© 2016 IJSRST | Volume 2 | Issue 3 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Synthesis, Characterization of Iron Oxide Nanoparticles and Their Applications as Nano-fertilizers on Some Quality Characters of Ginger (*Zingiber officinale* Rosc.)

Ganesan Vijaiyan Siva*, Lazer Fany John Benita

Department of Biotechnology, University of Madras, Maraimalai (Guindy) Campus, Chennai, Tamil Nadu, India

ABSTRACT

Iron oxide nanoparticles were synthesized by co–precipitation method. FE-SEM revealed the morphology of synthesized iron oxide nanoparticles were spherical in shape with a size range of 20–30 nm, EDS confirms the elemental composition of ferric oxide nanoparticles. The synthesized nanoparticles were characterized using X-ray diffraction analysis which confirmed the crystal structure corresponding to magnetite (Fe3O4). FTIR reveals the functional group of active compounds present in the synthesized iron oxide nanoparticles. The shift in the Raman spectra testifies the presence of iron oxide nanoparticles. The iron oxide nanoparticles are given to ginger plants and their performance based on biochemical parameters showed promising response, indicating good cellular health of the plants when compared to iron chelated supplementation. The simplicity and versatility of the present approach using nano-fertilizers as plant supplements is a smart farming approach. Increasing the iron content in food may also help in biofortification as it enhances iron storage in edible plants therefore addresses the problem of iron-deficiency anemia, one of the most prevalent human micronutrient deficiencies globally.

Keywords: Nanobiotechnology, nanofertilizer, iron oxide nanoparticles and ginger.

I. INTRODUCTION

Nanobiotechnology was born as a hybrid discipline, a combination of biotechnology and nanoscience. It may results in efficient delivery, abundant production of nutritious food and can bring about a rapid and significant progress in the agricultural industry. Nanoparticles (NPs) have unique physicochemical properties high surface area, high reactivity, pore size, and particle morphology. Nanoparticles can serve as magic bullets, containing herbicides, nano-pesticide fertilizers, or genes, which target specific cellular organelles in plant to release their content (Siddiqui *et al.*, 2015). Iron oxide nanoparticles in crystalline or amorphous state find varied applications in the field of magnetic liquids, storage media, electronic drug delivery, biological separation, magnetic and catalysis. Biofortification of staple foods with iron is a promising approach to fighting iron-deficiency anemia in developing countries. Anemia is still one of the major public health challenges on a global scale with an estimated 2 billion people affected worldwide. One billion of these suffer from iron-deficiency anemia. Supplementation with pharmaceutical iron preparations, food fortification, and dietary diversification are possible strategies to combat iron deficiency (Gibson 1997).

Biofortification can reach all population groups, including people with low incomes (Hoppler *et al.*, 2008). Biofortification through iron oxide nanoparticles is definitely a promising strategy. Iron oxide nanoparticles are efficiently absorbed by the roots of the plants because of their small dimension in nanometer scale. By increasing the iron content in plants will definitely increase the iron storage proteins. Recovery of iron-chlorotic plants by application of iron of iron has been known for over a century (Seckbach 1969).

The use of nanoparticles for the targeted delivery of substances has been given special attention and is of particular interest in the treatment of plant diseases. Iron-carbon nanoparticles in plant cells have been analyzed in living plants of *Cucurbita pepo*. Nanoparticles were capable of penetrating living plant tissues and migrating to different regions of the plant, although movements over short distances seemed to be favoured.

Methodological improvements are required to make the system suitable for agronomical purposes, one of them being the functionalization of the nanoparticles with organic groups that can help their penetration into the phloem or make their internalization into cells more efficiently (Corredor *et al.,* 2009). The most important thing in preparation of iron nanoparticles is to make them water soluble so it is efficiently absorbed by plants. Nanoparticles are materials that are small enough to fall within the nanometric range, with at least one of their dimensions being less than a few hundred nanometres. This reduction in size brings about significant changes in their physical properties with respect to those observed in bulk materials. A number of microscopy tools to identify the tissue and subcellular location of nanoparticles in plants. These cover a range of reliable methodological approaches based on fluorescence, confocal, light and electron microscopy which can be selected by the research teams to achieve their scientific goals within their technical availabilities and skill (Gonzalezmelendi 2008).

