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Effect of spraying nano iron and ascorbic acid on the physical and chemical characteristics of Mint plant (Mentha Pipreta L.)

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Abstract
This study was conducted in the agricultural research station- College of Agriculture and Marshlands, University of Thi Qar, Iraq. The paper presents the effect of spraying nano-iron and Ascorbic acid on some of the vegetative characteristics, chemical components and oil of the mint plant (Mentha pipreta L.), with three variants (0 control, 30 and 60) mg/l as nano-Iron source were tested, and three variants (0 control, 50 and 60) mg/l as Ascorbic acid source were tested. Results shown that the plants treated at a concentration of 60 mg/l with nano -iron fertilizer and Ascorbic acid were superior in plant height (28.11, 25.96) cm; leaves number (73.59,69.24)leaf/plant; branches number(16.90, 14.28)branch/plant; chlorophyll content in leaves (11.85, 10.77)mg/100 mg; total soluble carbohydrates (1.873, 1.563)mg/g; percentage of volatile oil in the leaves(0.6794, 0.6083)%; amount of volatile oil per plant (0.286, 0.309)g. respectively.
Keywords: Lamiaceae, Peppermint, Japanese mint, Spearmint, Bergamot mint.

1. Introduction

Mint plant (Mentha pipereta L.) is belonging to the Lamiaceae family. Mint plant is one of the most important aromatic medicinal perennial plants, which is widespread in the tropical and subtropical regions of the world, (Gupta et al., 2017). There are 200 genera and more than 4,000 species belonging to the Lamiaceae family, and many of the genera are medicinal plants used in the treatment of diseases and food (Leporatti and Ghedira, 2009).There are four species of Mentha: M. piperita (Peppermint); M. arvensis (Japanese mint); M. spicata (Spearmint); M. citrata (Bergamot mint)(Gholamipourfard et al., 2021). Many Lamiaceous species are used against bacterial and fungal pathogens through the oils they contain showing biological activity (Hajlaoui et al., 2009). India comes at the forefront of the countries producing oil mint in the world. India, China and Brazil exports mint oil to many countries such as United States of America, Japan and Germany (FAO, 2020). The content of essential oils in mint leaves and tissues (menthol, menthone, menthofuran and pulegone) is affected by environmental conditions such as climatic factors: wind, temperature, humidity, and soil factors such as: nutrients, soil revival, salinity, and drought (De Sousa Barros et al., 2015). Nutrients play an essential role in improving plant growth and productivity (Machiani et al., 2019). The N and P macronutrients with the micronutrients (Fe, Mn, Zn) and vitamins have a positive role on the quality of peppermint oil (Amooaghaie and Golmohammadi, 2017).
Nanotechnology is a science, engineering, and technology that is conducted at the nanoscale, which ranges from 1 to 100 nanometers. By various methods (physical, chemical and biological) depending on the morphology, size and chemical composition of that substance. (Kim and Kim 2019, Al ghasheem and Abood, 2023). Recently, nanotechnology applications have been widely used in the development of agriculture by finding preventive and curative ways for diseases that affect plants, as well as improving the absorption of nutrients by the plant, through the production of nano-fertilizers, where many researches and studies were conducted that confirmed the effectiveness of nanotechnology in increasing production and improve its quality (Hasan et al., 2022). Nano-fertilizers have many unique characteristics and advantages due to their small size and have a large surface area that increases the absorption of nutrients in a higher way

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and thus enhances the physiological processes in the plant, including programming the release of nutrients. According to the plant's need, also nano-fertilizers contribute to protecting the environment by reducing the use of conventional fertilizers, and thus reducing soil toxicity and improving its agricultural properties (Abobatta, 2019). Foliar feeding with microelements plays an important role in plant growth especially in different physiological processes within the plant such as Carbon construction and building proteins and carbohydrates (Hasan et al., 2022). Previous studies have confirmed that the foliar spraying of nano-fertilizers on fruit plants works to increase the plant tissue content of nutrients and thus increase the vegetative and root growth of the plant (Hussein and Abdel Aal 2018).

This study aims to investigate the effect of foliar spraying solutions nano-iron and Ascorbic acid on improving vegetative growth, mineral content and oil quantity in mint plants.

2. Materials and Methods

A study was conducted in the agricultural research station- College of Agriculture and Marshlands, University of Thi Qar, Iraq. During 2022-2023, the paper presents the effect of spraying with nano-iron and Ascorbic acid on some of the vegetative characteristics, chemical components and oil of the mint plant (Mentha pipreta L.)

A soil with a sandy clay was used, where random soil samples were taken from several areas in the experimental field before planting at a depth of 0-30 cm, then the samples were mixed well, dried then sifted with a sieve with an opening of 2 mm., after which some physical and chemical properties of the field soil were estimated in the College of Agriculture and Marshlands laboratories (Table 1).

