International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Volume 3, Issue 3, March - 2016

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-3-29

Iron Oxide Nanoparticles Promotes Agronomic Traits of Ginger (*Zingiber officinale* Rosc.)

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Abstract

Iron is an element essential for plant growth and development. Iron is involved in chlorophyll formation its deficiency will cause a plant disorder known as chlorosis. Nanoparticles like iron oxide nanoparticles are being investigated as plant supplements for its promising targeted delivery approach. The experiment is designed in a hydroponic system where along with the Hoagland solution 100ppm concentration of iron oxide nanoparticles are added to evaluate whether it gives beneficial results when compared to EDTA chelated iron. The experimental data showed ginger roots absorbed iron oxide nanoparticles also showed an increase with respect to protein levels and iron content of rhizome. Iron oxide nanoparticles are an effective supplement for chlorosis.

Keywords: Iron oxide nanoparticles, ginger, fertilizer, chlorosis.

Introduction

Co- precipitation of iron salts in the presence of alkaline aqueous solution are used to synthesize iron oxide nanoparticles which are water soluble and is also the economically best way to synthesize iron oxide nanoparticles. The highlights of the synthesis are that nanoparticles are prepared at room temperature and no solvents used in this synthesis. The synthesis requires only less cost and they have great potential in agricultural application. The above synthesis doesn't require hazardous chemicals that are toxic to plant growth. Iron deficiency of plants will cause chlorosis and have further impact on animals through food chain. Iron is taken up by plants as ferrous ions serving as an activator for several essential biochemical processes like photosynthesis, respiration and symbiotic nitrogen fixation while iron deficiency can induce serious troubles in plant metabolism even with lethal consequences (Kirk and Allen, 1965). Researches on the effects of nano-ferric oxide on the ecosystem are of great importance (Kirk and Allen, 1965.) Iron chelate, fe-EDTA is absorbed and useable by plants however it depends on soil conditions particularly soil pH (Pozveh, *et al.,* 2014). With production of nano fertilizers, this nano compounds rapidly and completely absorbed by plants and fix its nutrients shortages and growing needs (Pozveh, *et al.,* 2014). To reduce the consumption of excessive fertilizers and frequency of application nano – fertilizers would be a great solution. Nano iron fertilizer in addition to cost-effectiveness cost much less than the imported fertilizers also reducing the harmful effects of chemical fertilizers such as FeSo4 on the environment. (Pozveh, *et al.,* 2014). Even though iron is present in large amounts in earth crust mostly it is insoluble and only iron in some forms can be utilized by plants. Exposure of seeds to 100 ppm iron oxide nanoparticles had the greatest germination rate (Hassan, *et al.,* 2013). Bioferrofluids can be

introduced into whole living plants, can travel using the vascular system and can be concentrated in specific areas by application of magnetic gradients (Gonza, *et al*., 2008). Plants grown with Fe3O4 or TiO2 nanoparticles, indicating that the particles did not pose any toxicological effects to the plants at the concentration level tested (Tommaso, *et al.*, 2012). Nanoparticles uptake by roots and translocation to aerial organs including leaves were reported in pumpkin (*Cucurbita maxima*), using hydroponic condition (Tommaso, *et al.*, 2012). The changes in the contents of chlorophyll A, chlorophyll B and carotene like pigments were evidenced by spectral measurements in an experimental investigation on sunflower seedling supplied with low concentration of magnetic nanoparticles (Manuela, *et al.*, 2011). In recent decades, Sala was one of the first who evidenced the effects of magnetic nanoparticles in plants, the increase of chlorophyll levels and photosynthesis rate in seven days old beans seedlings, following the addition of 0.1% magnetite based magnetic fluid in the culture medium (Sala, 1999). Antioxidase activity assay shows watermelon plants with 20 mg/L of nano-ferric oxide treatment has a good capacity of scavenging oxygen radicals. Watermelon plants treated with 20 mg/L of nano-ferric oxide have relatively lower (Malondialdehyde) MDA content, and relatively higher content of chlorophyll which indicates a better cell health condition. Superparamagnetic iron oxide nanoparticles were translocated into soybean which increased chlorophyll levels and significantly enhanced the chlorophyll content in subapical leaves (Mohammad, *et al*., 2013). A data suggest that foliar application of Fe during vegetative growth stages can maximize plant growth and development also significantly increases plant height, number of plants, flag leaf area and flag leaf chlorophyll content (Rawashdeh, and Sala, , 2014).