In the last few years the unique properties of iron containing nanomaterials and derived nanoclusters are widely used in various areas (Zhu *et al.,* 2008). Small sizes of these particles (from 1 to 100 nm) and a large surface area determine specific physicochemical properties of nanoparticles that differ from properties of the respective macroscopic forms (Kane *et al.*, 2005 and Altavilla and Ciliberto 2011).

Influence ferric oxide nanoparticles on the content of photosynthetic pigments in *Triticum vulgare* were used to study seed germination, leaf elongation, and the content of photosynthetic pigments (chlorophylls a, b and carotenoids) as affected by five concentrations of iron containing nanoparticles. The parameters examined varied as a function of the exogenous agent applied, the agent concentration, and the exposure duration. Metal nanoparticles smaller than 10 nm are highly reactive owing to energy excess. It is known that metabolic permeation of nanoparticles into the plant cell is restricted by the diameter of cell wall pores from 5 to 20 nm (Fleischer *et al.,* 1999 (Rico *et al.,* 2011). However, the permeation of nanoparticles can also occur by means of endocytosis, transporter proteins, as well as through ion channels. After entering the cytoplasm, nanoparticles can bind to various organelles, thus altering physiological and intracellular plant functions (Da Silva *et al.,* 2006). Complex assessment of physiological parameters of *Triticum vulgare* seedlings grown in the presence of different forms of iron nanoparticles led us to conclude that the plant organism is an intricate flexible system readily reacting to the presence of iron nanoparticle in the medium (Lebedev *et al.,* 2014).

Therefore, the aim of this study was to prepare water soluble iron nanoparticles and to examine the influence of iron nanoparticles on the following parameters starch, photosynthetic pigments, phenolic and iron content help us to understand the health of the plant. Biochemical parameter gives us the understanding of plants response in relation to nanoparticles. These parameters help us to us to decide whether iron in nanoforms can improve the agronomic traits of the plants and whether its application could be a better alternate choice as fertilizers.

II. METHODS AND MATERIAL

Rhizome seeds were acquired from Indian institute of spices research, (IISR) Kerala.

Synthesis of water soluble iron oxide nanoparticles:

2 mM $FeCl_3.2H_2O$ (96.0%), 1mM of $FeSO_4.7H_2O$ (99.0%) and 0.5 g of sodium citrate (99.0%) were thoroughly mixed and crushed. 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 (Zhitao *et al.*, 2014). 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, water soluble nanoparticles dispersion.

Growing medium and nutrient solution preparation:

Perlite is mainly composed of minerals that are subjected to very high heat, which becomes very light weight, porous and absorbent and has a neutral pH. Hoagland solution is a hydroponic nutrient solution that was developed by Hoagland and Arnon in 1933.

The experiment was conducted in a non-circulating hydroponic method for cultivating edible ginger. Selected ginger rhizome seeds were washed with running tap water and surface sterilized with savlon and sodium hypochloride (2%v/v).

Treatment of iron oxide nanoparticles to ginger plants:

Then they were transplanted to perlite with Hoagland solution (Fig. 1). Three different solutions containing the complete combinations of macronutrients but presence of micronutrient iron and iron oxide nanoparticles supplement varies according to treatments. Plants are supplemented with Hoagland solution lacking iron as control group. Another group treated with Complete Hoagland solution with Fe –EDTA as iron source and Hoagland solution with iron oxide nanoparticles as other group.

Figure 1: Treatment of iron oxide nanoparticles to ginger plants in hydroponic system.

Characterization of Synthesized Iron Oxide Nanoparticles:

Field effect scanning electron microscope (FE-SEM) studies:

Morphology of iron oxide NPs were recorded on FE-SEM instrument (Hitachi model no: SU6600 Singapore).

The electron dispersive spectroscopy (EDS):

It is an analytical technique used for the elemental analysis or chemical characterization of a sample. EDAX HORIBA MODEL 8121–H displayed the elemental composition of synthesized nanoparticles.

