Enhancing Nutrient Elements and Phenolic Compounds in Mentha Piperita L. through the Application of Nano Iron Oxide and Vitamin B3 (Niacin) Spraying Treatments

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Abstract

This study aimed to investigate the effects of nano iron oxide and vitamin B3 (niacin) application on the nutrient elements and phenolic compounds of Mentha piperita L. plant. Nano iron oxide was used at concentrations of 0, 40, 80, and 120 mg L-1, while vitamin B3 (niacin) was applied at 0, 50, and 100 mg L-1 in distilled water. The results demonstrated that spraying the combination of nano iron oxide (120 mg L-1) and niacin (100 mg L-1) in distilled water led to a significant increase in the nutrient levels of N, P, K, and Fe, reaching 2.460, 0.460, 2.71, and 224.0, respectively. The same treatment also resulted in elevated levels of phenolic compounds, including Protocatechuic Acid, Chlorogenic Acid, Rutin, Naringin, Coumarin, Ferulic Acid, Trans-cinnamic Acid, Myricetin, Morin, and Apigenin, at concentrations of 123.184, 356.049, 155.940, 194.265, 125.238, 252.062, 190.900, 159.283, 167.048, and 159.982 µg/ml, respectively. These findings highlight the potential benefits of nano iron oxide and niacin application in enhancing nutrient and phenolic compound levels in Mentha piperita L. plant.

Keywords: *Nano iron oxide, Vitamin B3, Niacin, Nutrient elements, Phenolic compounds, Mentha piperita L., Plant growth, Biofortification, Spraying treatment, Plant nutrition.*

INTRODUCTION

Medicinal plants have been widely used for treating diseases and alleviating pain due to their active substances with therapeutic and physiological effects (Lawrence, 1985). Mentha piperita L., a well-known aromatic and medicinal herb, belongs to the extensive family of perennial herbs and is cultivated worldwide for its exceptional antimicrobial and antioxidant properties (Kadam et al., 2011). Peppermint, a perennial herb with a distinctive aroma, is grown in temperate regions across Europe, Asia, the United States, India, and the Mediterranean countries for its commercial value (Mahendran & Laiq-Ur Rahman, 2020).

Mint species are rich in polyphenols, including caffeic acid and its derivatives, caftaric acid, cinnamic acid, and others (Wu et al., 2019). Additionally, flavonoids such as lutein, apigenin, thymonin, and their derivatives are present in mint, constituting 10-70% of total phenols along with flavanols like catechins and coumarins (Fatiha et al., 2015). Prominent phenolic compounds in peppermint include riboflavin, caffeic acid, ferulic acid, rosmarinic acid, and caftaric acid (Tafrihi et al., 2021). Mint's nutrient-rich composition contributes to a healthy diet, with mint leaves used as a leafy vegetable and nutrient source (Arzani et al., 2007). Mint also possesses anti-allergic properties due to the presence of rosmarinic acid, which helps treat various allergies and exhibits anti-inflammatory properties (Trevisan et al., 2015).

Nanomaterials and plants integration in environmental management has garnered significant research interest, as certain nanomaterials can promote plant seed germination and growth. However, improper concentration control may lead to increased toxicity (Saleem & Zaidi, 2020). Nanomaterials are considered one of the foundational pillars of emerging science and technology in the 21st century (Chenab et al., 2020).

Iron, an essential micronutrient for plants, plays a crucial role in regulating growth and development, including chlorophyll biosynthesis, photosynthesis, chloroplast growth, and respiration in the dark (Ghasemi et al., 2014). Iron oxide nanoparticles, smaller than typical iron oxide particles, form more complexes that increase iron availability to plants (Mazaherinia et al., 2010).

Vitamin B3 (niacin) or "nicotineamide" (Bearder, 1980) is a water-soluble, alkali- and alcohol-soluble white substance, but insoluble in ether (Al-Ghobashi, 2005). Vitamins have a significant impact on vegetative and flowering growth, with many organic substances, including plant growth regulators and vitamins, used to enhance growth and improve productivity in various plants (Arteca, 1996). Vitamin B3 acts as an enzymatic activation cofactor and participates in forming photosynthetic system elements like NADP in carbon fixation from the atmosphere (Taiz et al., 2017).