Materials and Methods

Rhizome seeds were acquired from Indian institute of species research, (IISR) Kerala. Carefully preserved seed rhizomes are cut into small pieces of 2.5-5.0 cm length weighing 20-25 g each having one or two good buds.

Chemicals:

All chemicals were purchased from Sigma-Aldrich and Hi media unless otherwise noted.

Preparation of iron oxide nanoparticles:

Coprecipitation method was used in the preparation of nano-ferric oxide according to the method followed by Zhitao *et al.*, 2014. 2 mM FeCl3.2H2O (96.0%), 1 mM of FeSO4.7H2O (99.0%) and 0.5 g of sodium citrate (99.0%) were thoroughly mixed and crushed by grinding in a ceramic mortar with 12 cm of diameter. Then, 0.32 g of NaOH (99.0%) was directly added with continuous grinding for 10 min. After the mixtures reacted completely, the product was washed with distilled water, and the unreacted starting materials were removed from the iron oxide nanoparticles by centrifugation (12000 rpm for 30 min) several times, and naturally dried at room temperature and in air atmosphere. The above procedure was slightly modified by further addition of 1mg ascorbic acid was mixed for 10ml of 1mg iron oxide nanoparticles solution. The mixture was stirred for 1hr at room temperature to obtain stable, ascorbic acid coated magnetite nanoparticles dispersion. (Figure 1)

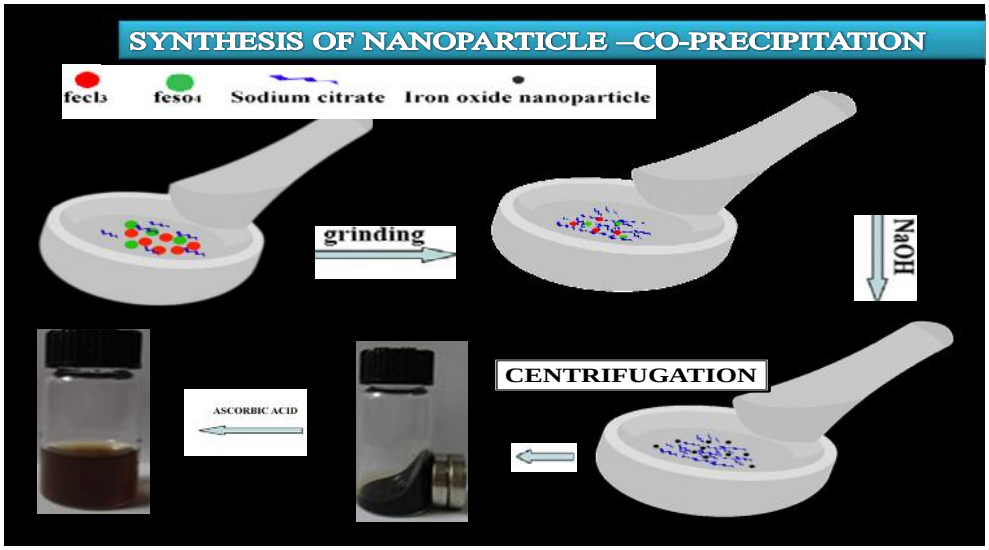

Figure 1: Ferric oxide nanoparticles synthesis.