X-ray Diffraction:

X-ray diffraction can be used to determine which iron oxide compounds are present in nanoparticles by calculating or comparing with the standard value of lattice parameters, crystal structures and crystallinity**.**

Fourier Transform Infrared (FTIR) analysis studies:

FTIR spectroscopy was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. FTIR analysis was conducted on a FTIR Spectrometer in the frequency range in the wave number range from 4000 to 400 cm-1. Spectroscopic grade potassium was used as background for IR analysis.

Raman Spectroscopy

Raman measurements were carried out using laser light excitation source using confocal Raman spectroscopy (Nanophoton Raman-11i). The Raman spectra of the samples were obtained in the spectral range from 1400 to 200 cm^{-1}**.** Raman microscopy was used to measure the Raman shift of synthesized iron nanoparticles.

Influence of Iron Oxide Nanoparticles on Various Biochemical Parameters of Ginger Plant:

Starch Analysis:

Starch preparation by acid hydrolysis method followed by Andreas Richter *et al.*, 2009 and starch concentration determined by Spectrophotometry.

Measurement of Total Chlorophyll:

The leaf samples were collected and absorbance was read at 645 and 663 nm in the UV spectrophotometer (Arnon, 1949).

Estimation of Carotenoid Content:

The carotenoid content of ginger leaves were determined by the method of Kirk and Allen, 1965.

Total Phenolic Determination:

Total phenolic content was determined using the Folin-Ciocalteu reagent and the absorbance was read at 765 nm using the method followed by Kaur and Mondall, 2014. Gallic acid (0–200 μg/mL) was used for calibration of standard curve. The total phenolic content was expressed as milligram gallic acid equivalent (mg GAE)/g dry weight of plant material.

Total iron Content:

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).

III. RESULT AND DISCUSSION

FESEM EDS, XRD, FT-IR and Raman analysis of ferric oxide nanoparticles

FESEM Studies:

It revealed that the synthesized nanoparticles possess almost spherical shape (Fig. 2). Magnified images of nanoparticles synthesized by co-precipitation process. The average size of iron oxide nanoparticles was analyzed using image J 1.50e software was found to be 20 - 30 nm.

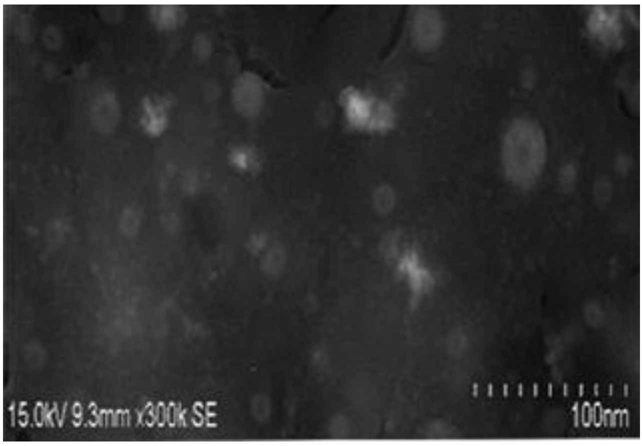

Figure 2: FE-SEM image of iron oxide nanoparticles.

EDS peak analysis:

The electron dispersive spectroscopy (EDS) analysis of the sample indicates the presence of Fe and O. No other peak indicating impurity has been detected (Fig. 3). This confirms the synthesized iron oxide nanoparticles are composed only with iron oxide nanoparticles (Table 1).

Table 1: Elemental compositions (weight% and atomic %).

Element	Weight%	Atomic%
Fe K	77.82	87.89
O K	22.18	12.11
Totals	100.00	

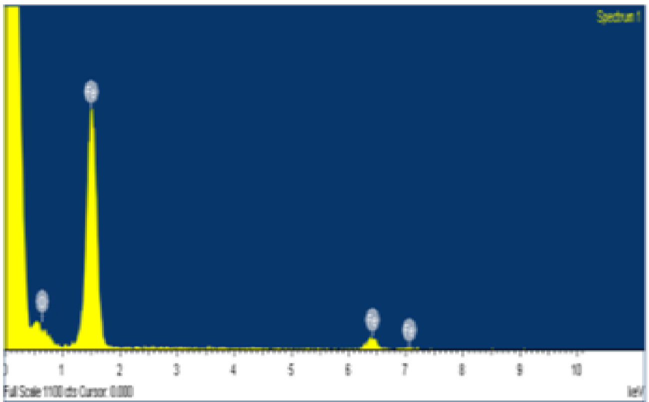

Figure 3: EDS outcomes from the selected area of FE-SEM image.