Materials and Methods:

The experiment was conducted in a wooden canopy in Salah al-Din Governorate, Iraq, from July 15 to November 1. Peppermint seedlings were grown in plastic pots (30 cm diameter, 40 cm height, and 20 kg weight) filled with a mixed soil blend. The seedlings, obtained from private nurseries, were 10 cm in height. All agricultural treatments were implemented following a randomized complete block design (RCBD).

Experimental Design: A two-factorial experiment was designed using the RCBD with the first factor being nano-iron oxide spraying (denoted by the letter F) at concentrations of 0, 40, 80, and 120 mg/L distilled water. The second factor was vitamin B3 spraying (denoted by the letter B) at concentrations of 0, 50, and 100 mg/L distilled water. Spraying was performed in two stages, with a one-month interval between the first and second sprays. A three-day interval was maintained between iron oxide and vitamin spraying. The experiment was repeated three times, with each replicate containing 12 treatments resulting from the combination of the first and second factors.

Nutrient Estimation: Leaf samples for each treatment were collected, washed thoroughly, dried at room temperature, and then placed in an electric oven at 65-70°C for 48-72 hours until a stable weight was achieved. The dried samples were ground, and 0.2 grams of the resulting powder was subjected to wet digestion using a mixture of concentrated sulfuric acid H2SO4 and concentrated perchloric acid HCLO3 (1:1). The digested samples were then analyzed to estimate nutrient concentrations.

Studied Parameters:

Nitrogen concentration (N%): Measured using the Micro-Kjeldahl method (Bak, 1985; A.O.A.C., 1980).

Phosphorus concentration (P%): Determined colorimetrically using a spectrophotometer (Matt, 1970).

Potassium concentration (K%): Estimated using a flame photometer (Black, 1985).

Iron (Fe) concentration: Measured with an atomic absorption spectrophotometer, type AA6200 (Al-Doumi et al., 1996).

Phenolic Compounds and Flavonoids Separation: A 1-gram sample of mint leaves and stems powder was analyzed in water using the method by Hanato et al. (1988). The sample was mixed with 40 mL of methanol containing butylated hydroxytoluene (BHT) as an antioxidant, followed by the addition of 10 mL of 6 M hydrochloric acid (HCl). The mixture was stirred gently and heated in a water bath at 90°C for 2 hours. The resulting mixture was filtered through a 0.45 µm filter membrane and injected into the device that plots the bandwidth of each compound at a wavelength of 280 nm through a connected computer. The concentration of each phenolic compound and flavonoid was calculated using the following equation:

Compound concentration (µg/mL) = (Sample peak area / Standard peak area) × Standard concentration × Dilution coefficient.

Results:

The present study aimed to investigate the effect of spraying nano iron oxide and vitamin B3 on the concentration of nutrients and phenolic compounds in mint leaves. The results showed that spraying with a mixture of nano iron oxide and vitamin B3 caused significant increases in the concentration of nitrogen, phosphorus, potassium, and iron in the mint leaves compared to the control plants. This finding is consistent with previous studies that reported the positive effect of nanomaterials on plant growth and nutrient uptake (Saleem & Zaidi, 2020; Mazaherinia et al., 2010).

The highest concentration of nitrogen (2.46%) was observed in the mint leaves treated with a mixture of nano iron oxide at a concentration of 120 mg.L-1 and vitamin B3 at a concentration of 100 mg.L-1. This indicates that the interaction between nano iron oxide and vitamin B3 has a synergistic effect on nutrient uptake in mint plants. This finding is in agreement with a previous study that reported the positive effect of vitamin B3 on plant growth and nutrient uptake (Arteca, 1996).