SEM Analysis of Synthesized Iron Oxide Nanoparticles:

The powdered sample was analyzed for the structure and morphology of synthesized iron oxide nanoparticles using FESEM at different magnification levels. The FESEM image shows the distribution of individual iron oxide nanoparticles. From the FESEM image it is clear that the biosynthesized nanoparticles are spherical in morphology and the average size of iron oxide nanoparticles was analyzed using image J 1.50e software. (Figure 2)

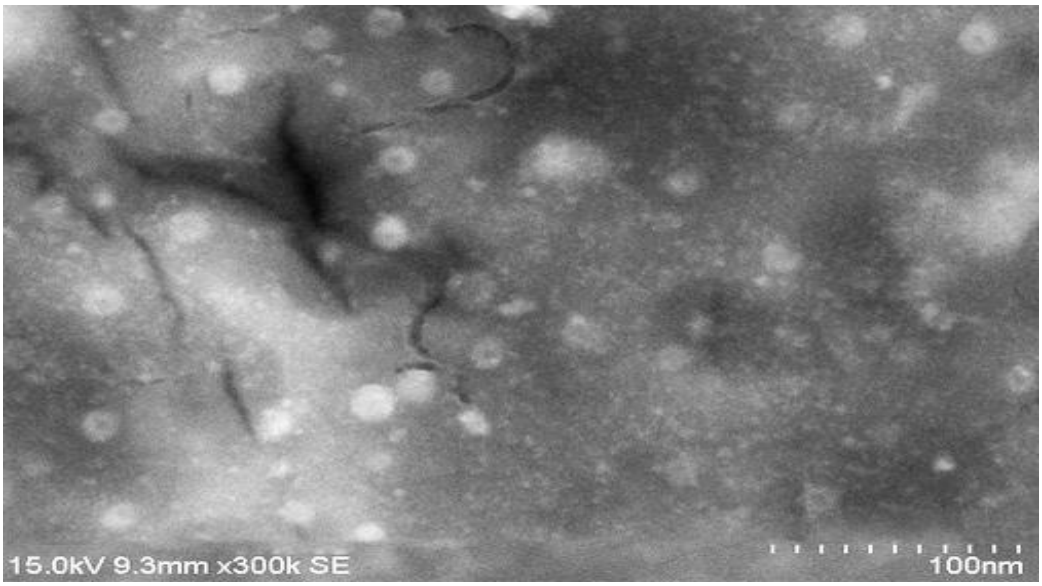

Figure 2: FESEM image of iron oxide nanoparticles.

Treatment of iron oxide nanoparticles to ginger plants:

Ginger rhizome seeds are chosen considering its economic importance for agriculture and food industry. Selected rhizomes were washed with running tap water and surface sterilized with savlon and sodium hypochloride (2%v/v). Then they were transplanted to perlite with Hoagland solution supplemented with 100 ppm iron oxide nanoparticles as one group and Hoagland nutrient solution with EDTA (Ethylenediaminetetraacetic acid) chelated ferric as another group and control supplemented with Hoagland solution without iron supplements for three weeks (Figure 3).

Figure 3 : Treatment of iron oxide nanoparticles to ginger plants in hydroponic system.

Estimation of protein:

Quantification of total protein was done using BCA (Bicinchoninic acid) protein assay kit following the manufacture's instruction using bovine serum albumin as standard protein. The reaction results in the development of an intense purple color and the tubes was incubated for 30 min at 37 °C and the absorbance measured at 562 nm. A set of protein standards ranging from 5ug/mL to 0.1ug/mL was prepared simply by diluting the standard stock containing 2mg/mL bovine serum albumin. A Standard curve is calibrated to determine the protein concentration of the rhizome samples. (Figure 4)

