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Effect of Magnetite Nanoparticles (Fe_3O_4) as Nutritive Supplement on Pear Saplings

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ABSTRACT

This study was performed during the two successive season of 2013 and 2014 to determine the effect of foliar sprays with magnetite nanoparticles (MNPs) as nutrient supplement on some growth parameters and some chemical components of pear saplings (*Pyrus serotina* L. X *Pyrus communis* L.) cultivar Le-Conte. Results showed an increase in biomass parameters i.e. sapling height, stem diameter, leaf area and dry weight. Additionally leaf bio-chemicals showed a tremendous increase in total carbohydrates percentage, total amino acids concentrate, nitrogen and iron content, total and refraction of chlorophyll and carotenoids content using magnetite treatments compared to traditional iron chelate treatment. 250 ppm MNPs proved to be the highly effective than the same concentration of traditional iron chelate. MNPs proved to have unique physicochemical properties and superparamagnetism that boosted over all plant metabolism that affected by biomass and bio-chemical properties.

Key words: Magnetite, nanoparticles, Fe_3O_4, nutrient, supplement, foliar spray, pear, saplings.

Introduction

Le-Conte pear cv. which belongs to Rosaceae family, is the main cultivar grown in Egypt it's a hybrid between *Pyrus serotina* L. X *Pyrus communis* L. (Lee, 1948).

Iron is one of the essential micronutrients elements for plant growth and development needed by plants in small quantities, consequent deficiency of iron causes physiology malfunction and plays an important role in the photosynthetic process reactions, including chlorophyll synthesis and chloroplast development (Bozorgi, 2012). Iron activates several enzymes and it is very important for chlorophyll formation and improves the performance of photosystems and many other vital processes of plants (Nadi *et al.,* 2013).

Nanotechnology has various functions in all stages from production, processing, storage, postharvest and transportation of agricultural products (Scott and Chen, 2003). Nanotechnology introduced a large scope of new application in agriculture. Using Nano fertilzers to plants is one of the critical importance due to its unique properties and activities in size brings about substantial changes in their physical properties with respect to bulk materials in terms of the small size of the particles and increase the surface area consequently for its higher resonance (Xia *et al*., 2009). The nanotechnology increases the application efficiency of fertilizers, reduces soil pollution and environmental risks of chemical fertilizers (Bakhtiari *et al*., 2015). Previous studies showed that nanoparticles can have a beneficial effect on plants growth and development (Zhu *et al.,* 2008; Roghayyeh *et al.,* 2010).Nutrients are very important for plant growth and development; there are many factors that reduce their availability to plants. Thus, it is necessary to reduce nutrient losses to increase crop yields through using new applications such as nanotechnology. Nano fertilzers or nano-encapsulated nutrients might have properties that are effective to crops, released the nutrients on-demand, controlled release of chemicals fertilizers that regulate plant growth and enhanced target activity (DeRosa *et al*., 2010; Nair *et al*., 2010). This effect maybe also due to superparamagnetism behavior of MNPs as describes (Shouhu *et al.* 2007). Magnetic field improved the plant growth characteristics (Abou El-Yazied *et al*., 2012; Esitken and Turan, 2004; Carbonell *et al*., 2011; Radhakrishnan and Kumari, 2012), influenced the chemical composition of plants (Radhakrishnan and Kumari, 2012) and activate plant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) (Alikamanoglu and Sen, 2011; Shabrangi *et al*., 2011)

This work was conducted to evaluate the possible effect of MNPs as nutrient supplement for pear saplings to help early growth of sapling and increase sapling vigor.

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Materials and Methods

Plant materials

One year old pear saplings (*Pyrus serotina* L. X *Pyrus communis* L.) Le-Conte cv. grafted on (*Pyrus betulifolia* L.) betulifolia rootstock was used as plant material and were chosen as uniform as possible grown in lath house located at Faculty of Agriculture Experimental farm, Ain Shams University, Cairo, Egypt.

Each sapling was placed in plastic Pot (30 cm) filled with a mixture of sand and peat moss rate 4:1.Saplings were subjected to regular and recommended fertilizer program under Egyptian condition.