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

Type of analysis	**Unit of measure**	**Soil**	**Method**
EC	$ds.m^{-1}$	4.7	Page et al. (1982)
PH	----	7.20	
Total nitrogen	$mg. kg^{-1}$	15.4	
Ready phosphorous	$mg. kg^{-1}$	11.45	
Ready Potassium	$meq . l^{-1}$	22.2	
Organic matter	$g.kg^{-1}$	10.00	
Percentage of clay	%	40.2	Black (1965)
Percentage of silt	%	10.3	
Percentage of sand	%	49.5	
Soil texture	---	sandy clay	

The cultivation operations of the land were carried out from plowing, smoothing and leveling, and decomposed organic animal manure (cow droppings) was added 36 meters / hectare, and the land was divided into equal slabs. The experiment included 27 experimental units with a length of 3 m. Each experimental unit contained 15 pots. The distance between one plant and another was 20 cm, and between the experimental unit and another 40 cm. The experiment was designed according to a randomized complete block design with three replications in 9 variants with three concentration (0 control, 30 and 60) mg/l as nano-Iron(Fe) source were tested, and three concentration (0 control, 50 and 60) mg/l as Ascorbic acid (C) source were tested (Table 2).

Table 2. Scheme of variants and components in study

Variants	**Components**
V1	Fe0C0
V2	Fe0C1
V3	Fe0C2

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V4	Fe1C0
V5	Fe1C1
V6	Fe1C2
V7	Fe2C0
V8	Fe2C1
V9	Fe2C2

Studied parameters

1. Plant height (cm): we were measured from base of plant to the apex of the plant using only a metric tape.
2. Estimating the number of leaves and branches: Use hand counting to calculate the number of leaves and branches per plant.
3. Total chlorophyll (mg/100 g fresh weight): Total chlorophyll pigment in leaves was determined according to the method of Goodwin (1976). We were took 1 gm of leaves was taken and crushed with 10 ml of acetone, then placed in a centrifuge for 5 minutes at a speed of 3000 revolutions/min. Spectrum readings for wavelengths 663 and 665 nm were recorded using a spectrophotometer. The total chlorophyll estimated by using the following equation: Total chlorophyll (mg/l) = 20.2 x (665) D x 8.02 + (645) D.
4. The carbohydrate content of the leaves: we were determined by the method of phenol-sulfuric acid according to Dobois et al.,(1956).
5. Estimate the amount and percentage of volatile oil % in the leaves:

The volatile oils were extracted using water distillation by using the Clevenger apparatus based on British Pharmacopoeia (1968). Estimated amount of volatile oil = weight of the can with oil - weight of the empty can.
The percentage of oil in the treatment leaves was estimated according to Guenther (1972).
The results of the experiment were analyzed statistically by using the statistical analysis program SPSS version 14, via Variance analysis (ANOVA) depend the least significant difference (LSD) test between the means at the probability level of 0.05 (Al-Rawi and Khalaf, 2000).

3. Results and Discussion:

3.1. Plant height (cm):

The results of the statistical analysis in (Table. 3) showed that there were significant differences in plant height when nano-Fe was added, nano -Fe 60 mg/l concentration was gave high value 28.11 cm on rest of the treatments in the experiment, while nano -Fe 0 mg/l concentration(control) was gave least value 19.40 cm. Also, the results of the statistical analysis in (Table. 3) showed that there were significant differences in plant height when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration was gave high value 25.96 cm on rest of the treatments in the experiment, while the Ascorbic acid 0 mg/l concentration (control) was gave least value 18.99 cm. Also, The interference between spraying with nano-Fe solution and ascorbic acid had a significant effect if the results of the interference gave Fe2C2 high value on plant height reached 31.23 cm compared to the lowest height of 15.10 cm for the interference Fe0C0 (Table. 3).

Table 3: Effect of spraying nano -Fe and Ascorbic acid and their interactions on plant height (cm) of the mint plant

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	15.10	19.20	23.90	18.99	19.40
30	17.33	20.60	22.73	22.79	20.22
60	24.53	28.57	31.23	25.96	28.11
LSD	1.411			0.815	0.815

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3.2. Leaves number(leaf/plant):

The results of the statistical analysis in (Table. 4) showed that there were significant differences in leaves number when nano- Fe was added, nano- Fe 60 mg/l concentration was gave high value 73.59 leaf/plant on rest of the treatments in the experiment, while nano- Fe 0 mg/l concentration (control) was gave least value 52.56 leaf/plant. Also, the results of the statistical analysis in (Table. 4) showed that there were significant differences in leaves number when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration was gave high value 69.24 leaf/plant on the rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave least value 60.04 leaf/plant. Also, the interference between spraying with nano-Fe solution and ascorbic acid had insignificant effect, results of the interference gave Fe2C2 high value on leaves number reached 78.20 leaf/plant compared to the least leaves number of 46.00 leaf/plant for the interference Fe0C0(Table. 4).

