



Response of mint plant (*Mentha Piperita* L.) to nano spraying and its effect on vegetative growth characteristics and volatile oil

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Abstract

The study was conducted at the Agricultural Research Station, Faculty of Agriculture and Marshes, Thi Qar University, Iraq. The work describes the effects of spraying Nano-ZINIC and Boron on some plant characteristics, chemical composition and oil of mint plants (*Mentha piperita* L.) using three variants (0 control, 20 and 60) mg/l as Nano-ZINIC. ZINIC source and three variants (0 control, 20 and 60) mg/l as Boron source were tested Randomized Completely Block Design (R.C.B.D) were used with three replicates. The means were compared according to L.S.D test at the level 0.05... The results showed that the plants treated with Nano-Zanic fertilizer and Boron concentration of 60 mg/L had higher plant height (24.86, 29.11) cm, more leaves (73.57, 66.30), higher carbohydrate content (2.873 - 2.563) mg/g, and higher volatile oil content in leaves (0.5794 - 0.7083)%, respectively.

Keywords: Lamiaceae, Peppermint, chlorophyll, carbohydrates, volatile oil

1. Introduction

Mint (*Mentha Piperita* L.) belongs to the mint family (Lamiaceae). Mint is one of the most important aromatic perennial medicinal plants, widely distributed in tropical and subtropical regions of the world (Gupta et al., 2017). The mint family includes 200 genera and more than 4,000 species, many of which are medicinal plants used to treat diseases and as food (Leporatti and Ghedira, 2009). There are four species of mint:

M. piperita (peppermint); *M. arvensis* (Japanese mint); *M. spicata* (spearmint); *M. citrata* (bergamot mint) (Gholamipourfard et al., 2021). Many Lamiaceae are used for their bioactive oils to combat bacterial and fungal pathogens (Hajlaoui et al., 2009). Nanotechnology is a branch of science, engineering, and technology that operates at the nanoscale of 1 to 100 nanometers. Different methods (physical, chemical, and biological) are

used depending on the morphology, size, and chemical composition of the substance. (Kim and Kim 2019, Al Ghashem and Abood, 2023). In recent years, the application of nanotechnology has been widely used in agricultural development, finding preventive and therapeutic methods for plant diseases, and improving plant nutrient absorption by producing nanofertilizers. A large number of studies and research that have been conducted have confirmed the effectiveness of nanotechnology in increasing yield and improving quality (Hasan et al., 2022). Nano Zrtilizers have many unique properties and advantages due to their small size and have a large surface area that can increase nutrient absorption, thereby improving plant physiological processes, including programmed nutrient release. Boron is considered one of the most important macronutrients as it supports life processes by stimulating physiological processes during the flowering period, especially in terms of fruit setting, which leads to increased vitality, vegetativeness, and tube growth (Vasil, 1964) Zinc is considered one of the most important micronutrients in all living organisms because of its role in the construction of proteins, as well as its essential role in the operation of plant metabolism. It's also important in the creation of most enzymes, and it's

crucial in the detachment of the cell from the oxygen radical (ROS), then it's protected several components of the cell, including the plasma membrane, lipids, chlorophyll, and DNA. (Cakmak, 2000). .. Boron changes the diversity of regions during the reproductive stage of plants and also helps in transporting sugars to plants (Al-Emadi, 1991; Bidwell, 1979; Pilbeam and Kirkby, 1983).

The aim of this study was to investigate the effects of foliar spray solutions containing nano-zink and boron fertilizer and there interaction on vegetative growth characteristics and volatile oil for mint plants

2. Materials and Methods

A study was conducted at the Agricultural Research Station of the Faculty of Agriculture and Marshes, Thi Qar University, Iraq. This paper presents the effects of spraying Nano-ZINIC and boron on some nutritional characteristics, chemical composition and oils of mint plants (*Mentha Pipereta* L.) during 2022-2023. The soil was sandy loam and soil samples were randomly taken from the experimental field at a depth of 0-30 cm before planting. The samples were then mixed thoroughly, dried and then sieved using a sieve with a pore size of 2 mm. Subsequently, some physical and chemical properties of the field soil were evaluated in the laboratory of the Faculty of Agriculture and Marshes. (Table 1).

Table (1): Some of physical and chemical properties of soil.