Preparation of magnetite nanoparticles (MNPs)

MNPs were synthesized by hydrothermal method using ascorbic acid reduction of $FeCl_3$ according to (Shouhu *et al.,* 2007). In brief: 0.25 g of $FeCl_3$ powder was dissolved in 25 ml distilled water. Then, 0.6 g $NaHCO_3$ powder dissolved in 10 ml distilled water was added to $FeCl_3$ solution drop by drop with continued stirring for 10 minutes. The solution becomes viscous with brown color. 0.12 g powder of ascorbic acid was added to the mixture with vigorous stirring for 15 minutes, the color of solution turned black, and nanoparticles capped with $C_6H_6O_6$ (The oxidation state of ascorbic acid) were formed. Finally, the volume of the solution was completed to 50 ml with sterile distilled water and transferred to stainless steel autoclave tube at 160^{o}c for 3 hr. The final product was separated from the reaction medium by using filter paper (Watman # 1) with washing several time, then the precipitate was dried at 60^{o}c for 12 hr.

Characterization of the prepared nanoparticles

Physico-chemical properties of nanoparticles were characterized using High-Resolution Transmission Electron Microscope (HR-TEM, Tecnia G20, FEI, Netherlands), X-ray Diffraction (XRD, X'pert Pro, PanAnalytical, Netherlands), Vibrating Sample Magnetometer (VSM, Lakeshore 7410), Particle size analyzer (Zeta sizer ananoseries'zs', Malvern, UK) Atomic absorption Spectroscopy (AAS, 3300, Perkin Elmar, Germany). All preparation procedures and characterization of MNPs were done at the Nanotechnology & Advanced Materials Central Lab, Agriculture Research Center, Giza, Egypt.

The experimental design and statistical analysis

Sapling Le-Conte cultivar was subjected to five treatments, twelve saplings for each with six replicates used as follows:

1-Control (sprayed with distilled water).
2-Foliar spraying with chelate iron on EDDHA 6% at 250 ppm (produced by Growtech ® Company).
3-Foliar spraying with magnetite nanoparticles at 250 ppm.
4-Foliar spraying with magnetite nanoparticles at 125 ppm.
5-Foliar spraying with magnetite nanoparticles at 25 ppm.

All treatments were applied as foliar spray on sapling using hand operated compressed air sprayer at the rate of one liter/treatment. The experiment was carried out in complete randomized design with six replicates. The spray occurred in three time for each concentrate (April 6, April 20 and April 27). All statistical analysis for the different traits was performed using SAS program (SAS, 2011). Differences among experimental groups were tested by Duncan's Multiple Range test (Duncan, 1955).

Vegetative measurements

- Sapling height (cm) estimated by using meter scale starting from soil surface and up to the plant tip, measurement was carried out every week during the growth season.
- Stem diameter (mm) measured by using vernier caliper (Tri circle, Shanghai-China) at; 10 cm above grafting zone every week during the growth season**.**
- Leaf area(cm^2) recording each sapling leaves using the recent full expanded fourth leaf from the plant top as a relation between unit area and leaf fresh weight (Koller, 1972).

$$\text{Leaf area (cm}^2\text{)} = \frac{\text{Disk area} \times \text{No. disks} \times \text{Leaf f.w.}}{\text{Disk f.w.}}$$

- Leaf dry weight (g/plant) by using hot air up to 70^{o}C for 6hr by hot air drying oven (WT-binder, 7200 tuttlingen / Germany) and repeated several times at the same temperature till constant dried weight was measure.

Leaf bio-chemical components

- Chlorophyll a (Chl a), Chlorophyll b (Chl b) and Carotenoids content were determined according to the method described by (Lichtenthaler, 1987). The samples were taken from fresh fully expanded mature leaves, 1 g of each sample was extracted using 10 mL 80% acetone, the absorbance of extract was estimated spectrophotometrically at wave lengths 660 nm, 640 nm and 440nm for Chl a, Chl b and Carotenoids respectively.

- Total amino acids were determined according to Moor and Stein, (1954).
- Nitrogen was determined by Microkjeldahl method (Jakson, 1973).
- Iron was estimated by using Atomic Absorption spectrophotometer (Cottenie, 1980).
- Total carbohydrates were determined by colorimetric method according to (Shaffer and Hartmann, 1921).

Results and discussion

Characterization of synthesized magnetite nanoparticles

Transmission electron microscope

MNPs were synthesized in a hydrothermal system by reduction reactions between $FeCl_3$ and ascorbic acid. The HR-TEM image shows that particles average size was within 20 nm spherical shape as shown in Figure 1.

