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# Magnetite- Nanoparticles Effects on Growth and essential oil of Peppermint

**Mohamed S. Khater**

*National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt*

**ABSTRACT**

The present study aimed to assess the effects of magnetite nanoparticles (MNPs) on growth and essential oil composition of *Mentha piperita* L. The experimental design was sprayed *Mentha piperita* L. plant with magnetite nanoparticles (MNPs) at concentrations 5, 10 and 15 ppm twice with different concentrations first one after 30 days from cultivation and the second one after 60 days in the first season and in the same time in the second season. The results showed that magnetite nanoparticles (MNPs) increased significantly plant growth and the essential oil composition of *Mentha piperita* L. plants.

***Key words:*** MNPs, *Mentha piperita*, Peppermint, essential oil composition

## Introduction

*Mentha piperita* L. is used for medicinal and food purposes (Lorenzi and Matos, 2002). Its cultivation has economic importance, peppermint essential oil ranks high in terms of total sales volume (Moraes, 2000). The main constituent is menthol, used in oral hygiene products, pharmaceuticals, cosmetics, and foods. Menthol also has high antifungal and antibacterial potentials, thus becoming one of the most demanded substances by the scents and essences industry (Souza *et al.,* 1991). Menthol stimulates cold receptors in the respiratory tract, which inhibits cough and improves nasal airflow (Shah and Mello, 2005). Nano technology is one of the main advancement in the science technology of the last decade. Nano particles are considered as molecular or atomic aggregates between 1 and 100 nm (Ball, 2002 and Roco, 2003a). In the present work we used magnetite nanoparticles (MNPs) which have concerned a great attention in the end of four decades and considered an active research field in the field of magnetism. Magnetite nano crystals have been extensively studied because it used in applications in biomedical fields such as tracking, biological labeling, detection and separations (Bulte *et al.,* 2001). An important phase of the hazard of ENPs is to recognize relations of ENPs with plants and also all ecosystems. Plants are main base component of all ecosystems and considered as an important role in fate and transfer of ENPs in environment through plant uptake and bioaccumulation in plant biomass, in addition ENPs could stick to plant roots and caused change in physical or chemical toxicity in plants (Battke *et al.,* 2009).

Iron deficiency is widespread and is one of the most concerned to healthcare officials among almost all developing countries (Buyckx, 1993). Iron is one of the essential elements for plant growth and plays an important role in the photosynthetic reactions. Iron activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems (Malakouti and Tehrani, 2005). Iron deficiency has increased from 30% in the 1960s to 40% in the 1990s among the world population (Welch and Graham, 2002). Nano-technology can present solution to increasing the value of agricultural products and environmental problems. With using of nano-particles and nano-powders, we can produce controlled or delayed releasing fertilizers. Nano-particles have high reactivity because of more specific surface area, more density of reactive areas, or increased reactivity of these areas on the particle surfaces. These features simplify the absorption of fertilizers and pesticides that produced in nano scale (Anonymous, 2009). Nanotechnology with materials having unique properties has promised applications in various fields. It has provided new solutions to problems in plants and food science (post-harvest products) and offers novel approaches to the rational selection of raw materials, the processing of such towards applying disease control molecules, slow-release pesticides and developing diagnostic tools. Nanosilver, a new class of material with remarkably different physicochemical and biological characteristics from convenient silver-containing substances, has been shown to have antibacterial, antifungal and antiviral effects and it can reduce damage and losses caused by diseases (Choi *et al.,* 2009). The alternative approach is to apply these micronutrients as foliar sprays. Six micronutrients including Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants (Mortvedt, 1991) iron is one of the essential elements for plant growth and plays an important role in the photosynthetic reactions. Iron activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems (Malakouti and Tehrani, 2005). Abbas *et al.* (2009) reported that iron contents enhanced wheat growth. Zeidan *et al.* (2010) applied foliar Fe fertilizer (1.0% $FeSO_4$) and reported that Fe application increased protein and Fe contents of wheat grain.

The present study aims to investigate the effect of MNPs on the on growth and essential oil of *Mentha piperita* L. plants.