XRD Pattern Analysis:

Nanoparticles characterization by X-ray powder diffraction revealed the phase identification and crystalline structures of the nanoparticles synthesized by co-precipitation method. It was noted a strong diffraction peak with with 2θ values of 33.1°, 35.5°and 53°, corresponding to the crystal planes of (012), (014) and (110) of crystalline Fe_3O_4-NPs, respectively. The peaks matching with the XRD standard for the magnetite nanoparticles and the results found to be a cubic spinel (JCPDS file number 33-0664) (Fig. 4).

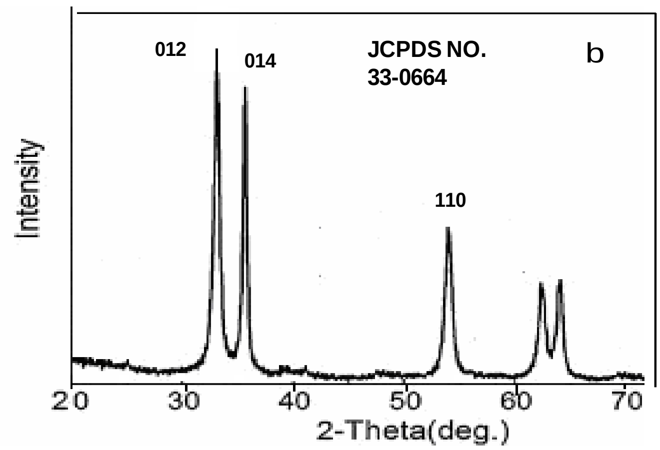

Figure 4: XRD patterns of synthesized iron oxide nanoparticles.

FT-IR spectral analysis:

The infrared portion of the electromagnetic spectrum is usually divided into three regions, near, mid and far infrared, named for their relation to the visible spectrum. The higher energy near IR, approximately 14000–4000 cm^{-1} displays bands at 1100, 1570, and 3422 cm^{-1} (Fig.5). The one at 1100 cm^{-1} was caused by stretching of the C-O group. Another band at 3422 cm^{-1} occurred, which may be attributed to the formation of new NH bonds. The absorption peaks are observed at around 1570 cm^{-1} are assigned to the C=O stretching vibration of the ionic carboxyl group (COO−).

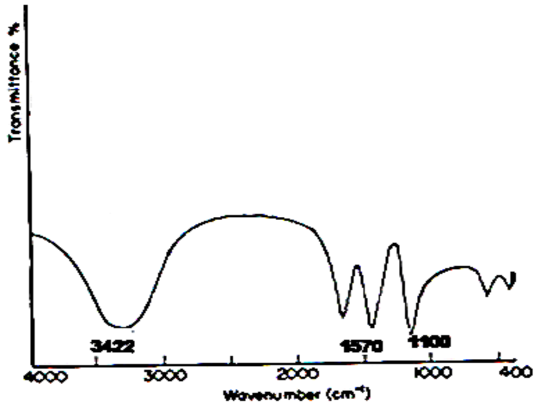

Figure 5: FT-IR spectra of iron oxide nanoparticles

Raman Studies:

The low laser power is used, to avoid sample degradation where main features of the wave-number are observed at about 200, 290, 400, 500, and 608 cm^{-1} for Fe-O stretching vibration. The majority of authors take as indicative the three broad maxima at around 360–380, 500 and 660– 720 cm^{-1}. In practice, these bands are most often accompanied by bands testifying to the presence of iron oxide or oxyhydroxide species (Fig. 6). The exact position of this band is difficult to establish from the literature, since it varies depending on sample preparation.