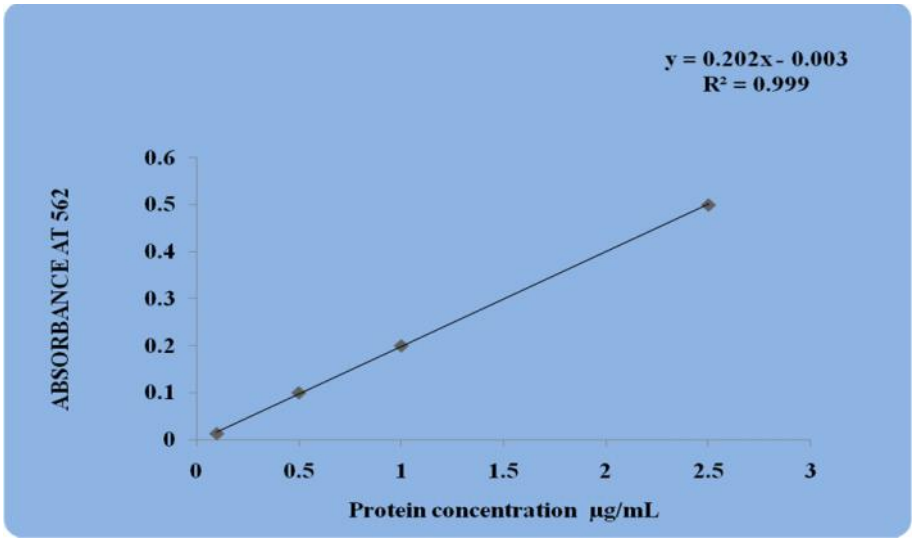

Figure 4 Calibration curve for BSA standards using BCA method.

Protein extraction:

Approximately 0.3 gram of fresh rhizome from four-week-old plants were homogenized in protein extraction was carried out according to the method of (Rostami and Ehsanpour, 2009) using extraction buffer (50 mM Tris-HCL, 1m M DTT, 2 mM EDTA, 2 mM 2-Mercaptoethanol, pH 7.5). SDS-PAGE was performed using 12% separating and 5% stacking gels (Laemmeli, 1970). After ele ctrophoresis at 100 V, protein bands were stained using CBB.

Plant height:

Plant height was significantly elevated by application of iron oxide nanoparticles for three weeks. The highest plant average height (13 cm) was achieved by treatment with 100ppm iron oxide nanoparticle. Fe plays a role in energy transfer within the plant, component of enzymes and proteins, involved in nitrogen fixation and enters in root cells, these reasons may be leads to an increase in plant height. (Abbas *et al*., 2009; Ali, 2012; Bameri et al., 2013) The lowest plant height of mean average (3cm) was recorded in plant groups without ferric supplement. In compared with iron oxide nanoparticles treated plants the Fe – EDTA treated plants groups showed less increase in plant height of (11 cm).

Number of leaves:

Application of nano-iron oxide for three weeks at the concentration of 100ppm caused an increase in number of leaves per plant with an average mean of (6 leaves) iron nano-particles causes increasing in pod and dry leaf weight and finally will increase total yield. (Ricardo, *et al.*, 2010). Whereas fe-Edta treated plant groups showed slightly lesser number of leaves with an average of about 4 leaves and control with no iron supplement showed only 2 leaves as average. They showed yellow chlorotic leaves when compared to other two groups. Fe is an important element in crops, because it is necessary for synthesize chlorophyll, keeps up the structure of chloroplasts, involved in nitrogen fixation which lead to higher crop production and leaf area increase (Pablo, *et al.*, 2014)

Measurement of total chlorophyll:

100 mg of fresh leaf material was taken and ground with help of pestle and mortar with 4ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes the supernatant was stored. The residues were re-extracted with 1ml of 80% acetone. The extract was utilized for chlorophyll estimation. Absorbance was read at 645 and 663 nm in the UV spectrophotometer (Arnon, 1949).

Total Chlorophyll (mg/g.fr.wt.) =

$$\frac{(20.2 \times \Delta A645 - 8.02 \times \Delta A663)}{1000 \times W} \times V$$

ΔA = Absorbance at respective wavelength
V = Volume of extract (ml)
W = Fresh weight of the sample (g)

Estimation of carotenoid content:

The carotenoid content of ginger leaves were determined by the method of Kirk and Allen, 1965. The extract that was used for the chlorophyll estimation was used for carotenoid estimation also. The same chlorophyll extract was measured at 480nm in UV-spectrophotometer to estimate the carotenoid content.

