Mn either alone or in combination on oregano growth characters; plant height, number of shoots and number of branches as compared to control (Table 3). All treatments showed significantly incrementin both seasons compared to control, except treatment with Mn at 100 ppm and Zn 100 ppm combined with Mn 50 ppm in the first season. The tallest plant was shown in the treatment with 50 ppm Zn in the first season, and with 50 ppm Zn combined with 100 ppm Mn followed by 50 ppm Zn in the second season. On the other hand, the greatest number of shoots per plant were shown under treatment with 50 ppm Zn combined with 50 ppm Mn followed by 100 ppm Zn in the first season, and under treatment with 100 ppm Zn followed by 50 ppm Zn combined with 100 ppm Mn in the second season. Meanwhile 50 ppm Zn combined with 50 ppm Mn led to the greatest number of branches in both seasons.

Table (3): Plant height, number of shoots and number of branches of oregano as affected by Zn and/or Mn.

Treatments	Plant height (cm)		Number of shoots		Number of branches	
	1st	2nd	1st	2nd	1st	2nd
Control	36.67^{de}	40.67^{e}	24.67^{d}	27.33^{f}	9.00^{c}	9.67^{d}
Zn 50 ppm	50.33^{a}	51.67^{ab}	31.33^{c}	35.33^{d}	10.33^{c}	11.67^{c}
Zn 100 ppm	41.00^{b}	42.33^{de}	36.33^{ab}	41.33^{a}	15.33^{ab}	16.33^{b}
Mn 50 ppm	35.00^{e}	45.33^{cd}	35.33^{ab}	38.33^{bc}	14.67^{b}	16.00^{b}
Mn 100 ppm	39.00^{bcd}	45.33^{cd}	30.67^{c}	35.67^{d}	14.00^{b}	15.33^{b}
Zn 50 ppm+ Mn 50 ppm	41.67^{b}	49.33^{bc}	37.33^{a}	37.33^{c}	17.33^{a}	18.33^{a}
Zn 50 ppm+ Mn 100 ppm	40.67^{bc}	53.67^{a}	34.67^{b}	39.67^{b}	15.67^{ab}	16.67^{ab}
Zn 100 ppm+ Mn 50 ppm	37.67^{cde}	43.33^{de}	30.67^{c}	34.67^{d}	13.67^{b}	15.67^{b}
Zn 100 ppm+ Mn 100 ppm	40.33^{bc}	43.67^{de}	29.33^{c}	32.33^{e}	13.67^{b}	15.33^{b}

Mean within the columns that are followed by different letter (s) are significantly different at p= 0.05.

In addition, Zn and Mn alone or in combination led to significant increases of herb fresh and dry weight as well as essential oil percentage (Table 4). In term of herb yield, the highest herb fresh and dry weights were shown under treatment with 100 ppm Mn in the first season, and under treatment with 50 ppm Zn combined with 100 ppm Mn followed by 50 ppm Mn in the second season. In term of essential oil content (%), the differences were not significant in the first season, and was highly significant in the second season. The highest essential oil percentage was shown under treatment with Zn at 50 or 100 ppm in the first season, and under treatment with 100 ppm Zn combined with 50 ppm Mn in the second season.

Table (4): Herb fresh and dry weight of oregano as well as essential oil content (%) as affected by Zn and/or Mn.

Treatments	Fresh weight/plant (g)		Dry weight/plant (g)		Essential oil (%)	
	1st	2nd	1st	2nd	1st	2nd
Control	26.33^{c}	67.16^{d}	7.13^{c}	17.27^{d}	0.057^{c}	0.073^{b}
Zn 50 ppm	39.93^{ab}	114.67^{ab}	9.90^{b}	33.20^{a}	0.087^{a}	0.103^{b}
Zn 100 ppm	38.27^{b}	96.27^{bc}	10.40^{ab}	22.91^{bc}	0.080^{ab}	0.097^{b}
Mn 50 ppm	34.67^{b}	118.27^{a}	9.77^{b}	33.77^{a}	0.067^{bc}	0.117^{b}
Mn 100 ppm	47.00^{a}	82.07^{cd}	12.47^{a}	22.80^{bc}	0.073^{abc}	0.083^{b}
Zn 50 ppm+ Mn 50 ppm	35.00^{b}	72.80^{d}	10.27^{b}	20.93^{c}	0.067^{bc}	0.040^{b}
Zn 50 ppm+ Mn 100 ppm	40.07^{ab}	119.37^{a}	11.07^{ab}	35.00^{a}	0.070^{abc}	0.107^{b}
Zn 100 ppm+ Mn 50 ppm	37.67^{b}	80.80^{cd}	11.40^{ab}	25.00^{b}	0.077^{ab}	0.130^{a}
Zn 100 ppm+ Mn 100 ppm	40.33^{ab}	75.60^{cd}	11.73^{ab}	23.23^{bc}	0.073^{abc}	0.113^{ab}

Mean within the columns that are followed by different letter (s) are significantly different at p= 0.05.