Table (4): Effect of spraying nano- Fe and Ascorbic acid and their interactions on the number of leaves of the mint plant

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	46.00	53.00	58.67	60.04	52.56
30	64.00	68.00	70.87	64.48	67.62
60	70.13	72.43	78.20	69.24	73.59
LSD	N.S			3.159	3.159

3.3. Branches number(branch/plant):

The results of the statistical analysis in (Table. 5) showed that there were significant differences in branches number when nano-Fe was added, nano-Fe 60 mg/L concentration was gave high value 16.90 branch/ plant on the rest of the treatments in the experiment, while nano-Fe 0 mg/l concentration (control) was gave least value 7.91 branch/ plant. Also, the results of the statistical analysis in (Table. 5) showed that there are significant differences in branches number when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration was gave high value14.28 branch/ plant on the rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave least value 10.35 branch/ plant. Also, the interference between spraying with nano-Fe solution and ascorbic acid had insignificant effect, results of the interference gave Fe2C2 high value on branches number reached 78.20 branch/plant compared to the least branches number 46.00 branch/plant for the interference Fe0C0 (Table. 5).

Table (5): Effect of spraying nano- Fe and Ascorbic acid and their interactions on branches number of mint plant

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	5.57	7.80	10.38	10.35	7.91
30	10.52	12.03	13.37	12.15	11.97
60	14.97	16.62	19.10	14.28	16.90
LSD	N.S			0.732	0.732

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

The results of the statistical analysis in (Table. 6) showed that there were significant differences in chlorophyll content in leaves when nano-Fe was added, nano -Fe 60 mg/l concentration was gave high value 11.85 mg/ 100 g fresh weight compare on the rest of the treatments in the experiment, while nano -Fe 0 mg/l concentration (control) was gave least value 9.04 mg /100 g fresh weight. Also, the results of the statistical analysis in (Table. 6) not there insignificant effect in chlorophyll content in leaves when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration 10.77 mg /100 g fresh weight was gave least compared on rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave high value 10.82 mg /100 g fresh weight. Also, the interference between spraying with nano-Fe solution and ascorbic acid had insignificant effect, results of the interference gave Fe2C2 high value on chlorophyll content reached 12.37 mg /100 g fresh weight compared to the least chlorophyll content 10.97 mg /100 g fresh weight for the interference Fe0C0 (Table. 6).

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

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	10.97	7.47	8.70	10.82	9.04
30	10.16	11.06	11.23	10.13	10.82
60	11.33	11.85	12.37	10.77	11.85
LSD	Ns			Ns	1.209

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

The results of the statistical analysis in (Table. 7) showed that there were significant differences in carbohydrate content when nano-Fe was added, nano -Fe 60 mg/l concentration was gave high value1.873 mg/g dry matter compared on the rest of the treatments in the experiment, while nano -Fe 0 mg/l concentration (control) was gave least value 0.824 mg/g dry matter. Also, the results of the statistical analysis in (Table. 7) showed that there are significant differences in carbohydrate content when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration 1.563 mg/g dry matter was superior than the rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave least value 1.308 mg/g dry matter. Also, the interference between spraying with nano-Fe solution and ascorbic acid had significant effect, results of the interference gave Fe2C2 high value on carbohydrate content reached 2.137 mg/g dry matter compared to the least carbohydrate content 0.733 mg/g dry matter for the interference Fe0C0 (Table. 7).

Table (7): Effect of spraying nano- Fe and Ascorbic acid and their interactions on total soluble carbohydrates content of mint leaves (mg/g dry matter)

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	0.733	0.833	0.907	1.308	0.824
30	1.490	1.587	1.647	1.401	1.574
60	1.700	1.783	2.137	1.563	1.873
LSD	0.1524			0.0880	0.0880

3.6. Percentage of volatile oil in the leaves(%):

The results of the statistical analysis in (Table. 8) showed that there were significant differences in Percentage of volatile oil in the leaves when nano -Fe was added, nano -Fe 60 mg/l concentration

0.6794% was superior to the rest of the treatments in the experiment, while nano -Fe 0 mg/l concentration (control) was gave least value 0.2779 %. Also, the results of the statistical analysis in (Table. 8) showed that there are significant differences in Percentage of volatile oil in the leaves when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration was gave high value 0.6083 % compared on the rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave least value 0.3739 %. Also, the interference between spraying with nano-Fe solution and ascorbic acid had significant effect, results of the interference gave Fe2C2 high value on percentage of volatile oil reached 0.7217% compared to the least percentage of volatile oil 0.1167 % for the interference Fe0C0 (Table. 8).