**Corresponding Author:** Mohamed S. Khater, National Institute of Laser Enhanced Sciences (NILES), Cairo University, Egypt
E-mail: saskhater@hotmail.com

## Material and Methods

*Field Experiment*

Field experiments were conducted during two successive seasons of 2010-2011 and 2011-2012 at research center of medicinal, aromatic and toxic plants (R.C.M.A.T.P). Rhizomes of Mentha x *piperita* L. were cultivated in early march month, the experimental plot area was 2 m x 3m, the planting distance was 25 cm apart and it was 50 cm between lines. The layout of the Experiment was randomized complete block design with three replicates. The experiment was consisting of 4 treatments 00, 5, 10 and 15 ppm of MNPs. Each treatment was represented by three plots. The plants were harvested two times in June 2010 and June 2011 during two successive seasons. At each harvesting time, fresh and dry weights of herb (g/plant) were recorded .The soil chemical properties were determined according to Jackson, (1973) and Cottenie *et al.* (1982). (Table 1).

**Table 1:** The soil chemical analysis for the two seasons.

| Measurements | Season 2010-2011 | | | | Season 2011-2012 | | | |
|---|---|---|---|---|---|---|---|---|
| Soil depth (Cm) | 0-30 | | | | 0-30 | | | |
| pH (1:2.5) | 7.5 | | | | 7.3 | | | |
| E.C. (mmhos/Cm) | 0.9 | | | | 0.7 | | | |
| Calcium Carbonates (%) | 7.1 | | | | 7.4 | | | |
| Soluble cations (meq/L) | $K^+$ | $Na^+$ | $Mg^{+2}$ | $Ca^{+2}$ | $K^+$ | $Na^+$ | $Mg^{+2}$ | $Ca^{+2}$ |
| | 0.47 | 4.5 | 0.83 | 1.3 | 0.57 | 5.6 | 0.80 | 1.8 |
| Soluble anions (meq/L) | $SO_4^{-2}$ | $Cl^-$ | $HCO3^-$ | $CO3^{-2}$ | $SO4^{-2}$ | $Cl^-$ | $HCO3^-$ | $CO3^{-2}$ |
| | 6.3 | 0.82 | 0.53 | - | 7.5 | 1.7 | 0.67 | - |
| Available micronutrients (ppm) | Fe | Mn | Cu | Zn | Fe | Mn | Cu | Zn |
| | 6.4 | 12 | 3.2 | 1.8 | 7.6 | 13.5 | 4.2 | 2.4 |

*Synthesis of magnetite nanoparticles*

Magnetite nanospheres 20±2nm size is synthesized by co-precipitation method using ascorbic acid, reduction of $FeCl_3$ (Jakubovics, 1994).

*Characterization of magnetite nanoparticles*

Magnetite nanoparticles can be characterized via TEM imaging. The TEM images of the synthesized magnetite nanoparticles shows that these particles has average size of 20 ± 2.0 nm with spherical shape as shown in fig (1)**.**