In contrast to growth and yield characters, photosynthetic pigments; chlorophyll “a”, “b” and carotenoids showed significant decreases when plants treated with Zn and Mn alone or in combination compared to control (Table 5). The highest values of chlorophyll “a”, “b” and carotenoids were shown under control conditions in both seasons. The lowest chlorophyll “a” was shown under treatment with 50 ppm Zn in both seasons. The lowest chlorophyll “b” was shown under treatment with 100 ppm Zn combined with 50 ppm Mn in the first season, and under treatment with 50 ppm Zn combined with 100 ppm Mn in the second season. Meanwhile the lowest carotenoids content was shown under treatment with 100 ppm Zn combined with 50 ppm Mn in both seasons.

Meanwhile application of Zn and Mg alone or in combination led to highly significant increases of total phenolic, total flavonoids and antioxidant activity(Table 6). In term of total phenolic and total flavonoids, the highest values were shown under the treatment of 100 ppm Zn combined with either 50 ppm Mn or 100 ppm Mn, respectively. In term of antioxidant activity, the highest values were shown under treatment with 100 ppm Zn combined with 100 ppm Mn.

Table (5): Chlorophyll "a", "b" and carotenoids contents of oregano leaves as affected by Zn and/or Mn.

Treatments	Chlorophyll "a"		Chlorophyll "b"		Carotenoids	
	1st	2nd	1st	2nd	1st	2nd
Control	1.721[a]	1.780[a]	0.548[a]	0.613[a]	0.173[a]	0.177[a]
Zn 50 ppm	1.160[f]	1.185[d]	0.368[c]	0.373[f]	0.134[de]	0.135[d]
Zn 100 ppm	1.523[b]	1.589[ab]	0.448[b]	0.470[c]	0.164[b]	0.165[b]
Mn 50 ppm	1.192[ef]	1.455[bc]	0.376[c]	0.382[ef]	0.131[e]	0.132[e]
Mn 100 ppm	1.374[c]	1.481[bc]	0.437[b]	0.515[b]	0.139[c]	0.141[c]
Zn 50 ppm+ Mn 50 ppm	1.257[de]	1.323[cd]	0.373[c]	0.427[d]	0.126[f]	0.127[f]
Zn 50 ppm+ Mn 100 ppm	1.370[c]	1.323[cd]	0.440[b]	0.372[f]	0.136[cd]	0.128[f]
Zn 100 ppm+ Mn 50 ppm	1.178[ef]	1.226[cd]	0.335[d]	0.418[de]	0.123[f]	0.123[g]
Zn 100 ppm+ Mn 100 ppm	1.304[cd]	1.390[bcd]	0.425[b]	0.534[b]	0.131[e]	0.133[e]

Mean within the columns that are followed by different letter (s) are significantly different at p= 0.05.

Table (6): Total phenolic, total flavonoids and antioxidant activity of oregano as affected by Zn and/or Mn.

Treatments	Total phenolic (mg GAE/g)	Total flavonoids (mg CE/g)	DPPH (mg TE/g)
Control	2.635[f]	0.200[f]	0.220[c]
Zn 50 ppm	2.927[e]	0.250[ef]	0.267[bc]
Zn 100 ppm	3.281[d]	0.300[e]	0.201[c]
Mn 50 ppm	3.979[c]	0.450[d]	0.258[bc]
Mn 100 ppm	3.854[c]	0.500[d]	0.286[bc]
Zn 50 ppm+ Mn 50 ppm	4.042[c]	0.600[c]	0.351[bc]
Zn 50 ppm+ Mn 100 ppm	4.104[c]	0.650[c]	0.342[bc]
Zn 100 ppm+ Mn 50 ppm	5.010[a]	1.150[a]	0.417[b]
Zn 100 ppm+ Mn 100 ppm	4.521[b]	0.800[b]	1.174[a]

Mean within the columns that are followed by different letter (s) are significantly different at p= 0.05.

In the current study, oregano plants exhibited significant variations in their essential oil composition under different treatments. Gas Chromatography Mass Spectrometry (GC-MS) analysis identified approximately 22 compounds (Table 7), with carvacrol and thymol emerging as the major constituents in the essential oil. The highest Carvacrol level was pointed out in plants fertilized with Zn at 50 ppm (50%), followed by treatment with Mn at 50 ppm (49%). The highest thymol concentration was observed in plants fertilized with 50 ppm Zn combined with 100 ppm Mn (31%), and the lowest was observed under control (16.05%).