Type of analysis	Unit measure	of Soil	Method
EC	ds.m ⁻¹	4.5	Page <i>et al.</i> (1982)
PH	----	7.33	

Total nitrogen	mg. kg ⁻¹	14.4	
Available of phosphorous	mg. kg ⁻¹	10.45	
Available of Potassium	meq . l ⁻¹	20.2	
Organic matter	g.kg ⁻¹	11.00	
Clay	%	42	Black (1965)
Silt	%	11	
Sand	%	47	
Soil texture	---	sandy clay	

Tillage operations included ploughing, leveling and grading. In addition, well-rotted organic animal manure (cow dung) was added at a rate of 36 m/ha and the land was divided into equal-sized blocks. The experiment consisted of 27 experimental units, each of which was 3 m in length and contained 15 pots. The distance between plants was 20 cm and the distance between experimental units

was 40 cm. The experiment was conducted according to a randomized block design. The means were compared according to L.S.D test at the level 0.05, with 3 replications in 9 variations, testing three concentrations (0 control, 20 and 60) mg/L as a source of nano ZINIC(Z), and three concentrations (0 control, 20 and 60) mg/l as a source of boron (B). (Table 2).

Table 2. Scheme of variants and components in study

Variants	Components
V1	Z0B0
V2	Z0B1
V3	Z0B2
V4	Z1B0
V5	Z1B1
V6	Z1B2
V7	Z2B0
V8	Z2B1
V9	Z2B2

Studied parameters

1. Plant height (cm): We only measured the height from the base of the plant to the top of the plant using a metric tape measure.
2. Estimation of the number of leaves and branches: Manual counting was

used to count the number of leaves and branches per plant.

3. Total chlorophyll (mg/100g fresh weight): The total chlorophyll pigment in the leaves was determined according to the method of Goodwin (1976). We took 1 gram of leaves, crushed them with 10 ml of acetone,

and then placed them in a centrifuge at 3000 rpm for 5 minutes. The spectral values at wavelengths of 663 and 665 nm were recorded using a spectrophotometer. The total chlorophyll was estimated using the following formula: Total chlorophyll (mg/L) = 20.2 x (665) D x 8.02 + (645) D.

4. Estimation of the content and percentage of volatile oils in leaves:

According to the British Pharmacopoeia (1968), volatile oils were extracted by water distillation using a Clevenger apparatus. The estimated amount of volatile oil = weight of the oil-containing tank - weight of the empty tank.

The percentage of oil in the treated leaves was estimated according to Guenther (1972).

The experimental results were statistically evaluated using the SPSS version 14 statistical analysis program. Analysis of variance (ANOVA) was performed based on the least significant difference (LSD) between means at a probability level of 0.05 (Al-Rawi and Khalaf, 20004).

5. The carbohydrate content of the leaves was determined using the

phenol-sulfuric acid method according to the method of Dobois et al. (1956).

3. Results and Discussion:

3.1. Plant height (cm):

(Table 3)The statistical analysis results showed that there was a significant difference in plant height after adding Nano-Zanic. Among the rest of the treatments in the experiment, the concentration of Nano-Zanic 60 mg/l gave the highest value of 29.11 cm³, while the concentration of Nano-Zanic 0 mg/l (control) gave the lowest value of 17.40 cm³. (Table 3)The statistical analysis results also showed that there was a significant difference in plant height after adding boron. The highest value of boron concentration 60 mg/L in the rest of the treatments in the experiment was 24.86 cm, while the lowest value of boron concentration 0 mg/L (control) was 17.69 cm. In addition, the interference effect between spraying Nano-Zanic solution and boron was significant when the Z2B2 value was higher and the plant height reached 30.44 cm, while the lowest value of Z2B2 was 13.10 cm. (Table. 3).

Table 3: Effect of spraying nano -Z and Boron and their interactions on plant height (cm) of the mint plant

Boron \ Nano -Zinc	B2 (60 mg L ⁻¹)	B1 20 mg L ⁻¹)	B0 0 mg L ⁻¹)	Mean of Nano -Zinc
Z0(0 mg L ⁻¹)	23.90	19.20	13.10	18.70

Z1(20 mg L ⁻¹)	22.73	20.60	17.33	20.22
Z2(60 mg L ⁻¹)	30.44	28.57	24.53	27.84
Mean of Boron	25.69	22.79	18.32	
L.S.D (0.05)	Z=0.815	B=0.815	Z*B=1.411	

3.2. Leaves number(leaf/plant):

(Table 4) The statistical analysis results showed that there was a significant difference in the number of leaves after adding Nano-Z. Among the rest of the treatments in the experiment, the 60 mg/l Nano-Z concentration gave the highest value of 66.30 leaves/plant, while the 0 mg/l Nano-Z concentration (control) gave the lowest value of 50, 07 leaves/plant results. (Table 4) The statistical analysis results also showed that there was a significant difference in the number of leaves after adding boron.