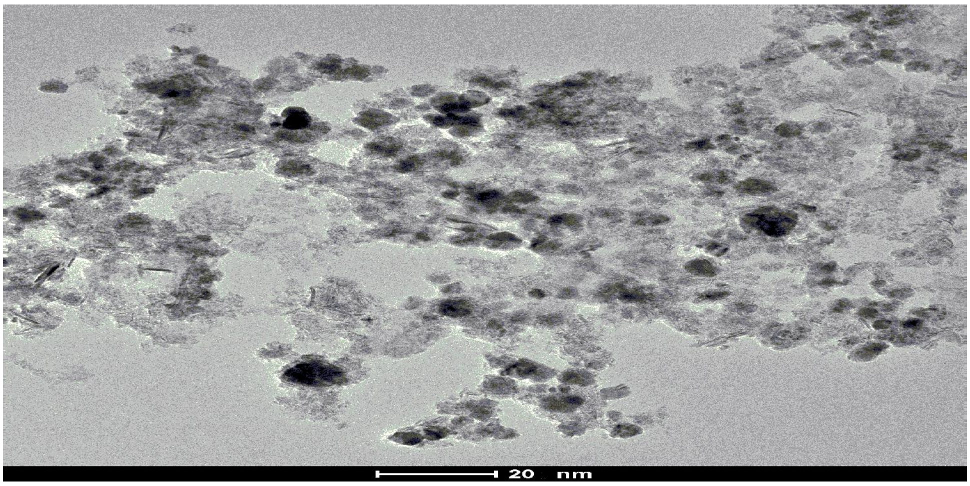


**Fig. 1:** TEM image of the magnetite nanoparticles capped with ascorbic acid shows that these particles has spherical shape with average size of 20± 2.0 nm

*Essential-oil extraction and analysis*

The oil was extracted from the fresh plants by hydrodistillation in a Clevenger-type apparatus for 3 according to the Egyptian Pharmacopeia (1984). The essential oil extracted were dried over anhydrous sodium sulfate and analyzed by gas chromatography using Agilent 7000 triple Quad GCMs.

*Determination of Fe, Mn, Zn and Cu*

Micronutrients (Fe, Mn, Zn and Cu) were determined according to Cottenie *et al.* (1982).

*Statistical Analyses*

All statistical analyses were carried out by SAS version 9 software for all data. R- Squared values ($R^2$) are considered significant (p-values <0.05) for the analysis of variance test (ANOVA).

## Results and Discussion

*Plant growth*

Data presented in (Table 2) showed that spraying peppermint plants with MNPs at 5, 10 and 15 ppm had a significantly promotive effect on all growth parameters i.e. fresh and dry weights of peppermint herb (gm/plant ). MNPs at 15 ppm gave the greatest fresh and dry herb yield 103.8, 22.8 and 400,89.4 gm/plant for first and second season, respectively. These data are harmony with those obtained by Roghayyeh *et al.,* (2010) who found that nano-iron oxide had significant effects on the dry pod weight; leaf plus dry pod and yield. Application of nano-iron oxide at 0.75 g /L compare to other treatments had maximum effect on dry pod weight. It seems that the use of iron nano-particles causes increasing in pod and dry leaf weight and finally will increase total yield. Moreover, Iron is one of the essential elements for plant growth and plays an important role in the photosynthetic reactions. Iron activates several enzymes and contributes in RNA synthesis and improves the performance of photosystems (Malakouti and Tehrani, 2005).

**Table 2:** Effect of different concentrations of MNPs on fresh and dry weight of *Mentha piperita* plants during 2011 and 2012.

| Treatment | Fresh weight (g/ plant) | | Dry weight (g/ plant) | |
|---|---|---|---|---|
| | 2010/ 2011 | 2011/2012 | 2010/ 2011 | 2011/ 2012 |
| Control | 90.6 | 315.6 | 20.25 | 70.5 |
| 5ppm | 97.3 | 357.3 | 21.52 | 80.3 |
| 10 ppm | 102 | 383.7 | 21.7 | 85.1 |
| 15ppm | 103.8 | 400 | 22.8 | 89.4 |
| R2 | 0.94 | 0.96 | 0.93 | 0.95 |

*Essential oil Composition*

As shown in (Table 3) The foliar treatment of *mentha piperita* L. with MNPs at 5, 10 and 15 ppm increased significantly the principal component of peppermint essential oil as compeared with the control ones. These finding agrees with souad *et al.* (2013) who find that Basil plants treated with 3 mg/L of iron NP had the lowest total carbohydrate concentrations and the highest essential oil content. Dubey *et al.* (2003) showed that any increase of total plant carbohydrates will be utilized in the biosynthesis of essential oil, consequently increasing the production of essential oil.