Carvacrol showed clearly higher concentration compared to thymol under all treatments (Table 7). The effect of low concentration of Zn or Mn alone was most clearly compared to the combination in term of carvacrol. On the other hand, Mn showed clear effect on thymol compared to Zn, followed by Mn combined with low concentration of Zn. The highest total percentageof (carvacrol + thymol) were shown under treatment with Mn alone at 50 ppm (77.8%), followed by Mn at 100 ppm (70.7%) and Zn at 50 ppm (69.9). these results suggest the important role of Zn and Mn not only on the essential oil content, but also on its chemical composition and the balance between carvacrol and thymol.

4. Discussion

Manganese (Mn) and zinc (Zn) are crucial micronutrients that plants require for their growth [34, 35]. Zinc plays a significant role in promoting the generation of plant biomass [36, 37]. It serves as an essential component of enzymes and functions as a vital regulator and cofactor for various enzyme activities [37, 38]. Additionally, Mn has the ability to stimulate plant growth and enhance biomass production [39]. Mn plays a pivotal role as a micronutrient essential for supporting plant growth and development. It serves diverse metabolic functions within various compartments of plant cells. One of its indispensable functions is acting as a cofactor for the oxygen-evolving complex within the photosynthetic machinery. This complex catalyzes

the water-splitting reaction in photosystem II, a crucial process in the photosynthetic pathway[24].

Table (7): The main constituents of the essential oil of oregano plant as affected with Zn and/ or Mn that analyzed with GC-MS.

	Component	RI	C	T1	T2	T3	T4	T5	T6	T7	T8
1	àThujene	872	0.40	0.57	0.49	0.61	0.23	0.47	0.29	0.28	0.21
2	àPINENE	903	0.42	0.64	0.30	0.44	1.94	0.60	1.04	0.39	0.22
3	Sabinene	926	1.19	1.57	1.39	1.21	1.28	1.60	1.23	1.88	1.62
4	1-Octen-3-ol	931	0.37	0.75	0.58	0.50	1.03	0.81	0.82	1.69	1.60
5	á Myrcene	952	1.00	1.59	1.12	1.85	1.80	1.96	0.90	0.70	0.30
6	β-(Z)-Ocimene	957	0.83	0.69	0.65	0.30	0.45	0.24	0.74	0.71	0.64
7	à Phellandrene	860	0.51	0.38	0.77	0.27	0.38	0.65	0.80	0.32	0.22
8	à Terpinene	989	3.59	4.42	6.08	7.89	2.77	1.56	2.33	3.33	1.60
9	β-Ocimene	996	14.25	10.80	9.82	12.30	9.40	10.99	9.33	11.88	13.48
10	Delta 3Caren2ol	1006	0.24	0.34	0.19	0.24	0.30	0.27	0.70	0.38	0.30
11	ç Terpinene	1030	10.13	10.71	15.27	10.30	5.30	6.89	1.55	3.85	4.65
12	à Terpinolene	1037	0.16	0.48	0.34	0.95	0.89	---	0.58	0.64	0.51
13	Allo-Ocimene	1043	0.76	0.08	0.10	0.33	0.69	0.40	0.53	0.33	0.41
14	Terpinen-4-ol	1049	0.85	0.62	0.49	0.30	0.48	0.61	1.53	0.85	0.74
15	Linalool	1073	0.58	0.81	0.63	0.67	0.72	0.52	0.71	0.73	0.65
16	Thymol methyl ether	1196	1.11	1.28	3.81	2.27	1.57	1.78	1.85	2.27	1.95
17	Carvacrol	1201	33.0	50.0	29.5	49.0	42.0	39.0	35.0	35.9	36.0
18	Carvacrol methyl ether	1210	2.33	2.75	1.11	2.05	1.37	1.71	2.62	2.31	2.11
19	Thymol	1251	16.05	19.9	21.0	28.8	28.7	28.8	31.0	24.5	23.4
20	à Bourbonene	1299	0.33	0.47	0.28	0.63	0.87	0.60	0.81	0.68	0.74
21	Aromadendrene	1320	0.70	0.53	0.98	0.55	0.53	0.19	0.21	0.54	0.45
22	Trans Caryophyllene	1383	4.42	6.31	4.27	4.17	4.32	3.39	4.32	2.82	2.56

C: control; T1: 50 ppm Zn; T2: 100 ppm Zn; T3: 50 ppm Mn; T4: 100 ppm Mn; T5: 50 ppm Zn+ 50 ppm Mn; T6: 50 ppm Zn+ 100 ppm Mn; T7: 100 ppm Zn+ 50 ppm Mn; T8: 100 ppm Zn+ 100 ppm Mn.