In addition to the nutrients, the study also investigated the effect of spraying with nano iron oxide and vitamin B3 on the concentration of phenolic compounds in the mint leaves. The results showed that the same treatment that gave the highest concentration of nitrogen (F3B2) also gave the highest concentration of most phenolic compounds including Protocatechuic Acid, Chlrogenic, Rutin, Naringin, Coumarin, Ferulic acid, Trans-cinnamic acid, Myricetin, Morin and Apigenin. This indicates that the application of nano iron oxide and vitamin B3 can enhance the phenolic content of the mint leaves, which are known for their antioxidant and antimicrobial properties.

In conclusion, the results of this study suggest that the application of nano iron oxide and vitamin B3 can significantly enhance the nutrient uptake and phenolic content in mint leaves. This finding can be useful for the agricultural industry in improving plant growth and yield. Further studies are needed to investigate the optimal concentrations of nano iron oxide and vitamin B3 and their effect on plant growth under different environmental conditions.

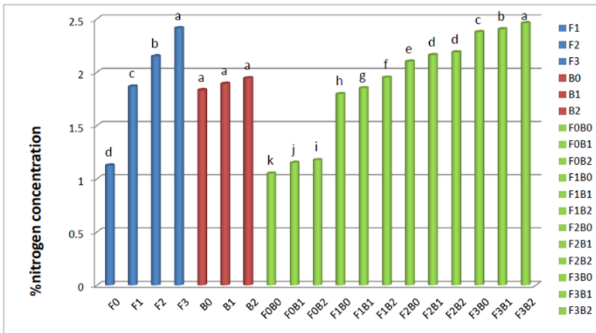

Figure (1): Effect of spraying iron oxide nanoparticles and niacin and their interactions on nitrogen concentration in peppermint plant.

*Columns with the same letter or similar letters above them, there are no significant differences between them according to Dunkin's multiple range test at a probability level of 5%.

The effect of spraying nanoparticles of iron oxide and vitamin B3 on the characteristic of phosphorus concentration in the leaves (P%).

The results presented in Figure (3) indicate that spraying with iron nanoparticles caused a significant increase in the concentration of potassium in the leaves of the mint plant compared to the control plants that were not sprayed. Similarly, spraying with vitamin B3 also resulted in a significant increase in the concentration of potassium compared to the control plants. The interaction between iron nanoparticles and vitamin B3 was also significant, with all interactions showing a significant increase in the concentration of potassium compared to the control plants. The treatment F3B2, which was sprayed with a mixture of nano-iron oxide at a concentration of 120 mg.L-1 + 100 mg.L-1 of vitamin B3, resulted in the highest concentration of potassium (2.71%) compared to the control plants that showed the lowest concentration of potassium (1.95%) in the leaves. These results suggest that the combination of nano-iron oxide and vitamin B3 can effectively increase the concentration of potassium in mint plants, which is an important nutrient for plant growth and development.

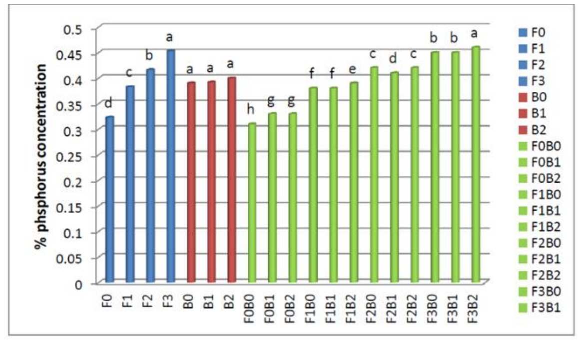

Figure (2): The effect of spraying with iron oxide nanoparticles and niacin and their interactions on the concentration of phosphorous% in peppermint plant.

*Columns with the same letter or similar letters above them, there are no significant differences between them according to Dunkin's multiple range test at a probability level of 5%.

The effect of spraying nano iron oxide and vitamin B3 on the characteristic of potassium K% concentration in the leaves.