Carotenoid (µg/g.fr.wt) =
ΔA480 + (0.114 × ΔA663) – (0.638 × ΔA645)
ΔA = Absorbance at respective wavelength.

Total iron content:

Ginger rhizome is shade dried and powdered using a mechanical blender. Accurately weigh one grams and the total iron content was measured by wet-ashing method followed by ferrozine method for quantification of total iron in ppm present in the rhizome sample. (Min *et al*., 2008)

Results and Discussion

Protein variation is an essential part of plant response to stress as well as for adaptaion to environmental conditions (Vierstra, 1933; Hieng *et al.,* 2004). Proteins are final products of informational pathways in cells that produce in response to cellular needs and transfer to proper location in cells (Rostami and Ehsanpour, 2009). Soluble proteins were studied to measure the physiological effects of iron oxide nanoparticles, might have on ginger. Protein will lose its physiological activity when denatured and cellular aging. Protein content of (16.44%) were achieved in 0.04% Fe concentration and the lowest values were achieved in the control (Mitra, *et al.,* 2015). It is been found that application of iron fertilizer increased protein and Fe concentration in grains and straw of wheat and increased grain yield by 20%. The positive effect of spraying basil plants with iron nano fertilizer was also observed in the experiments of (Peyvandi *et al.,* 2010) and (Mitra, *et al.,* 2015) .The present study shows an increase of protein content in of rhizome with an average of 1.699 µg/ml in plant groups treated with iron oxide nanoparticles and comparatively lesser protein content in plant groups with fe –EDTA treated groups with 1.108 µg/ml as average and lesser content of protein as 0.208 µg/ml in plant groups with no ferric supplement added to their nutrient solution .

Exposure of ginger plants to iron oxide nanoparticles induces response at molecular level. Variation of protein pattern was visualized using SDS-PAGE. Some studies have demonstrated that, heavy metals such as Cd, Pb, Ni and Ag changed the total protein amount in plants (Rostami and Ehsanpour, 2009). This study showed slight increase in the amount of protein in 100ppm treated ginger plants and there is expression of some bands when is not present in control. When compared to Fe –EDTA treated plants the levels of expression of protein bands in iron oxide treated plants are more vivid indicating a positive response to iron oxide nanoparticles through the increase in the total protein amount in their rhizome (Figure 5)

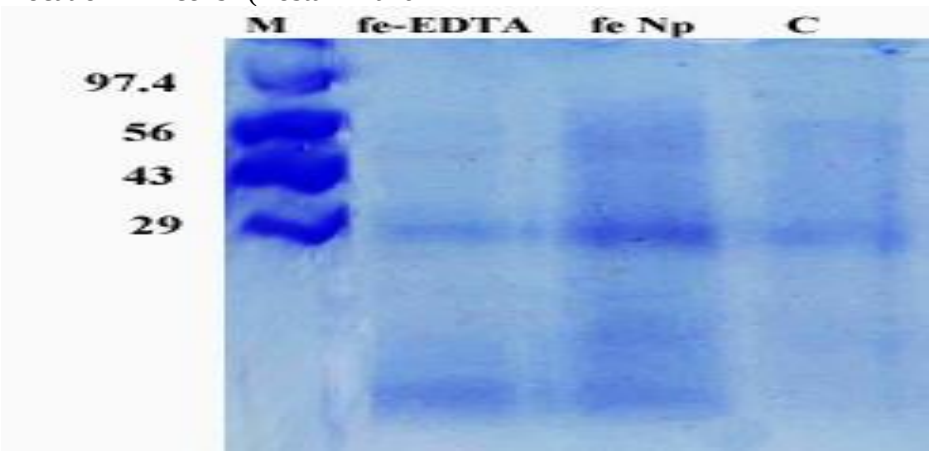

Figure 5: SDS-PAGE pattern of soluble proteins from the rhizone of fe –EDTA, iron oxide nanoparticles treated plants and control indicates the protein bands with changes of expression levels with the marker (M) in the first lane.