Zinc is essential for various critical processes in plants, including chlorophyll production, pollen function, and fertilization [36, 40]. On the other hand, Mn is a fundamental component of the Mn cluster structure within the oxygen-evolving complex in photosystem II, a key player in water splitting and electron provision for photosynthesis [41]. This importance of Mn is why it is crucial for plant growth and development [42]. However, it's important to note that excessive Mn can be toxic to plants, leading to damage to chloroplasts, as it disrupts the thylakoid structure and electron transport chain in photosynthesis. In such cases, chloroplasts appear to be the primary target of manganese toxicity [42]. In the overall metabolism of plants, Photosynthesis is a pivotal process in plant biology, encompassing four key stages: light perception, electron transfer, energy fixation, biosynthesis, and photo-assimilation transfer. It serves as the primary determinant of plant growth and yield [43]. Proper nutrition plays a crucial role in enhancing the rate of photosynthesis [44]. To meet a plant's nutritional needs, one effective strategy is the provision of Mn fertilizer. The application of Mn can optimize the photorespiration process in plants, leading to efficient photosynthesis and streamlining the overall photosynthetic mechanism [45].

In this study, Mn and Zn addition alone or in combination led to significant increases in growth parameters including plant height, number of branches, number of shoots as well as herb and essential oil yield. The results obtained are consistent with previously reported data, which have demonstrated a significant increase in essential oil yield in plants such as *Mentha piperita*, *Allium cepa* L., *Moringa peregrine*, Japanese mint, and roselle through the application of Zn fertilizer [46-48,23]. A similar positive effect of Zn supply on both vegetative growth parameters and essential oil yield

has also been observed in *Matricaria chamomilla* L., *Salvia farinacea*, and *Coriandrum sativum* [49,50]. Additionally, it's worth noting that a deficiency of Mn can lead to growth inhibition, chlorosis, necrosis, early leaf shedding, and reduced nutrient reutilization [51]. Several research studies have shown a positive impact of micronutrient application, including both Zn and Mn, on crop yield and various quantitative parameters, as noted by Mousavi et al. [52. In their study, Nazarovna et al. [53] found that the application of manganese fertilizer at low and medium levels resulted in a 9.6% increase in plant height compared to plants without manganese fertilizer. However, when manganese was applied at a high level, the plant height decreased by 7.4% compared to plants without manganese fertilizer.

In contrast, photosynthetic pigments represented by chlorophyll “a” and “b” as well as carotenoids content decreased with Mn and Zn addition alone or in combination. In previous study conducted by Arya and Roy [54], it was found that the total chlorophyll content increased by 22% when Mn fertilizer was provided. This increase in chlorophyll content is crucial as chlorophyll is responsible for converting sunlight into energy through photosynthesis. Nutritional deficiencies can result in chlorophyll insufficiency, leading to a reduction in quantum photosystem (II) efficiency. Manganese plays a significant role in maintaining the normal structure of chloroplast membranes, as well as in the photosynthetic process. Research conducted by Chun-xia et al. [55] demonstrated that the administration of Mn and gibberellin concentrations in wheat leaves led to an increase in total chlorophyll and chlorophyll a, ultimately improving photosynthesis parameters. This sustainable enhancement of photosynthesis can contribute to increased crop yields. Additionally, a study by Hisamitsu et al. [56] revealed that zinc deficiency can disrupt chlorophyll synthesis. Zinc serves as a structural and catalytic component of proteins and enzymes, and it acts as a cofactor for the normal development of pigment biosynthesis. Therefore, zinc is crucial for chlorophyll formation and, by extension, effective photosynthesis in plants.

Zn and Mn play essential roles in the synthesis of proteins, activation of enzymes, oxidation, revival reactions, and carbohydrate metabolism. According to Kelling and Speth [57], the combined utilization of elements such as Zn and Mn sourced from sulfate has been shown to enhance the efficiency and quality of potato crops. Mohamadi [58] similarly observed that the foliar application of Zn along with Mn led to an increase in the efficiency and quality of potato crops. These findings highlight the positive impact of coordinated Zn and Mn application on the overall performance of potato crops. In this study, Zn and Mn played an important role in increasing total phenolic, total flavonoid and DPPH activity, especially with combination of 100 ppm Zn with 50/100 ppm Mn.