The rest of the treatments in the experiment had a boron concentration of 60 mg/l with a highest value of 73.57 leaves/plant, while the boron concentration of 0 mg/l (control) gave a lowest value of 53.00 leaves/plant. There was also no significant effect of the interaction between spraying Nano-Zan solution and boron. The results of the interaction showed that the leaf number was as high as 97.60 leaves/plant compared to the lowest leaf number of 51.20 leaves/plant in the interaction of Nano-Z and Boron (Table 4).

Table (4): Effect of spraying Nano-Z and Boron and their interaction on the number of leaves in mint plan

Boron Nano -Zinc	B2 (60 mg L⁻¹)	B1 20 mg L⁻¹)	B0 0 mg L⁻¹)	Mean of Nano -Zinc
Z0(0 mg L⁻¹)	74.10	65.92	51.20	50.0 7
Z1(20 mg L⁻¹)	84.13	80.40	82.33	76.63
Z2(60 mg L⁻¹)	97.60	92.49	85.28	66.30
Mean of Boron	53.00	80.43	73.57	
L.S.D (0.05)	Z=2.023	2.023	Z*B=3.504	

3. Chlorophyll content in leaves (mg/100g fresh weight):

(Table 6) The statistical analysis results showed that there was a significant difference in leaf

chlorophyll content after adding Nano-Zanic. The highest value was 11.85 mg/100 g fresh weight at a concentration of 60 mg/l Nano-Zanic, while the lowest value was 9.82 mg/100 g fresh weight at a

concentration of 0 mg/l Nano-Zanic (control) compared to other treatments in the experiment. (Table 6) The statistical analysis results also showed that the addition of boron had no significant effect on leaf

chlorophyll content. The interaction between the spraying of Nano-Zanic solution with boron at a concentration of 60 mg/l and boron also had a significant effect compared to the lowest chlorophyll content (Table 6).

Table (6): Effect of spraying with nano zanic and Boron and their interactions on the chlorophyll content of mint leaves (mg/100 g fresh weight)

Boron Nano -Zinc	B2 (60 mg L⁻¹)	B1 20 mg L⁻¹)	B0 0 mg L⁻¹)	Mean of Nano -Zinc
Z0(0 mg L⁻¹)	8.70	7.47	9.97	9.04
Z1(20 mg L⁻¹)	12.23	11.06	9.16	10.82
Z2(60 mg L⁻¹)	12.37	11.85	10.33	11.85
Mean of Boron				
L.S.D (0.05)	Z=N.S	NS	Z*B=NS	

3.4. Total soluble carbohydrates (mg/g dry matter):

(Table 7) The statistical analysis results showed that there was a significant difference in carbohydrate content when Nano-Zanic was added. The Nano-Z concentration of 60 mg/L gave the highest value of 2.873 mg/g dry matter, while the Nano-Z concentration of 0 mg/L (control) gave the lowest result of 0.824 mg/g dry matter compared to the rest of the treatments in the experiment. (Table 7) The statistical analysis results also showed that there was a significant difference in carbohydrate content

after the addition of boron. The boron concentration of 60 mg/L (2.563 mg/g dry matter) outperformed the other treatments in the experiment, while the boron concentration of 0 mg/L (control) was the lowest at 1.308 mg/g dry matter. The interaction between the spraying of the nano-zinc solution and boron also produced a significant effect. The results of the interaction showed that the carbohydrate content was as high as 3.137 mg/g dry matter compared to the lowest carbohydrate content of 0.5-33 mg/g dry matter.

Interference Z0B0

Table (7): Effects of spraying nano zinc and boron and their interaction on the total amount of soluble carbohydrates in mint leaves (mg/g dry matter)

Boron Nano -Zinc	B2 (60 mg L⁻¹)	B1 20 mg L⁻¹)	B0 0 mg L⁻¹)	Mean of Nano -Zinc
Z0(0 mg L⁻¹)	0.907	0.833	0.5 33	0.824
Z1(20 mg L⁻¹)	1.647	1.587	1.2 90	1.574
Z2(60 mg L⁻¹)	3.137	1.783	1.700	2.873
Mean of Boron	1.308	1.308	1.308	
L.S.D (0.05)	Z=0.0880	0.0880	Z*B=0.1524	