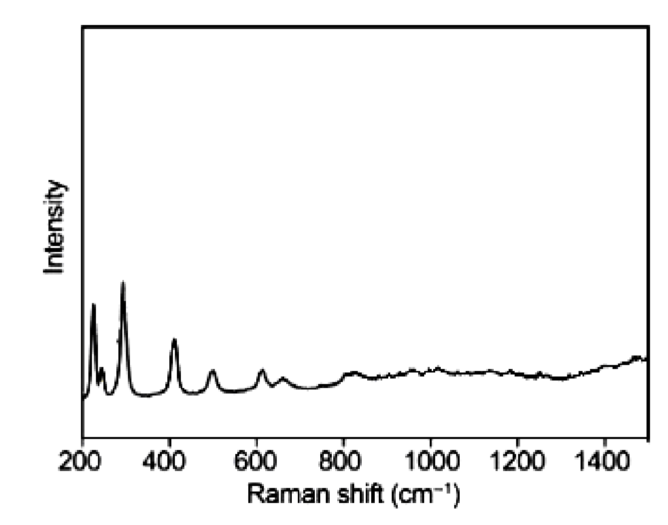

Figure 6: Raman spectra of ferric oxide nanoparticles

Analysis from biochemical studies:

Effect of iron oxide nanoparticles on ginger plants showed a positive response to starch content. According to the results of this study iron oxide treated plants showed a higher amount of starch comparind to other two groups (Fig. 7). There is an evident increase in chlorophyll and carotenoid content observed in iron oxide supplemented plant leaves (Fig.8&9). Control plants even showed chlorotic leaves indicating the lack of iron more essential in formation of photosynthetic pigments. The phenolic content is also high in iron oxide nanoparticles treated group than when compared to other treatment groups (Fig. 10). After application of nano-iron oxide, there is also significant increase in the iron content of rhizome in comparison to the rhizomes of Fe –EDTA and control (Fig. 11).

All results were presented as mean of the replicates ± standard deviations (SD). Statistical differences between the means were evaluated using t- test. Results were considered to be statistically significant at $p<0.05$.

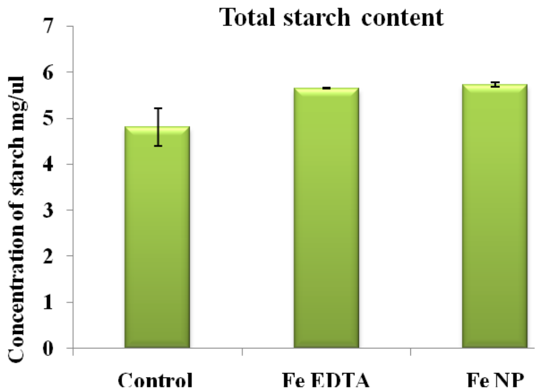

Figure 7: Concentration of starch in mg/ul from the rhizome of control, Fe –EDTA and iron oxide nanoparticles treated ginger plants.

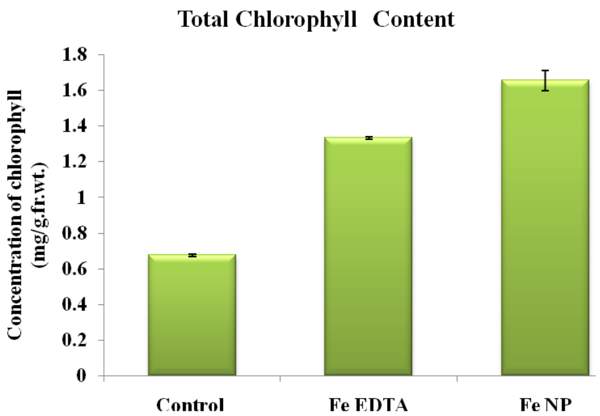

Figure 8: Concentration of chlorophyll in mg/gm from the leaves of control, Fe –EDTA and iron oxide nanoparticles treated ginger plants.