Earlier research has indicated that more than 50% of oregano oil is composed of phenolic compounds, with carvacrol and thymol being the primary constituents. Additionally, oregano oil contains sesquiterpenes, terpinenes, terpineol alcohol, flavonoids, and various other compounds, as reported by Arcila-Lozano et al. [59] and Ozkan et al. [60]. In this study, about 22 compounds were identified by GC-Mass analysis, mainly carvacrol and thymol. The chemical composition of essential oil differed greatly by addition of Zn and Mn alone or in combination.

5. Conclusion

It is concluded that growth parameters (plant height, number of branches and number of shoots) and yield parameters (fresh and dry herb and essential oil) as well as biochemical components (total phenolic, total flavonoid, and antioxidant activity) increased with addition of Zn and Mn alone or combination, with the best findings of growth and yield parameters obtained with combination of Zn and Mn. In contrast, the highest photosynthetic pigments had been shown under control conditions, and decreased with Zn and Mn addition alone or in combination. The chemical composition of essential oil differed greatly by addition of Zn and Mn alone or in combination. These results suggested the important role of Zn and Mn not only on the essential oil content, but also on its chemical composition and the balance between carvacrol and thymol.

6. Conflict of interest

The authors declare that there is no conflict of interest.

7. Data availability

The data supporting this study's findings are available at the request of the corresponding author.

8. Authorship Contribution Statement

Conceptualization, H.M.A., and W.S.S.; methodology, H.M.A. and J.L.; software, W.S.S.; validation, H.M.A., and W.S.S.; formal analysis, W.S.S.; investigation, H.M.A., S.F.H., and W.S.S.; resources, S.F.H.; data curation, H.M.A., J.L. and W.S.S.; writing—original draft preparation, H.M.A., and W.S.S.; writing—review and editing, H.M.A., W.S.S. and J.L.; visualization, H.M.A, and S.F.H.; supervision, H.M.A, and S.F.H.; project administration, H.M.A.; funding acquisition, H.M.A. All authors read and approved the final manuscript.

9. References

1. Sarrou, E.; Martinidou, E.; Palmieri, L.; Poulopoulou, I.; Trikka, F.; Masuero, D.; Matthias, G.; Ganopoulos, I.; Chatzopoulou, P.; Martens, S. High throughput pre-breeding evaluation of Greek oregano (Origanum vulgare L. subsp. hirtum) reveals multi-purpose genotypes for different industrial uses. J. Appl. Res. Med. Aromat. Plants. 2023, 37, 100516. https://doi.org/10.1016/j.jarmap.2023.100516.

2. Vokou, D.; Kokkini, S.; Bessiere, J-M.; Geographic variation of Greek oregano (Origanum vulgare ssp. hirtum) essential oils. Biochem. Syst. Ecol. 1993, 21 (2), 287-295. .https://doi.org/10.1016/0305-1978(93)90047-U.

3. Kokkini, S.; Karousou, R.; Vokou, D.; Pattern of geographic variations of Origanum vulgare trichomes and essential oil content in Greece. Biochem. Syst. Ecol. 1994, 22 (5), 517-528. https://doi.org/10.1016/0305-1978(94)90046-9.

4. Gutiérrez-Grijalva, E.P.; Picos-Salas, M.A.; Leyva-López, N.; Criollo-Mendoza, M.S.; Vazquez-Olivo, G.; Heredia, J.B.;. Flavonoids and phenolic acids from oregano: occurrence, biological activity and health benefits. Plants, 2018, 7, 2. https://doi.org/10.3390/plants7010002.

5. Kokkini, S., Karousou, R., Hanlidou, E. Herbs of the Labiatae. In Encyclopedia of Food Sciences and Nutrition, 2nd ed.; Academic Press: Cambridge, MA, USA, pp. 3082–3090. (2003).

6. Kintzios, S. E. Oregano. In Handbook of Herbs and Spices; Woodhead Publishing: Cambridge, UK, 2, 215–229. (2004).

7. Napoli, E., Siracusa, L., Ruberto, G. New tricks for old guys: Recent development in the chemistry, biochemistry, applications and exploitation of selected species from the Lamiaceae family. Chem. Biodivers. 17, e1900677. (2020).

8. Gavaric, N.; Mozina, S.S.; Kladar, N.; Bozin, B. Chemical profile, antioxidant and antibacterial activity of thyme and oregano essential oils, thymol and carvacrol and their possible synergism. J. Essent. Oil-Bear. Plants, 2015, 18(4), 1013-1021, .http://doi.org/10.1080/0972060X.2014.971069

9. Cui, H., Zhang, C., Li, C., Lin, L., Antibacterial mechanism of oregano essential oil. Ind. Crops Prod. 2019, 139, 111498. https://doi.org/10.1016/j.indcrop.2019.111498.