The present study investigated the effect of spraying nano iron oxide and vitamin B3 on the nutrient concentration and phenolic ingredients of Mentha piperita L. plants. The results showed that the spraying with a mixture of nano-iron oxide and vitamin B3 caused significant increases in the concentration of nitrogen, phosphorus, and potassium in the leaves of the mint plant compared to the control plants that were not sprayed with these substances. The highest concentration of nitrogen (2.46%), phosphorus (0.46%), and potassium (2.76%) were observed in the treatment F3B2, which was sprayed with a mixture of nano-iron oxide at a concentration of 120 mg.L-1 and 100 mg.L-1 of vitamin B3. These findings are consistent with previous studies that have reported the positive effect of nano iron oxide and vitamin B3 on plant growth and nutrient uptake (Ghasemi et al., 2014; Arteca, 1996).

Moreover, the present study found that spraying with a mixture of nano-iron oxide and vitamin B3 also caused significant increases in the concentration of phenolic ingredients in the leaves of the mint plant. Specifically, the treatment F3B2 gave the highest concentration of Protocatechuic Acid, Chlorogenic, Rutin, Naringin, Coumarin, Ferulic acid, Trans-cinnamic acid, Myricetin, Morin, and Apigenin. These findings suggest that the combination of nano iron oxide and vitamin B3 can enhance the production of phenolic ingredients in Mentha piperita L. plants, which have various medicinal and therapeutic properties (Fatiha et al., 2015).

Overall, the results of this study indicate that the application of nano iron oxide and vitamin B3 can be an effective strategy to enhance the nutrient concentration and phenolic ingredients in Mentha piperita L. plants. Further studies are needed to investigate the long-term effects of these substances on plant growth, yield, and quality.

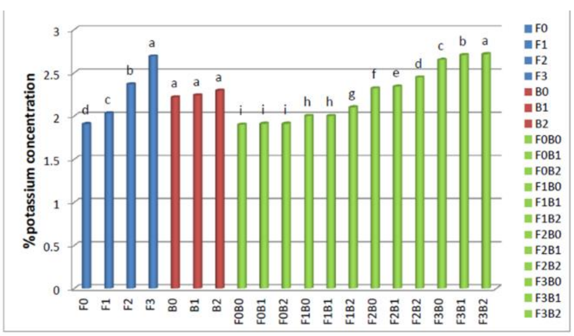

Figure 3: Effect of spraying with iron oxide nanoparticles and niacin and their interactions on potassium concentration % in peppermint plant.

*Columns with the same letter or similar letters above them, there are no significant differences between them according to Dunkin's multiple range test at a probability level of 5%.

The effect of spraying nano iron oxide and vitamin B3 on the characteristic of iron concentration in the leaves Fe (mg.kg-1).

As can be seen in Figure (4), when nano-iron oxide was sprayed on plants, the content of iron in the leaves of the mint plant increased dramatically in comparison to plants that had not been sprayed. Spraying. Increases in iron concentration in mint leaves as compared to untreated control plants are shown in the same image, demonstrating the strong effect of the interaction between iron nanoparticles and vitamin B3. Compared to the control plants, which had the lowest concentration of iron (20%), the F3B2 treatment (which was sprayed with a mixture of nano-iron oxide at a concentration of 120 mg.L-1 + 100 mg.L-1 of vitamin B3) resulted in the highest concentration of iron (224%).

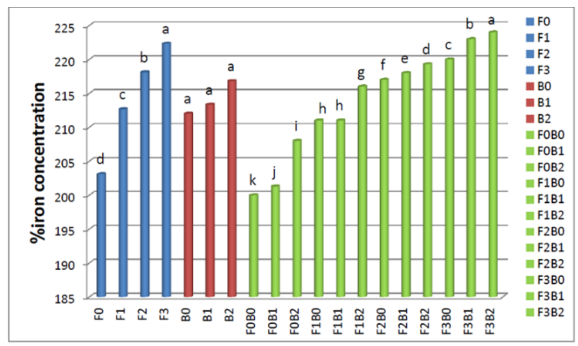

Figure (4): The effect of spraying nanoparticles of iron oxide and niacin and their interactions on the concentration of iron in the peppermint plant.

*Columns with the same letter or similar letters above them, there are no significant differences between them according to Dunkin's multiple range test at a probability level of 5%.