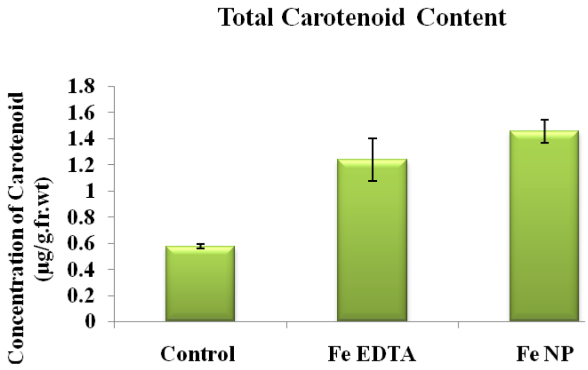

Figure 9: Concentration of carotenoid in ug/gm from the leaves of control, Fe –EDTA and iron oxide nanoparticles treated ginger plants.

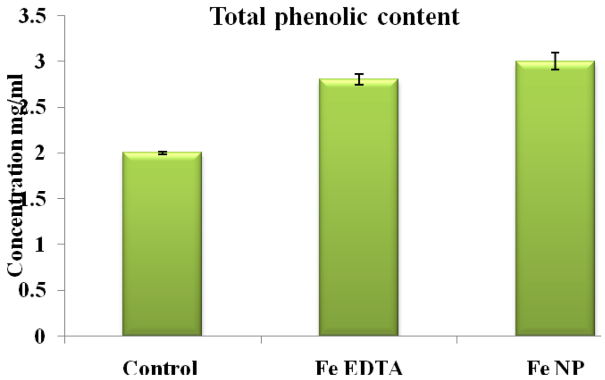

Figure 10: Concentration of total phenolic content in mg/ml from the leaves of control, Fe –EDTA and iron oxide nanoparticles treated ginger plants.

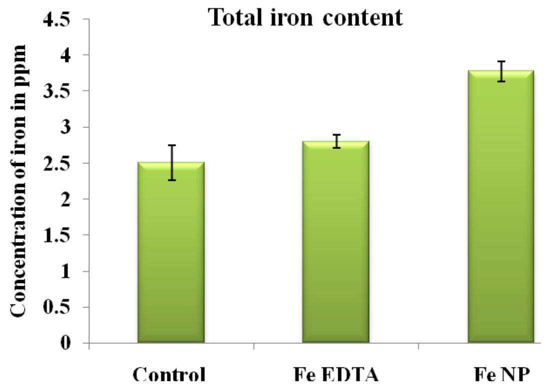

Figure 11: Concentration of iron in ppm from the rhizome of control, Fe –EDTA and iron oxide nanoparticles treated ginger plants.

IV. CONCLUSION

Ferric oxide nanoparticles had significant effects on the starch, total chlorophyll, carotenoid, phenolic and iron content of ginger plants. Iron oxide nanoparticles have shown improving agronomic traits of ginger, so implementation of iron nano-fertilizers have a greater scope and promises intelligent methods of application of fertilizers. The results of this work show strong evidence for the high efficiency of this new nanofertilizer on plant growth enhancement. These powerful and inexpensive nanoparticles could replace traditional methods of plant growth enhancement. But the effect of nanoparticles varies from plant to plant and depends on their mode of application, size, and concentrations.

The present work of nanoparticles to plants is in the beginning; more rigorous works are needed to understand physiological, biochemical, and molecular mechanisms of plants are essentially more important. Also, more studies are needed to explore the mode of action of NPs, their interaction with biomolecules, and their impact on the regulation of gene expressions in plants. (Siddiqui *et al*., 2015).

These powerful and inexpensive nanoparticles could replace traditional methods of plant growth enhancement. Further developments in nanotechnology in this sector could have large-scale economic implications and multiple benefits for consumers, producers, farmers, and the ecosystem.

V. ACKNOWLEDGMENT

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

VI. REFERENCES

[1] Altavilla, C., Cilibert, E. (2011). Inorganic nanoparticles: synthesis, applications, and perspectives. An overview, Inorganic Nanoparticles: Synthesis, Applications, and Perspective. CRC Press. Boca Raton (FL).

[2] Arnon, D.I (1949). Copper enzymes in isolated chloroplasts, polyphenol oxidase in beta vulgaris. Plant Physiol.2: 1-15.