10. Bedoya-Serna, C. M.; Dacanal, G. C.; Fernandes, A. M.; Pinho, S. C. Antifungal activity of nanoemulsions encapsulating oregano (Origanum vulgare) essential oil: in vitro study and application in Minas Padrão cheese. Braz. J. Microbiol. 2018, 49 (4), 929-935. https://doi.org/10.1016/j.bjm.2018.05.004.

11. Kapustová, M.; Granata, G.; Napoli, E.; Puškárová, A.; Bučková, M.; Pangallo, D.; Geraci, C. Nanoencapsulated essential oils with enhanced antifungal activity for potential application on agri-food, material and environmental fields. Antibiotics. 2021, 10(1), 31. http://doi.org/10.3390/antibiotics10010031

12. Plata-Rueda, A.; Zanuncio, J.C.; Serrão, J.E.; Martínez, L.C. Origanum vulgare essential oil against Tenebrio molitor (Coleoptera: Tenebrionidae): composition, insecticidal activity, and behavioral response. Plants. 2021, 10, 2513. https://doi.org/10.3390/plants10112513

13. Wu, M.; Zhou, Z.; Yang, J.; Zhang, M.; Cai, F.; Lu, P. ZnO nanoparticles stabilized oregano essential oil Pickering emulsion for functional cellulose nanofibrils packaging films with antimicrobial and antioxidant activity. Int. J. Biol. Macromol. 2021, 190, 433-440. https://doi.org/10.1016/j.ijbiomac.2021.08.210.

14. Bedini, S.; Farina, P.; Napoli, E.; Flamini, G.; Ascrizzi, R., Verzera, A.; Conti, B.; Zappalà, L., Bioactivity of different chemotypes of oregano essential oil against the blowfly Calliphora vomitoria vector of foodborne pathogens. Insects. 2021, 12(1), 52. https://doi.org/10.3390/insects12010052

15. Torabian, S.; Zahedi, M.; Khoshgoftar, A.H. Effects of foliar spray of two kinds of zinc oxide on the growth and ion concentration of sunflower cultivars under salt stress. J. Plant Nutr. 2016, 39(2), 172–180. https://doi.org/10.1080/01904167.2015.1009107

16. Hussain, A.; Ali, S.; Rizwan, M.; Rehman, M. Z. U.; Javed, M. R.; Imran, M.; Chatha, S. A. S.; Nazir, R. Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. Environ. Pollut. 2018, 242, 1518–1526. https://doi.org/10.1016/j.envpol.2018.08.036

17. Shahhoseini, R.; Azizi, M.; Asili, J.; Moshtaghi, N.; Samiei, L.; Effects of zinc oxide nanoelicitors on yield, secondary metabolites, zinc and iron absorption of Feverfew (Tanacetum parthenium (L.) Schultz Bip.). Acta Physiol. Plant. 2020, 42, 1–18. https://doi.org/10.1007/s11738-020-03043-x

18. Velasco, E.A.P.; Galindo, R.B.; Valdez-Aguilar, L.A.; González-Fuentes, J.A.; Urbina, B.A.P.; Lozano-Morales, S.A.; Sanchez-Valdes, S. Effects of the morphology, surface modification and application methods of ZnO-NPs on the growth and biomass of tomato plants. Mol. 2020, 25, 1282. https://doi.org/10.3390/molecules25061282

19. Pandey, A.C.S.; Sanjay, S.S.; Yadav, R. Application of ZnO nanoparticles in influencing the growth rate of Cicer arietinum J. Exp. Nanosci. 2010, 5, 488–497. https://doi.org/10.1080/17458081003649648

20. Rehman, H.U.; Aziz, T.; Farooq, M.; Wakeel, A. Zinc nutrition in rice production systems: A review. Plant and Soil. 2012, 361, 203–226. https://doi.org/10.1007/s11104-012-1346-9

21. Sadak, M.S.; Bakry, B.A. Zinc-oxide and nano-ZnO oxide effects on growth, some biochemical aspects, yield quantity, and quality of flax (Linum uitatissimum L.) in absence and presence of compost under sandy soil. Bull. Natl. Res. Cent. 2020, 44, 98. https://doi.org/10.1186/s42269-020-00348-2

22. Hassanein, Y.Z.; Abdel-Rahman, S.S.A.; Soliman, W.S.; Salaheldin, S. Growth, yield, and quality of roselle (Hibiscus sabdariffa L.) plants as affected by nano zinc and bio-stimulant treatments. Hortic. Environ. Biotechnol .62, 202, 1879–890. https://doi.org/10.1007/s13580-021-00371-w