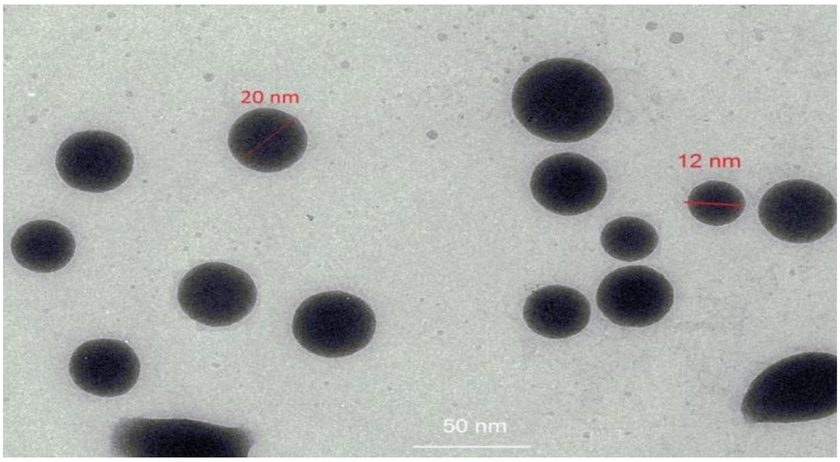

Fig. 1. HR-TEM image of the prepared Magnetite Nanoparticles capped with oxidation state of ascorbic acid shows that particles have spherical shape.

X-ray diffraction

Phase analysis was characterized by XRD which confirms the phase formation of nanoparticles, Fe_3O_4 and the crystal size was determined by Schere formula: D=kλ/bcosɵ (Klug and Alexander, 1962) as shown in Figure 2. The XRD of the sample shows the formation of Fe3O4 capped with oxidation state of ascorbic acid, based on the comparison of their XRD patterns with the standard pattern of Fe_3O_4(card #: 04-013-9808). The diffraction peaks at 2ɵ=30, 35, 43, 57.3 and 63 were corresponding to hkl= 220, 311, 400, 511, 440 respectively; were quite identical to characteristic peaks of the Fe_3O_4 crystal with the cubic spinal structure. The application of Scherer's formula to the (311) reflection peak indicated the formation of MNPs with crystal average size 20 nm in diameter.

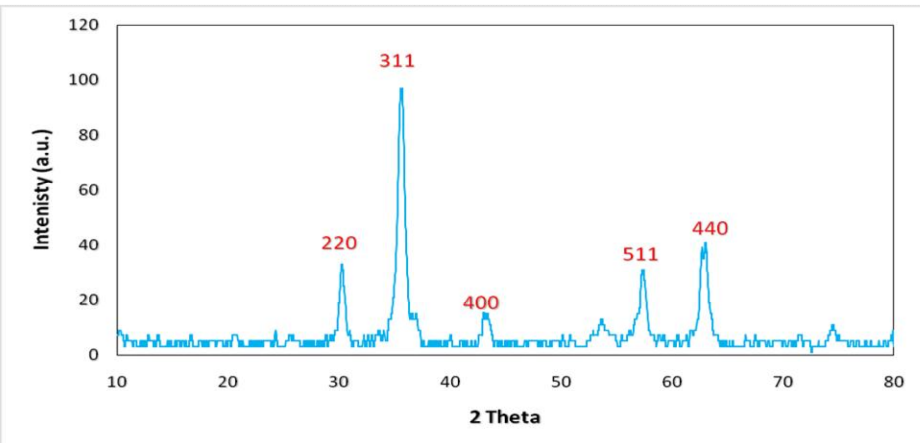

Fig. 2: Graph represents the XRD pattern of synthesized nanoparticles shows the formation of Fe_3O_4capped with oxidation state of ascorbic acid based on the comparison of their XRD patterns with the standard pattern of Fe_3O_4

Vibrating sample magnetometer

Figure 3. Shows magnetic hysteresis loops of pure Fe_3O_4 NPs measured at room temperature. It is obvious that, with increasing the applied field, the magnetization increase sharply and then gradually, and reach saturation at 3000 Oe. The saturation magnetization (Ms) of magnetite was 80.0 emu/g. therefore; Fe_3O_4 has magnetic properties.

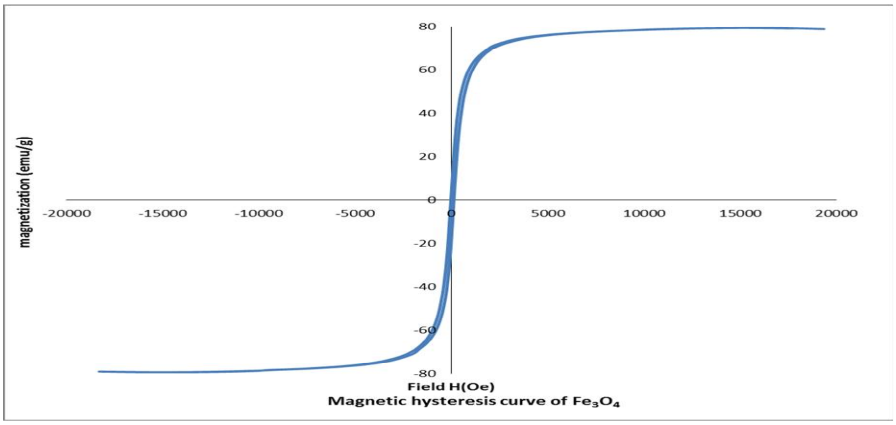

Fig. 3: Hysteresis loop obtained from VSM measurements of synthesized MNPs capped with state of ascorbic acid