[3] Corredor, E., Testillano, P.S., Coronado, MJ., González-Melendi, P., Fernandez-Pacheco, R., Marquina, C., Rubiales, D., PerezdeLuque, A., Risueno, M.C. (2009). Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. BMC plant biology.9:45

[4] DaSilva, L.C., Oliva, M.A., Azevedo, A.A., and de Araujo, J.M., (2006). Responses of restinga plant species to pollution from an iron pelletization factory, Water Air Soil Pollut., vol. 175:241–256.

[5] Fleischer, A., O'Neill, M.A., and Ehwald, R. (1999)The pore size of nongraminaceous plant cell wall is rapidly decreased by borate ester crosslinking of the pectic polysaccharide rhamnogalacturon .Plant Physiol. 121: 829–838.

[6] Gibson RS. (1997). Technological approaches to combatting iron deficiency. Eur J Clin Nutr. 51:25–7.

[7] Gonzalezmelendi, P., Fernandez-pacheco, R., Coronado, M. J., Corredor, E., Testillano,P. S., Risueno, M. C., Marquina, C., Ibarram, R., Rubiales, D., and Rezdeluquea,P.E., (2008). Nanoparticles as Smart Treatment-delivery Systems in Plants: Assessment of Different Techniques of Microscopy for their Visualization in Plant Tissues. Annals of Botany 101: 187–195.

[8] Hoppler, M., Schonbachler. M., Hurrell, RF., and Walczyk, T. (2008). Ferritin-iron is released during boiling and in vitro gastric digestion J. Nutr. 138: 878–884.

[9] Kanel, S.R., Manning, B., Charlet, L., and Choi, H., (2005) Removal of arsenic (III) from groundwater by nano scale zero valent iron, Environ. Sci. Technol.39: 1291–1298.

[10] Kirk, J.T.O., and Allen, R.L. (1965). Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. Biochem. Biophysics res. J. Canada.27: 523-530.

[11] Lebedev, S. V., Korotkova, A.M., Osipova E. (2014). A Influence of Fe0 nanoparticles, magnetite fe3o4 nanoparticles. Russian Journal of Plant Physiology 61: 603–608.

[12] Min, B., Nam, K.C., Cordray, J., Ahn, D.U.(2008). Endogenous factors affecting oxidative stability of beef loin, pork loin, and thigh meats .Journal of Food Science 73:439-446

[13] Richter, A., Wanek, W., Werner, R.A., Ghashghaie, J., Jaggi, M., Gessler, A., Brugnol, E., Hettmann, E., Gottlicher, SG., Salmon, Y., Bathellier, C., Kodama, N., Nogues, S., Soe, A ., Volders, F., Sorgel, K., Bloch, A., Siegwolf, R T., Buchmann, N., Gleixner, G. (2009). Preparation of starch and solu,ble sugars of plant material for the analysis of carbon isotope composition: a

comparison of methods Rapid Commun. Mass Spectrom. 23: 2476–2488

[14] Rico, C.M., Majumdar, S., DuarteGardea, M., PeraltaVidea, J.R., Gardeatorresdey, J.L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain, J. Agric. Food Chem., 59: 3485–3498. 13.

[15] Kaur S and Poonam Mondal (2014).Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants J Microbiol Exp 1(1): 00005.

[16] Seckbach, J (1969). Iron Content and Ferritin in Leaves of Iron Treated Xanthium pensylvanicum Plants .Plant Physiol. 44, 816-820

[17] Siddiqui. M.H., Al-whaibi, M.H., Mohammad, F. Nanotechnology and Plant Springer International Publishing xii ed. Switzerland.

[18] Zhitao, W., Lijun, Z., Ping, Y., Zhongwen, L.V.C., Huan, S., Qing, J. (2014). Water-soluble amorphous iron oxide nanoparticles synthesized by a quickly pestling and nontoxic method at room temperature as MRI contrast agents a Chemical Engineering Journal. 235: 231–235

[19] Zhu, H., Han, J., Xiao, J.Q., and Jin, Y. (2008). Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants, J. Environ. Monit., no. 10: 713–717.