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

Nano-Fe mg/l	**Ascorbic mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	0.1167	0.2470	0.4700	0.3739	0.2779
30	0.3617	0.4900	0.6333	0.4701	0.4950
60	0.6433	0.6733	0.7217	0.6083	0.6794
LSD	0.0646			0.0373	0.0373

3.7. Amount of volatile oil per plant (g /plant):

The results of the statistical analysis in (Table. 9) showed that there were significant differences in volatile oil per plant when nano -Fe was added, nano -Fe 60 mg/l concentration 0.286 g/plant was superior than the rest of the treatments in the experiment, while nano -Fe 0 mg/l concentration (control) was gave least value 0.173 g/plant. Also, the results of the statistical analysis in (Table. 9) showed that there are significant differences in volatile oil per plant when Ascorbic acid was added, Ascorbic acid 60 mg/l concentration 0.309 g/plant was superior than the rest of the treatments in the experiment, while Ascorbic acid 0 mg/l concentration (control) was gave least value 0.161 g/plant. Also, the interference between spraying with nano-Fe solution and ascorbic acid had significant effect, results of the interference gave Fe2C2 high value on amount of volatile oil reached 0.361 g/plant compared to the least amount of volatile oil 0.090 g/plant for the interference Fe0C0 (Table. 9).

Table (9): Effect of spraying nano- Fe and Ascorbic acid and their interactions on the amount of volatile oil of mint plant (g)

Nano-Fe mg/l	**Ascorbic acid mg/l**			**Means of Ascorbic acid**	**Means of nano-Fe**
	0	**50**	**60**		
0	0.090	0.188	0.240	0.161	0.173
30	0.160	0.228	0.327	0.227	0.239
60	0.231	0.266	0.361	0.309	0.286
LSD	0.090			0.070	0.070

The results of this study confirmed the existence of a response to spraying iron nanoparticles and Ascorbic acid solutions of alone or together, and the response was significant, in tables (3, 4 and 5) 60 mg / l concentration (nano-iron and Ascorbic acid) was superior on remaining concentrations in vegetative traits (plant height, number of leaves, number of branches), and in tables (6, 7, 8 and 9) in leaves content of total chlorophyll. Soluble carbohydrates and the amount and percentage of oil. Iron is one of the most important elements

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involved in the formation of chlorophyll pigment by entering into the construction of porphyrin compounds that make up chlorophyll (Gyana and Sunita, 2015). The vegetative growth and increase in the chemical content of the plant is attributed to the important role of iron in the formation of many compounds of cytochrome and ferredoxin, which are of great importance in the processes of photosynthesis and respiration through its role in receiving and transporting electrons that are of great importance in the process of photosynthesis, or because of the role of the iron element along with nitrogen, in the construction of RNA in the chloroplasts of leaves, or it may be due to the vital role of iron in the representation of auxins that increase cell division and thus increase the content of chlorophyll in the leaf, which increases the leaf area. Iron is also involved in the synthesis of several enzymes like, peroxidase, catalase and cytochrome oxidase, which inportant of vital processes within the plant (Havlin et al., 2005; Rui et al 2016; Hasan, 2019). The results were consistent with the results of Al-Mohammadi's studies (2020), where it was found that spraying with nano-iron concentration (160 mg / l) increased the growth and yield characteristics of fenugreek plants (Trigonella foenum-graecum L).

Also, Ascorbic acid serves many functions in plants. It acts as an effective reducing agent (Pignocchi and Foyer, 2003), and acts as an active factor for many enzymes and as an essential antioxidant. It also regulates cell division and growth and has a major role in transmitting electronic signals (Smirnoff and Wheeler, 2000). Ascorbic acid contributes to many physiological processes within the plant, 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 the results of the study conducted by Al-Rashedy et al., (2023) on bean plants (Vicia faba L.), where it was found that spraying of Ascorbic acid at a concentration of 250 mg / l was significantly superior on remaining concentrations in increase the percentage of magnesium, phosphorus, potassium and chloride in the seeds.

4. Conclusions

Results shown that the plants treated at a concentration of 60 mg/l with nano -iron fertilizer and Ascorbic acid were superior in plant height (28.11, 25.96)cm; leaves number (73.59,69.24)leaf/plant; branches number(16.90, 14.28)branch/plant; chlorophyll content in leaves (11.85, 10.77)mg/100 mg; total soluble carbohydrates (1.873, 1.563)mg/g; percentage of volatile oil in the leaves(0.6794, 0.6083)%; amount of volatile oil per plant (0.286, 0.309)g. respectively.

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