Particle size analyzer

The frequency number of particle size data is in linear scale as shown in (figure 4). The smooth curve drawn is a valid size-frequency curve if sufficient particles are counted and the size interval is at least ten. Hundreds of particles were measured to present statistically reliable mean size data. For instance, it has been proposed to measure 500-1000 grains for an optimum sample size. The peak shows that average size of MNPs is 20 nm.

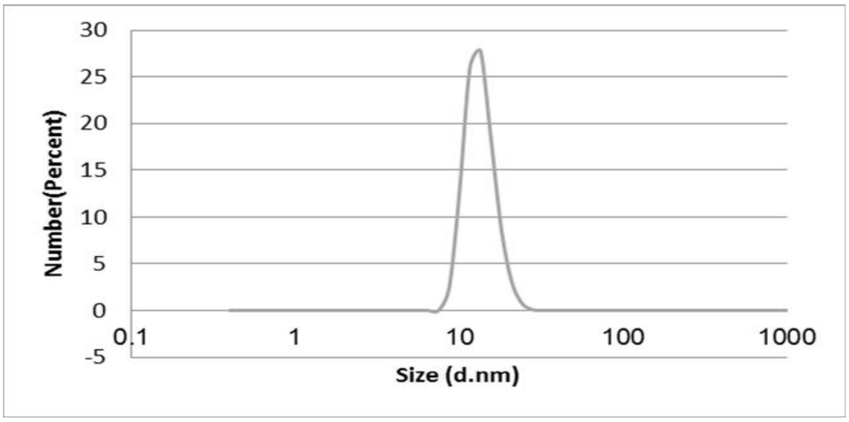

Fig. 4: Graph showing particle size distribution by number for synthesized nanoparticles that was obtained by particle size analyzer.

Vegetative growth parameters

Data presented in (Figure 5) demonstrated that application of magnetite nanoparticles at 250 ppm gave the highest significant values of plant height, leaf area per sapling and dry weight of sapling in both seasons; on the contrary, traditional chelate iron at 250 ppm gave the lowest values of plant height. Application of iron nanoparticles, regardless of the concentration, showed significant increase in leaf area in pear sapling in the second season, but the higher values obtained MNPs at 250 ppm. Sapling dry weight showed that application of magnetite nanoparticles at 25 and 125 ppm reduced dry weigh compared to control only in the first season. These results may be attributed to the function of iron element in cell metabolism and it is involved in photosynthesis due to enzymes activity**.** Researchers from their findings suggested both positive and negative effects on plant growth and development, and the impact of engineered nanoparticles (ENPs) on plants depends on the composition, concentration, size, and physical and chemical properties of ENPs as well as plant species (Siddiqui *et al.,*2015). In this respect, Abou El-Yazied *et al.* (2011) reported that the highest leaf area value and dry weight were obtained in tomato seedling under the application magnetic seed treatment and with magnetize water irrigation.