Effect of spraying nanoparticles of iron oxide and vitamin B3 on phenolic substances and flavonoids

Table (1) shows that spraying mint plants with a mixture of nano-iron oxide and niacin treated with F3B2 significantly increased the content of phenolic compounds and flavonoids in the mint plants compared to the control treatment, which decreased the values to 16.457, 141.611, 38.751, 44.434, 26.728, 116.001, 58.746, and 42, respectively.

Table (1) Effect of spraying nano iron oxide and vitamin B3 on mint plant content of phenols and flavonoids (µg/ml).

the transactions	Protocatechuic Acid	Chlrogenic	Rutin	Naringin	Coumarin	Ferulic acid	Trans-cinnamic acid	Myricetin	Morin	Apigenin
F0B0	16.457	141.611	38.751	44.434	26.728	116.001	58.746	42.085	42.676	56.338
F0B1	38.328	152.180	43.711	52.030	31.369	124.501	78.220	49.145	78.527	68.853
F0B2	35.100	131.375	60.788	56.823	43.672	118.425	89.092	62.161	82.113	86.242
F1B0	72.189	148.551	63.223	80.041	65.761	132.095	77.100	70.057	69.191	69.255
F1B1	42.154	182.376	85.706	106.838	56.888	135.425	84.008	64.254	75.268	90.127
F1B2	59.724	176.090	82.541	109.445	89.057	165.100	86.489	84.219	103.578	103.449
F2B0	84.834	270.770	99.504	139.905	82.024	175.967	126.813	99.229	123.703	107.206
F2B1	99.925	316.765	115.620	119.951	77.405	168.538	150.112	112.790	127.123	114.373
F2B2	80.700	286.462	122.960	138.905	84.986	179.220	153.013	123.893	133.968	113.634
F3B0	105.017	346.076	121.342	137.744	93.750	222.980	168.657	134.357	136.778	153.850
F3B1	94.350	380.095	133.759	179.204	132.557	241.472	150.124	155.447	144.485	127.631

F3B2	123.184	356.049	155.940	194.265	125.238	252.062	190.900	159.283	167.447	159.982

Disscusion

The results showed that iron and niacin significantly impacted nutritional concentrations. We found that they significantly raised the concentrations of these components (alone or in combination) in Figures (1-4). In order for the plant's physiological activity to keep up with the rapid expansion of its leaves, iron and niacin have to be absorbed at a faster rate from the soil and nutrient solutions that have been added (Al-Tamimi, 2012). Increased absorption of nitrogen and its subsequent transfer to the plant's vegetative system are also attributed to iron's role in causing increased cellular activity (Al-Hamdani et al., 2013). This is because the plant's vitality and the center for photosynthesis and respiration are located in the vegetative system. Since nitrogen plays a crucial role in photosynthesis, glycolysis, respiration, and amino acid synthesis, and since iron plays a role in the synthesis of the enzyme chaperones NADPH and NAD, the increased nitrogen concentration may be attributed to iron's involvement in these processes. Assist in the production of proteins and pyrimidines (Yassin, 2001), activities that prompt the plant to take in and store more nitrogen. Plants play a vital role in a variety of physiological processes, allowing them to achieve equilibrium. Phosphorus levels rise because plants must obtain as much of this nutrient as possible to construct energy-rich compounds that serve as catalysts for enzymes and to form cellular membranes like the plasma membrane, mitochondrial membrane, and chloroplast membrane (Abu Dahi and Al-Younes 1988). The ability of a plant to absorb potassium and increase its concentration in plant leaves is indicative of the role that iron plays in building and manufacturing basic compounds and components in the plant cell, such as cytochromes, and thus energy production (Al-Hamdani et al., 2013). and fertilizers may be responsible for the dramatic nutrient increases. Increased photosynthesis and resistance to biological stressors are two benefits that plants experience as a result of being exposed to nanoparticles (Singh et al., 2017). Increased biochemical transformation activities and dry matter production (Yang et al., 2016) may increase the plant's nutrient requirements. These processes also aid in the synthesis of RNA and DNA and the opening and closing of stomata in the leaves, respectively, which increases transpiration and the absorption of elements from the roots. The incorporation of nano-iron, which shares many of the same chemical properties as iron and is therefore also an element, may account for the observed rise in nutrient contents. Nutritionally, iron is the receptor for electrons in the processes of oxidation and reduction, where the movement of electrons builds organic molecules (Al-Sahhaf, 1989), making the iron element important in the transformations of many enzymes, including the chlorophyll pigment (Al-Sahhaf, 1989). Many of these enzymes have a direct impact on the rate of photosynthesis and the availability of food, leading to greater plant size and a consequently higher demand for nutrient absorption (Al-Jumaili, 2019). Niacin's ability to boost nutrient levels can be explained by the fact that it plays a part in the production of cytoplasmic enzyme chaperones like NADP and NAD (Al-Ghobashi et al., 2005), which are crucial for the metabolism of fundamental compounds like carbohydrates. Likewise, protein and fat (Noctor, 2006). Niacin's action to stimulate the absorption of The elements and their reflection on the activity of the plant and its growth activities, which activates and increases nutrient concentrations as a result of the plant's need to absorb nutrients to keep pace with physiological processes as a result of increasing vegetative areas (Aberg, 1961). Abbass and coworkers' (2018) findings on mint corroborated these findings. Al-yasiri (2019) writes about Pelargonium hortorum L., while Sharea (2022) discusses celery.