Table (8): Effect of spraying nano- ZINIC and Boron and their interactions on percentage of volatile oil of mint plant (%)

Boron Nano -Zinc	B2 (60 mg L⁻¹)	B1 20 mg L⁻¹)	B0 0 mg L⁻¹)	Mean of Nano -Zinc
Z0(0 mg L⁻¹)	0.4700	0.2470	0.1 167	0.267
Z1(20 mg L⁻¹)	0.6333	0.3800	0.4 617	0.4950
Z2(60 mg L⁻¹)	0.9 217	0.6733	0.5 433	0.5 794
Mean of Boron	0.3839	0.3839	0.3839	
L.S.D ₅)	Z=0.0373	0.0373	Z*B=0.0646	

(Table 8) The statistical analysis results showed that there was a significant difference in the volatile oil content in the leaves after the addition

of nano-Fe. The nano-Zn concentration of 60 mg/L (0.5794%) was better than the rest of the treatments in the experiment, while

the nano-Zn concentration of 0 mg/L (control) gave the lowest value of 0.267%. (Table 8) The statistical analysis results also showed that there was a significant difference in the percentage of volatile oil in the leaves after the addition of boron. The boron concentration of 60 mg/L gave a high value of 0.7083% compared to the rest of the treatments in the experiment, while the boron

The results of this study confirm the existence of a significant reaction between the spraying of ZINIC nanoparticles and boron solution alone or together. In Tables (3, 4 and 5), the concentration of 60 mg/L (Nano-ZINIC and boron) outperformed the other concentrations in terms of vegetative characteristics (plant height, number of leaves, number of branches), and in Tables (6, 7, 8 and 9) the content and percentage of total chlorophyll content, soluble carbohydrates and oil in leaves were higher. As for the superiority of plants treated with zinc, the increase is due to the action of zinc, which leads to the activation and formation of the internal growth regulator indoleacetic acid (IAA), preventing its oxidation, which has a positive effect on stimulating growth and elongation of plant cells (Mohamed and El-Younes; 1991). Zinc is not only a cofactor for many important enzymes in life processes, especially photosynthesis, conversion of sugars into starch and production of proteins, which has a positive effect on promoting vegetative growth. Zinc also affects the formation of chlorophyll pigments, which directly affects the production of nutrients required by plants (Taiz and Zeiger; 2002). This is consistent with the conclusions of Muhammad (2017) on

concentration of 0 mg/L (control) gave the lowest value of 0.3839%. The interference between the nano-Zan solution spraying and boron also had a significant effect. The interference results showed that the volatile oil content of zn2b2 was higher at 0.9,217%, while the interference zne0b0 had the lowest volatile oil content of 0.1,167%. (Table. 8).

coriander plants, who showed that zinc has a significant effect on the nutritional characteristics of plants. Zinc also helps to increase the efficiency of the photosynthesis process, thereby improving the characteristics of vegetative growth. Zinc also plays a role in the metabolism of carbohydrates, proteins and auxins. Zinc deficiency in plants reduces the production of these substances, which has a negative impact on secondary compounds, including volatile oils (Brown et al., 1993). Zinc also affects the primary metabolic processes that ultimately lead to the biosynthesis of volatile oils (Pirzadet al., 2013), a result that is consistent with the results of (Zahraet (2021)) on mint plants.

Boron also has many functions in plants. It is a potent reductant (Pignocchi and Foyer, 2003), an active factor for many enzymes, and an important antioxidant. It also regulates cell division and growth and plays an important role in the transmission of electronic signals (Smirnoff and Wheeler, 2000). Boron aids in many physiological processes within plants, such as cell division, photosynthesis, transpiration, and helps build proteins and lipids (Venkatesh and Park 2014; Podgórska et al. 2017;

Akram et al. 2017). The results of this study are consistent with those of Al-Rashedy et al. (2023) who conducted a study on legumes (*Vicia faba* L.) and found that spraying 250 mg/L of borate significantly outperformed the rest of the concentrations in increasing the ratio of magnesium, phosphorus, potassium, and chloride in seeds..

4. Conclusions

The results showed that the plants treated with nano-Zanic fertilizer and 60 mg/L boron concentration had a significant effect on plant height, number of leaves (leaves/plant), number of branches (branch/plant), leaf chlorophyll content (100 mg), total soluble carbohydrates (mg/g), and percentage of essential oil in leaves (amount of essential oil per plant)..

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