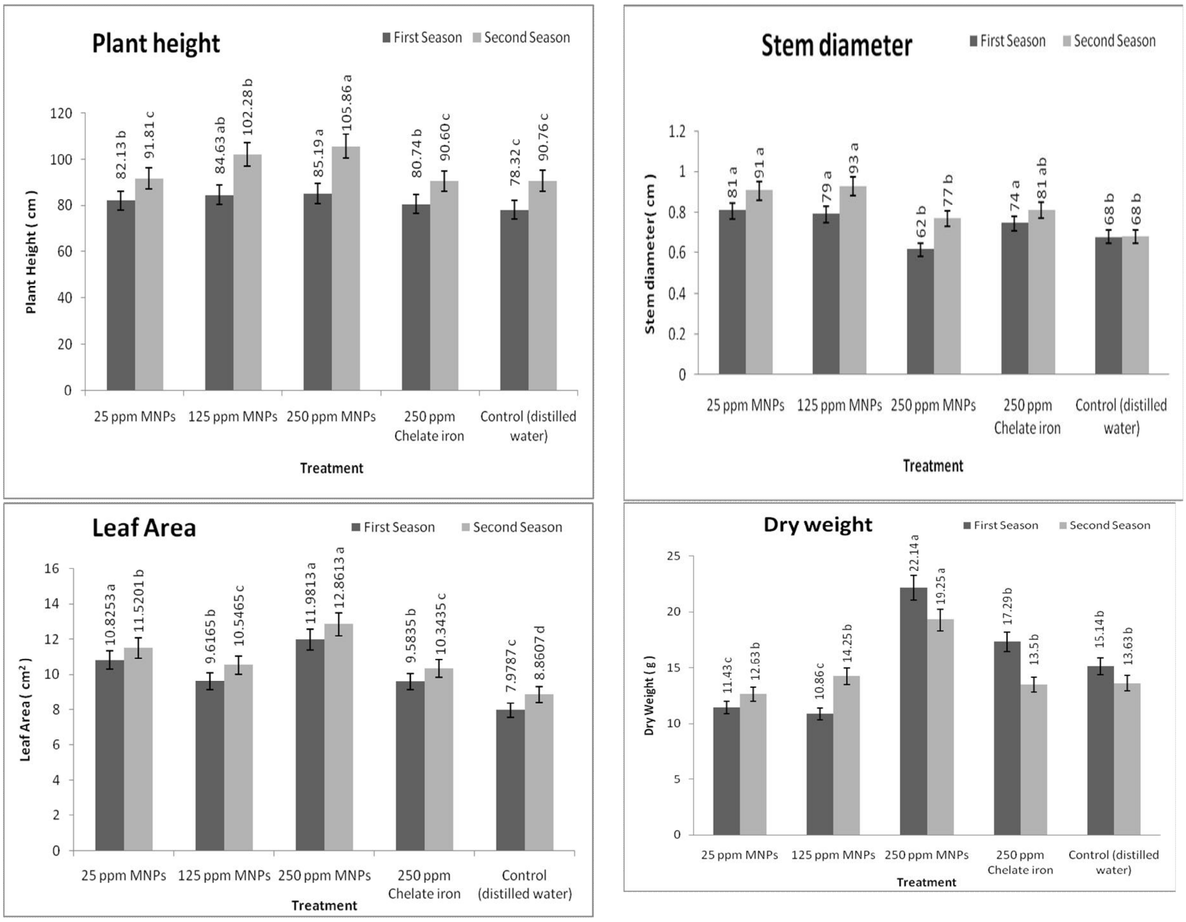

Fig. 5: Effect of foliar application of MNPs and chelated iron on some biomass parameters of pear saplings Le-Conte cultivar during the two seasons (2013 and 2014).

Leaf bio-chemical components

Pigments content

Data presented in (Figure 6) revealed that, foliar spray with magnetite nanoparticles significantly increased the values of pigments leaf content compared to foliar spray with iron chelated and control treatment. The content of chlorophyll-a and chlorophyll-b and total chlorophyll showed a significant increase by increasing MNPs concentration; whereas MNPs at 250 ppm was the most effective treatment in increasing chlorophyll content during both seasons. Carotenoids content showed that MNPs at 250 ppm was the most effective treatment in increasing carotenoids content during the second season. This increase may be due to the role of iron to encourage the activity of enzymes, needed for chlorophyll synthesis. Studies indicated that iron function through the installation of a specific type of RNA, which regulates the chlorophyll synthesis through a series of reactions that is not known yet (Jia *et al.,* 2012). Also, these finding may be due to the association of Fe with chlorophyll formation (Mazaherinia *et al.,* 2010).In this respect, Tomas *et al.,* (1998) reported that iron is a component of various enzymes and functions as a catalyst in the synthesis of chlorophyll; much of the iron in a plant is localized in the chloroplast.

Nitrogen and iron contents

An increase in nitrogen and iron leaf content was generally observed, due to foliar spray with magnetite nanoparticles treatments compared to control plants (Figure 7), magnetite nanoparticles at 125 and 250 ppm resulted in a maximum increase in nitrogen content in the leaves in both seasons compared to iron chelated and control treatments, where highest iron content was recorded in the first and the second season, respectively. Reasons for increasing nitrogen and iron contents may be due to the electromagnetic fields that modify the rate of ion transport across the plasma membrane or otherwise affect the structure of cell membrane lipid protein dynamics; this may cause the alteration in the permeability of the plasma membrane (Stange *et al.* 2002). As for the superiority of using magnetite nanoparticles on nitrogen and iron contents of pear saplings may be due to the main role of iron on vegetative growth as well as increasing chlorophyll content in the leaves.