23. Andresen, E.; Peiter, E.; Küpper, H. Trace metal metabolism in plants. J. Exp. Bot., 2018, 69(5), 909–954. http://doi.org/10.1093/jxb/erx465

24. Alejandro, S.; Höller, S.; Meier, B.; Peiter, E. Manganese in plants: from acquisition to subcellular allocation. Frontiers in plant science. 2020, 11, 300.http://doi.org/10.3389/fpls.2020.00300

25. Alloway, B.J. Micronutrients and Crop Production: An Introduction. In: Alloway, B.J. (Ed) Micronutrient Deficiencies in Global Crop Production. Springer, pp 1-40. 2008.

26. Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., Function of nutrients: micronutrients, in Marschner's Mineral Nutrition of Higher Plants, 3rd Edn, ed. P. Marschner (Oxford: Elsevier), 191–249. (2012).

27. AOAC. Official Methods of Analysis of AOAC International, vol. 14, William Horwitz. AOAC International, Washington, D.C (2012).

28. Rafea, H. S.; Ali, S. H.; El-Shiekh, T. A., Youssef, D. A., Amer, H. M.; El-Aasar, A. E. M. Toxicological effect of camphor oil nanoemulsion on cotton leaf worm and its safety evaluation on swiss albino mice. Egypt. J. Chem, 2022, 65(131), 1301-1312.

29. Costache A.M.; Campeanu G.; Neata G. Studies concerning the extraction of chlorophyll and total carotenoids from vegetables. Rom. Biotechnol. Lett. 2012, 17, 7702-7708.

30. Yousif, E. S.; Yaseen, A.; Abdel-Fatah, A. F.; Shouk, A. H., Gdallah, M.; Mohammad, A. Antioxidant and anticancer properties of nano and fermented-nano powders of wheat and rice by-products. Discover Food. 2022, 2(1), 33.

31. Mehaya, F. M.; Mohammad, A. A. Thermostability of bioactive compounds during roasting process of coffee beans. Heliyon, 2020, 6(11).

32. Snedecor, G.W.; Cochran, W.G. Statistical Methods. 8th ed., Iowa State University Press, Ames. Iowa, USA. 1989.

33. Gomez, K.A.; Gomez, A.A. Statistical Procedures for Agricultural Research. 2nd ed. John Wily, NY, pp. 680. (1984).

34. Das, S.K. Role of micronutrient in rice cultivation and management strategy in organic agriculture—a reappraisal. Agric. Sci. 2014, 05(09), 765–769. https://doi.org/10.4236/as.2014.59080

35. Shebl, A.; Hassan, A.A.; Salama, D.M.; Abd El-Aziz, M.E.; Abd Elwahed, M.S.A. Template-free microwave-assisted hydrothermal synthesis of manganese zinc ferrite as a nanofertilizer for squash plant (Cucurbita pepo L). Heliyon, 6(3), 2020, 1–13. https://doi.org/10.1016/j.heliyon.2020.e03596

36. Kaya, C.; Higgs, D. Response of tomato (Lycopersicon esculentum L.) cultivars to foliar application of zinc when grown in sand culture at low zinc. Hortic. Sci. 2002; 93, 53-64.

37. Cakmak, I., Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil, 2008, 302, 1-17.

38. Wissuwa, M.; Ismail, A.M.; Graham, R.D. Rice grain zinc concentrations as affected by genotype, native soil-zinc availability, and zinc fertilization. Plant Soil. 2008, 306, 37-48.

39. Ciurli, A., Di Baccio, D., Scartazza, A., Grifoni, M., Pezzarossa, B., Chiellini, C., Mariotti,

L., Pardossi, A. Influence of zinc and manganese enrichments on growth, biosorption and photosynthetic efficiency of Chlorella sp. Environmental Science and Pollution Research, 2021, 28(7), 8539–8555. https://doi.org/10.1007/s11356-020-11033-2

40. Pandey, N.,;Pathak, G.C.; Sharma, C.P. Zinc is critically required for pollen function and fertilization in lentil. J. Trace. Elem. Med. Biol. 2006, 20, 89-96.

41. Nickelsen, J.; Rengstl, B. Photosystem II Assembly: From Cyanobacteria to Plants. Annu. Rev. Plant Biol. 2013, 64, 609–635.