**Table 3:** Effect of MNPs on the essential oil constituents of peppermint plant

| Essential Oil Constituents | MNPs concentration (ppm) | | | |
|---|---|---|---|---|
| | Control | 5 | 10 | 15 |
| Myrcene | 0.15 | 0.16 | 0.18 | 0.18 |
| Linalool | 0.38 | 0.40 | 0.41 | 0.44 |
| 1,8 cineol | 4.2 | 4.5 | 4.8 | 5.2 |
| αterpinolene | 0.11 | 0.11 | 0.12 | 0.12 |
| Menthone | 16.6 | 16.8 | 17.2 | 17.4 |
| Isomenthone | 3.5 | 3.8 | 4.2 | 4.4 |
| Neo-menthol | 5.3 | 5.6 | 5.8 | 6.3 |
| menthol | 40.3 | 42.6 | 46.5 | 48.2 |
| Pulegone | 3.2 | 3.3 | 3.6 | 3.7 |
| Piperitone oxide | 0.44 | 0.46 | 0.48 | 0.49 |
| Isomenthyl acetate | 3.3 | 3.4 | 3.6 | 3.8 |
| Neomenthyl acetate | 0.14 | 0.14 | 0.16 | 0.17 |
| Caryophyllen oxid | 0.04 | 0.05 | 0.06 | 0.06 |
| Limonene | 1.8 | 2.0 | 2.1 | 2.3 |
| Linalool | 0.38 | 0.4 | 0.42 | 0.43 |

*Iron Content*

It is clear that spraying 15 ppm of MNPs on *Mentha piperita* L. plant exhibited the highest content of iron 5.6 and 5.9 ppm compared with 2.8 and 3.2 ppm for controal plants, in the first and second season, respectively (Table 4A,B). These results were in agreement with the findings of Moosavi and Ronaghi, (2011), who reported that foliar application of iron significantly increased shoot iron concentration and uptake of dry bean to levels higher than those observed with soil foliar application. Mohammed *et al.* (2013) shows that the spraying 30 ppm of $Fe_3O_4$ NPs on Basil plant increased tissue iron content to level two times higher than controls.

*Mn, Zn and Cu Content:*

Presented data in (Table 4 A,B) revealed that MNPs at 15 ppm gave the highest values of Mn, Zn and Cu in the first and second season on peppermint plants compared with the control and lower concentrations compared with the allowable concentrations. These results confirmed that MNPs at 5, 10 and 15 ppm are safe when sprayed on peppermint plants. Our results agreed with Larry *et al.,* (1979; Gupta, (1975); Radojevic and Vladimir, (1999) and Haider *et al.* (2004 )

**Table 4 a:** Effect of MNPs on Fe, Mn , Zn and Cu content in peppermint plant at first season (2010-2011)

| Concentration(ppm) | Fe | Allowable concentration (ppm) | Mn | Allowable concentration (ppm) | Zn | Allowable concentration (ppm) | Cu | Allowable concentration (ppm) |
|---|---|---|---|---|---|---|---|---|
| Control | 2.8 | 50 | 0.83 | 300 | 0.78 | 15 | 0.74 | 20 |
| 5 | 3.7 | | 0.111 | | 0.85 | | 0.78 | |
| 10 | 4.5 | | 0.187 | | 0.163 | | 0.94 | |
| 15 | 5.6 | | 0.194 | | 0.173 | | 0.104 | |
| main | 4 | | 0.207 | | 0.187 | | 0.108 | |

**Table 4 b:** Effect of MNPs on Fe, Mn , Zn and Cu content in peppermint plant at second season (2011-2012)

| Concentration(ppm) | Fe | Allowable concentration (ppm) | Mn | Allowable concentration (ppm) | Zn | Allowable concentration (ppm) | Cu | Allowable concentration (ppm) |
|---|---|---|---|---|---|---|---|---|
| Control | 3.2 | 50 | 0.93 | 300 | 0.82 | 15 | 0.78 | 20 |
| 5 | 3.9 | | 0.115 | | 0.94 | | 0.84 | |
| 10 | 4.8 | | 0.195 | | 0.167 | | 0.98 | |
| 15 | 5.9 | | 0.206 | | 0.178 | | 0.108 | |
| main | 4 | | 0.213 | | 0.193 | | 0.114 | |

**Conclusion**

In conclusion, magnetite nanoparticles (MNPs) had significant effects on growth and essential oil of Peppermint. The results of this work show strong evidence for the high efficiency of this new Nanofertilizer on plant growth enhancement

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