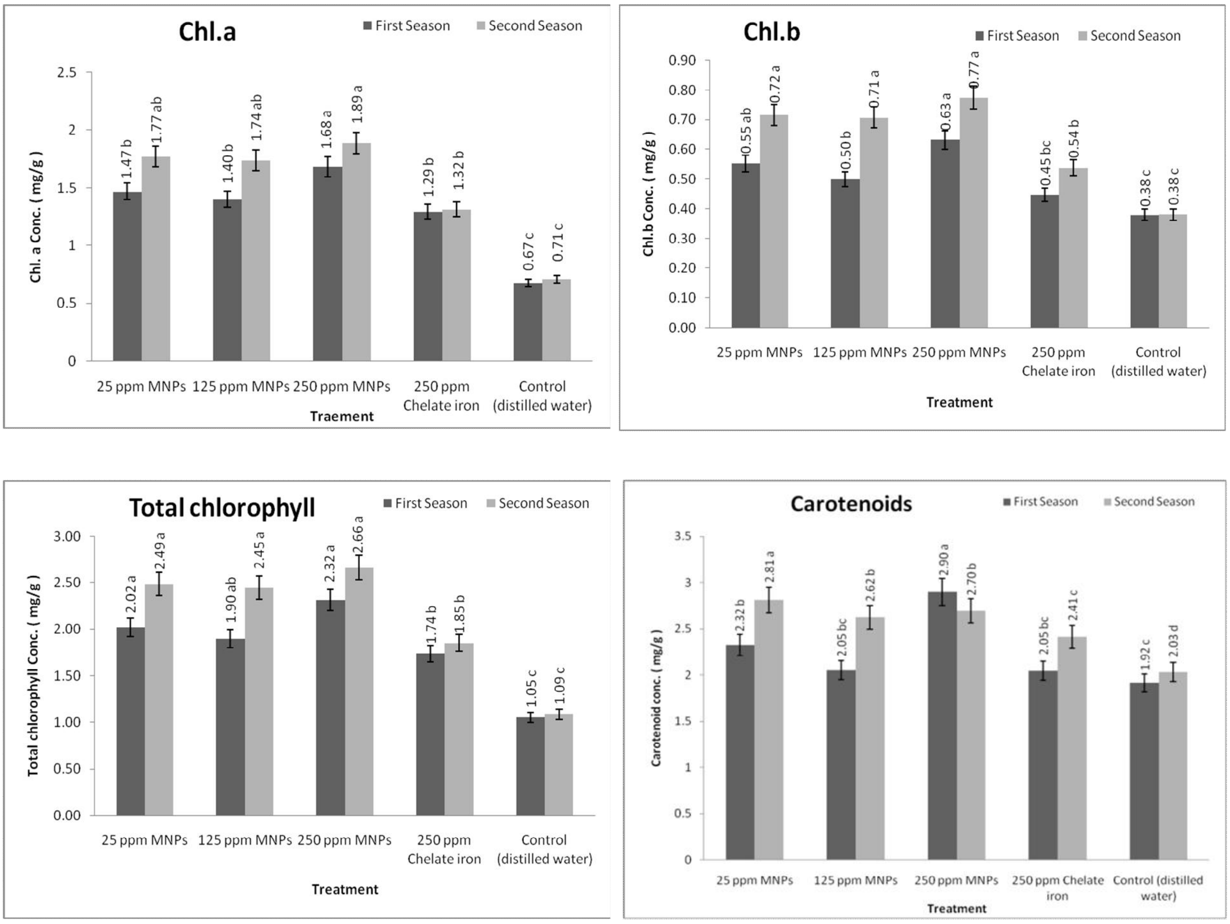

Fig. 6: Effect of foliar application of MNPs and chelated iron on pigments content of pear leaf saplings Le-Conte cultivar during the two seasons (2013 and 2014).