42. Chen, C.; Xia, S.; Deng, R.; Liu, C.; Shi, G. AhIRT1 and AhNRAMP1 metal transporter expression correlates with Cd uptake in peanuts under iron deficiency. PLoS One. 2017, 12(10), 1–15. https://doi.org/10.1371/journal.pone.0185144

43. Blankenship, R. E. Molecular Mechanisms of Photosynthesis (2nd ed.). John Wiley & Sons, (2014).

44. Guo, J.; Jia, Y.; Chen, H.; Zhang, L.; Yang, J.; Zhang, J.; Hu, X.; Ye, X.; Li, Y.; Zhou, Y. Growth, photosynthesis, and nutrient uptake in wheat are affected by differences in nitrogen levels and forms and potassium supply. Sci. Rep. 2019, 9(1), 1–12. https://doi.org/10.1038/s41598-018-37838-3

45. Bloom, A. J.; Lancaster, K. M. Manganese binding to Rubisco could drive a photorespiratory pathway that increases the energy efficiency of photosynthesis. Nat. Plants. 2018, 4(7), 414–422. https://doi.org/10.1038/s41477-018-0191-0

46. Akhtar, N.; Sarker, M.A.M.; Akhter, H.; Nada, M.K. Effect of planting time and micronutrient as zinc chloride on the growth, yield and oil content of Mentha piperita. Bangladesh Journal of Scientific and Industrial Research, 2009, 44(1), 125-130.

47. El-Tohamy, W.; Khalid, A.K.; El-Abagy, H.; Abou-Hussein, S. Essential oil, growth and yield of onion (Allium cepa L.) in response to foliar application of some micronutrients. Aust. J. Basic Appl. Sci. 2009, 3, 201-205.

48. Soliman, A.S.; El-feky, S.A.; Darwish, E. Alleviation of salt stress on Moringa peregrina using foliar application of nanofertilizers. J. Hortic. For. 2015, 7, 36-47.

49. Grejtovský, A.; Markušová, K.; Eliašová, A.; Šafárik, P. The response of chamomile (Matricaria chamomilla L.) plants to soil zinc supply. Plant Soil Environ. 2006, 52(1), 1-7.

50. Said-Al Ahl, H.; Omer, E. Effect of spraying with zinc and/or iron on growth and chemical composition of coriander (Coriandrum sativum L.) harvested at three stages of development. J. Med. Food Plants. 2009, 1, 30-46.

51. Kabata-Pendias, A.; Pendias, H., Biogeochemistry of trace elements. 2nd edn, Wyd Nauk PWN, WarSaw, Poland. (1999).

52. Mousavi, S.R.; Galavi, M.; Ahmadvand, G. Effect of zinc and manganese foliar application on yield, quality and enrichment on potato (Solanum tuberosum L). Asian J. Plant Sci. 2007, 6, 1256-1260.

53. Nazarovna, A.K.; Bakhromovich, N.F.; Alavkhonovich, K.A.; Ugli, K.S.S. Effects of Sulfur and Manganese Micronutrients on the Yield of Soybean Varieties. Agric. Sci. 2020, 11(11), 1048–1059. https://doi.org/10.4236/as.2020.1111068

54. Arya, S.K., Roy, B.K. Manganese induced changes in growth, chlorophyll content and antioxidants activity in seedlings of broad bean (Vicia faba L.). . Environ. Biol., 32(6), 707–711. (2011).

55. Chun-xia, L.; Xiang-ping, M.; Hai-yan, G.; Rui-xia, D.; Bao-ping, Y.; Qing-fang, H.; Zhikuan, J. Effects of manganese soaking and gibberellins spraying on wheat photosynthetic characteristics and yield. Nat. Environ. Pollut. Technol. 2018, 17(3), 791–798.

56. Hisamitsu, T.O.; Ryuichi, O.; Hidenobu, Y. Effect of zinc concentration in the solution culture on the growth and content of chlorophyll, zinc and nitrogen in corn plants (Zea mays L). J. Trop. Agric. 2001, 36(1), 58–66.

57. Kelling, K. A.; Speth, P. E. Effect of micronutrient on potato tuber yield and quality at Spooner. Department of Soil Science, University of Wisconsin, Madis. (2001).

58. Mohamadi, E. Study effects of nutrient elements utilization methods (Zn, Mn and Mg) on increase performance quantitative and quality of two potato species. Final Report, Research Institute Reformand Providing Sapling and Seed, Jehad and Agriculture Ministry. (2000).

59. Arcila-Lozano, C.C., Loarca-Pina, G., Lecona-Uribe, S., et al., Oregano: properties, composition and biological activity. Arch. Latinoam. Nutr. 2004, 54(1), 100–111.

60. Ozkan, G., Baydar, H., Erbas, S. The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (Origanum onites L.). J. Sci. Food Agric. 2010, 90(2), 205–209. http://dx.doi.org/10.1002/jsfa.3788