Under the treatment conditions the iron nanoparticles supplemented plant leaves shows the maximum total chlorophyll content (mg/g.fr.wt.) and carotenoid content (µg/g.fr.wt) was (1.605 and 1.36293) followed by those grown with fe –EDTA was (1.329 and 1.042) and the least for those growing without any iron supplement was (0.690 and 0.596) respectively (Table 1). This increase would undoutabaly helps to improve the photosynthetic efficiency. The applications of iron oxide nanoparticles may prove beneficial for improvement of growth and productivity of economically important crops.

Table 1: Total chlorophyll and carotenoid content of ginger leaves in different treatment conditions.

Treatment conditions	Total Chlorophyll (mg/g.fr.wt.)	Carotenoid content (µg/g.fr.wt)
Without Iron supplement	0.69 0	0.596588
Wth fe -EDTA	1.329	1.042834
Iron Nanoparticle supplement	1.605	1.36293

Each value is a mean of 5 replicates

According to the results of this study, after application of nano-iron oxide, iron content of rhizome was significantly increased to (3.77ppm) in comparison with fe -EDTA treated plants (2.80ppm) and control (2.50ppm) and Each value is a mean of three replicates (Figure 6).

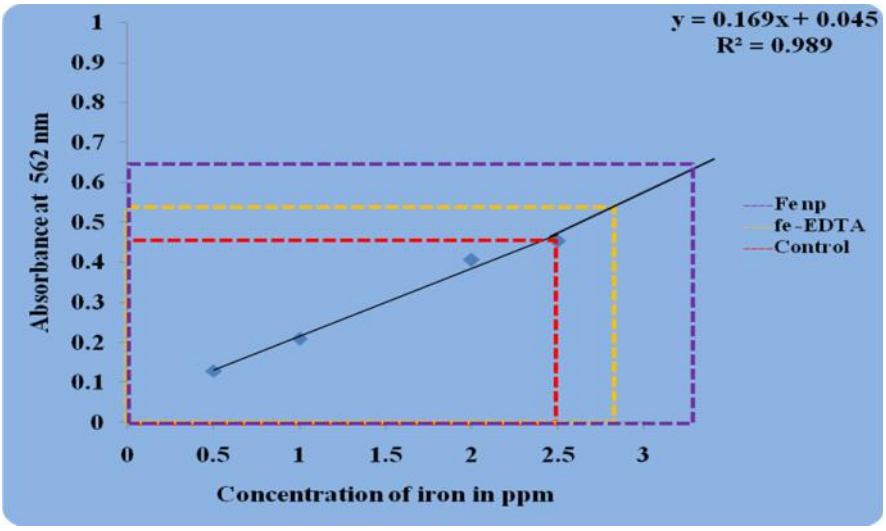

Figure 6: Concentration of iron in ppm from the rhizome treated with iron oxide nanoparticles, fe –EDTA and control plants.

This study was conducted to evaluate the effects and benefits of iron nanoparticles on plants growth and, the lack of information on this subject, this experiment was conducted to evaluate the effect of iron Nano-oxides on growth, yield, protein and iron content of ginger.

Because of small size (average size 20 - 30 nm) it can easily penetrate into the cell wall pores (1-20nm) also ascorbic acid coating acts as a antioxidant and a reducing agent.

From the above studies iron oxide nanoparticle is a potential fertilizer compared to fe –EDTA also showed a positive results to the below parameters: height, number of leaves, chlorophyll content, carotenoid content, protein content and iron content. It can yield efficient solution to chlorosis.

Acknowledgments

The authors are thankful to IISR (Indian Institute of Spices Research) for the supply of ginger rhizome seeds.

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How to cite this article:
Ganesan Vijaiyan Siva and Lazer Fany John Benita. (2016). Iron Oxide Nanoparticles Promotes Agronomic Traits of Ginger (*Zingiber officinale* Rosc.). Int. J. Adv. Res. Biol. Sci. 3(3): 230-237.