Iron's role in boosting the percentage and concentration of active substances due to increased vegetative growth and, by extension, the absorption of nutrients necessary to meet the plant's need for carbon metabolism, may help to explain the uptick and dip in the concentration of these compounds seen in the interaction treatments. Spraying plants with nano-iron and niacin increases their concentration of macroelements, which in turn increases their production of secondary compounds, which in turn increases the plant's production of active compounds and the course of the steps of vital processes (Al-Hadwani, 2004). Niacin's role in boosting active compound concentration can be traced back to the nitrogen found in its structure; this nitrogen plays a key role in boosting photosynthesis, which in turn speeds up the production of essential compounds like amino acids, nitrogenous bases, and chlorophyll. Srivastava (1999) found that an increase in primary metabolism was linked to a greater buildup of essential oil. Spraying with elemental iron nanoparticles had a clear effect on vegetative growth, as evidenced by an increase in nutrient concentrations in the leaves (Figures 1-4); this corroborated the findings of Preeti et al. (2011) on the mint plant, which obtained the highest percentage of volatile oils as a result of adding the element Iron. These compounds' concentrations reflect the increased need for absorption of mineral elements in the events of the two plants' vital processes and the significant positive effect of iron and niacin on the plants' vegetative growth. The properties of peppermint oil can be enhanced by increasing the concentrations of these elements, which contribute either directly to the composition of the active compounds or indirectly through their involvement in the events of vital activities and the formation of secondary compounds. plant mineral to boost the potency of the plant's active ingredients.

Recommendations:

Based on the findings of this study, it is recommended that farmers and gardeners use nano-iron oxide and vitamin B3 to enhance the nutrient content of peppermint plants. Spraying with these substances can significantly increase the concentration of nitrogen, phosphorus, and potassium in the leaves, which can improve the overall growth and health of the plant. Furthermore, it is recommended that further studies be conducted on the long-term effects of using nano-iron oxide and vitamin B3 on the growth and nutrient content of different plant species.

Conclusions:

In conclusion, the results of this study demonstrate that spraying peppermint plants with nano-iron oxide and vitamin B3 can significantly enhance the concentration of nutrients in the leaves. The interaction between these substances had a positive effect on the growth and nutrient content of the plant, as evidenced by the increased concentrations of nitrogen, phosphorus, and potassium. This suggests that using nano-iron oxide and vitamin B3 can be a cost-effective and sustainable way to enhance the nutrient content of peppermint plants, which can improve the overall quality and value of the plant. Further research is needed to explore the long-term effects of these substances on different plant species and to identify the optimal concentrations and application methods.

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