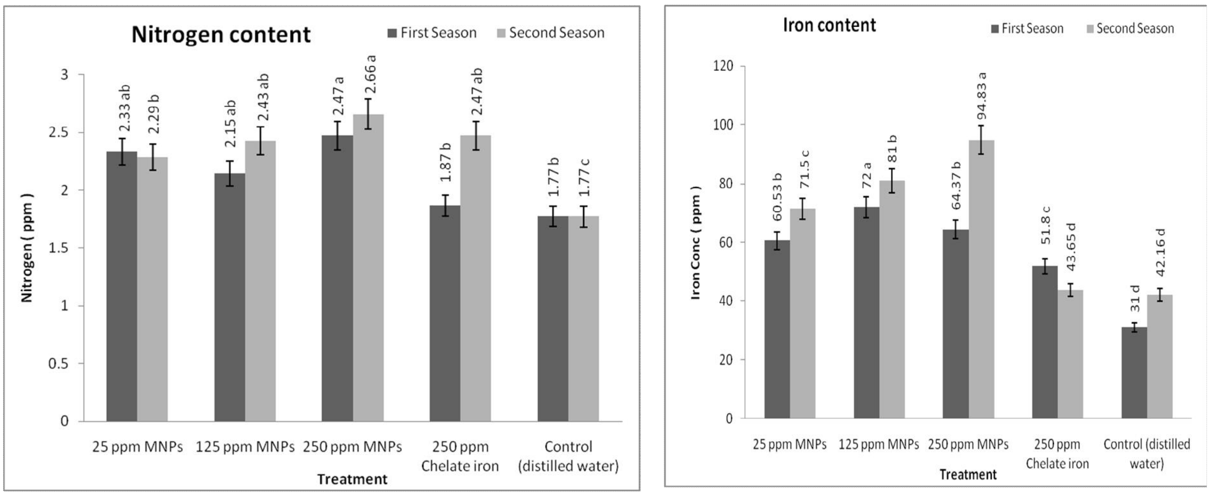

Fig. 7: Effect of foliar application of MNPs and chelated iron on nitrogen* and iron* contents in pear leaf saplings Le-Conte cultivar during the two seasons (2013 and 2014).
** Optimum nitrogen content of pear leaves (2.3-2.7 %) and iron content (60-100 ppm) according to Van den Ende and Leece, (1975).*

Total carbohydrates and amino acids content

Application of MNPs at 250 ppm increased leaf total carbohydrates percentage and amino acids content of pear saplings compared to other treatments during the two studied seasons (Figure 8). This may be due to NPs have unique physicochemical properties and the potential to boost plant metabolism (Giraldo *et al.* 2014) that positively affected metabolism and increased biosynthesis of carbohydrates and amino acids content.

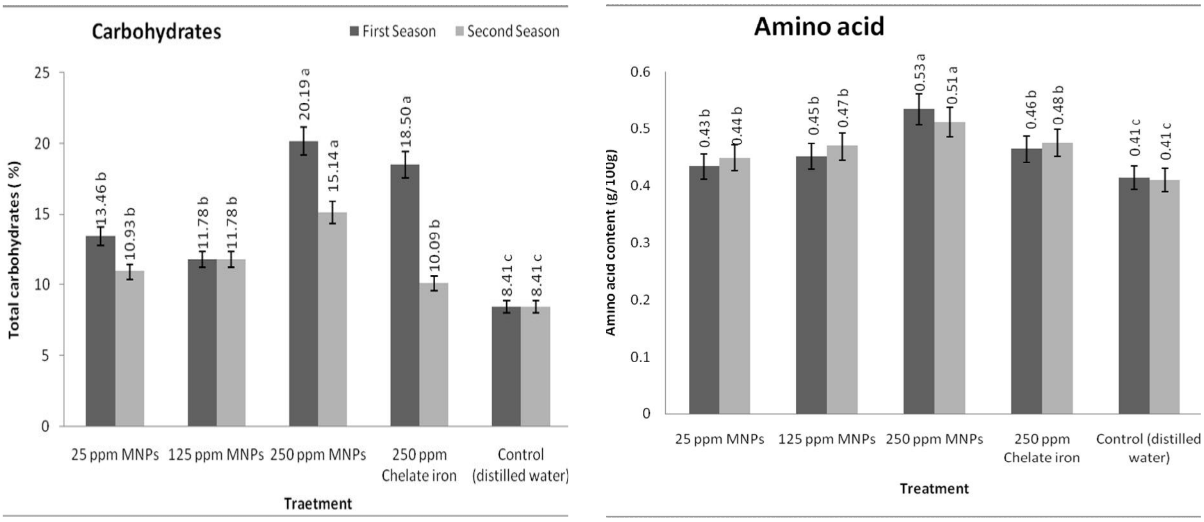

Fig. 8: Effect of foliar application of MNPs and chelated iron on total carbohydrates content and amino acids content in pear leaf saplings Le-Conte cultivar during the two seasons (2013 and 2014).

Correlation Analysis

The data in Figure (9) showed the linear relationship between leaf area, nitrogen content, iron content, total carbohydrates and chlorophyll content.

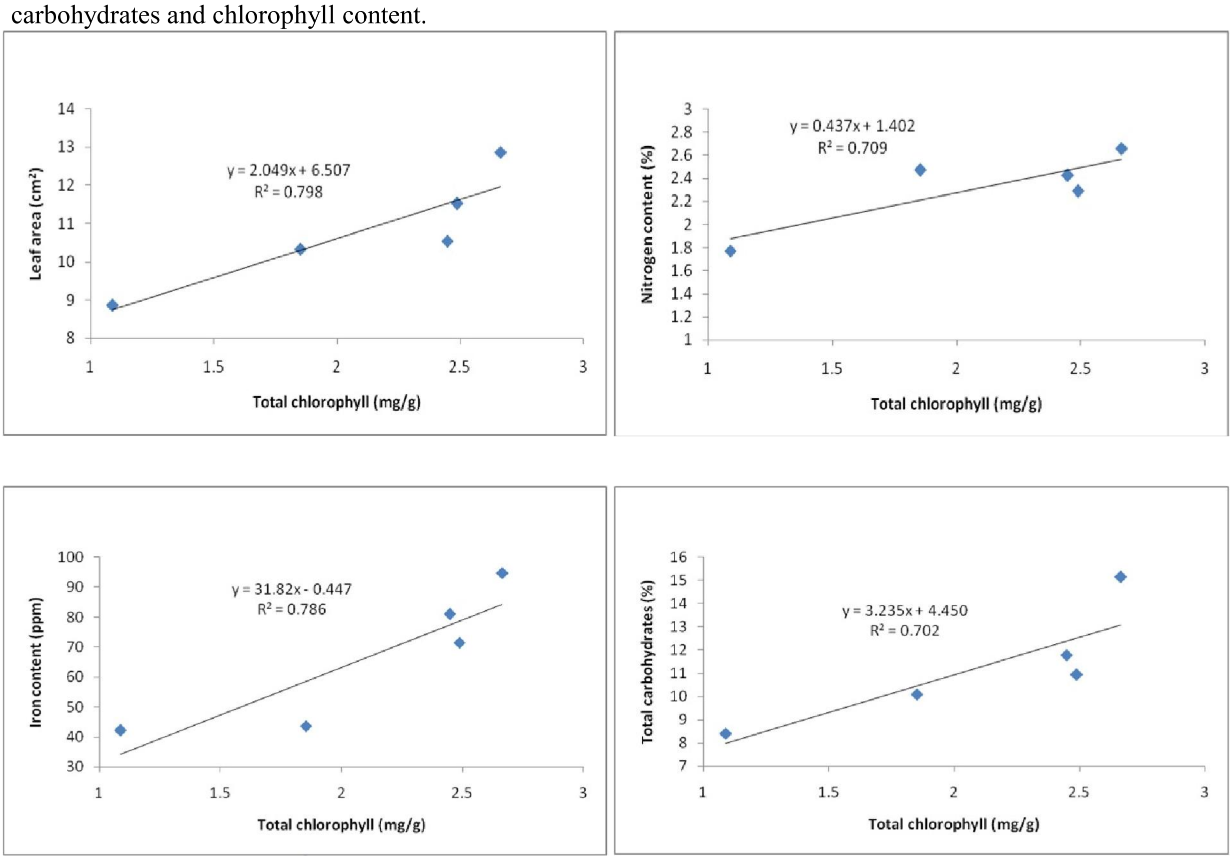

Fig. 9: Coefficients of correlation between the leaf area, nitrogen content, iron content, total carbohydrates and chlorophyll constituents of pear leaf saplings Le-Conte cultivar in 2014 seasons.

Table (1) revealed the correlation analysis between MNPs application and chemicals properties showed iron content with total chlorophyll and total carbohydrates have a positive and significant correlation. Also a positive correlation was obtained between nitrogen content and total amino acids and also between total carbohydrates and total amino acids.

Table 1: Correlation coefficients for effect of MNPs on studied chemicals properties of pear saplings.

	Total Chlorophyll	Nitrogen content	Iron content	Total carbohydrates	Total Amino acids
Total Chlorophyll	1				
Nitrogen content	0.840	1			
Iron content	0.887*	0.669	1		
Total carbohydrates	0.836	0.825	0.915*	1	
Total Amino acids	0.772	0.969**	0.689	0.898*	1

: Correlation significant at 5%;: Correlation significant at 1%*

Conclusion

Data of the current research showed a significant increase in iron content of pear sapling after foliar application of the MNPs even with a very low concentration at 25ppm. However, no significant effect was observed until the application rate reaches 250ppm. At this concentration plants showed a higher response to MNPs compared to the traditional chelate iron. The data demonstrated the effect of MNPs on different parameter of pear saplings might be due to the synergetic effect of nanoparticles as a nutrient supplement and its paramagnetite effect. Even though the nanotechnology has shown fast and noticeable results on plants, it still needs more detailed research**.**

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