BIOGENIC SYNTHESIZED Fe_3O_4 NANOPARTICLES AFFECT ON GROWTH PARAMETER OF MAIZE (ZEA MAYS L.)

N. JAYARAMBABU[a], K. V. RAO[a,] , S.H. PARK[b], V. RAJENDAR[b*]
[a]*Centre for Nano Science and Technology, Jawaharlal Nehru Technological University Hyderabad, Telangana, India*
[b]*Department of Electronisc Engineering, College of Engineering, Yeungnam University, Republic Korea*

In the present research manuscript investigation, nanoscale iron oxide nanoparticles were prepared using biocombustion method. The biogenic iron oxide nanoparticles (Fe_3O_4 NPs) are developed as a novel method for application in agriculture field. However, its uptake and translocation require further study prior to large – scale field application. The synthesized Fe_3O_4 NPs (mean size 21nm) were characterized using techniques like, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and particle size analyzer. The aim of this investigation was to determine the effect of biogenic iron oxide nano particles (Fe_3O_4 NPs) on yield and quality traits of maize. The crop growth of maize and translocation of iron were measured to test the effect of Fe_3O_4 NPs on yield of maize. The results showed that pretreatment sowing of maize with Fe_3O_4 NPs at 0.3 mg concentration enhanced 20% germination and improved yield by 20% and 10% yield over control. Similarly, compared to control, Fe_3O_4 NPs pretreatment sowing caused an increase by 20% in cob weight, 10% in average tassel length and 15 % in the average plant height. Therefore, the present study showed that Fe_3O_4 NPs at 0.3 mg results in an enhanced yield, seed quality traits of maize under field conditions.

(Received June 19, 2018; Accepted October 2, 2018)

Keywords: Fe_3O_4 NPs, Biocombustion method, Characterization, Maize germination, Yield

1. Introduction

Nanotechnology has numerous applications in other sciences like medicines, pharmaceuticals and their effect is still at its initial stages in agricultural field in India. The nano scale particles (size 1-100 nm) less than one dimension pose beneficial applications [1]. Nano technology based fertilizers are promising to promote sustainable development of agriculture. Nano particles play an important role because of its solubility, stability and degradability in the environment and the effectiveness of binding firmly to the plant surfaces and reduced deposition of agrochemicals by preventing runoff [2]. Nps can be effectively used in plant growth, seed germination [3] also as regulators of seed germination, have the ability to penetrate, regulate cell division and plant growth by a unique molecular mechanism that is related to the extract of water channels, major gene regulators of cell division, extension [4]. The biogenic iron oxide nanoparticles (Fe3O4 NPs) are developed using a novel and promising method of application in agriculture field and because of its unique properties, being super paramagnetic, biocompatible [5], biodegradable and non toxic to humans. The iron is an important transitional metal and cofactor in the synthesis of several enzymes essential for the synthesis of ethylene and absicic acid [6]. In many crops Fe_2O_3 NPs can physiologically enhance seed germination, root growth, chlorophyll content and induced oxidative stress in certain plants viz., water melon [7] and mungbean [8] and increased root length in soybean [2] and rice [3] in the presence of Fe2O3 NPs. However, NPs

[*]Corresponding author: rajendar.nano@gmail.com

impact on germination, antioxidant enzyme activity malondialdehyde (MDA) content in corn roots was earlier reported by many workers. Of late a simple rapid green method was introduced to synthesize magnetic Fe2O3 NP [9] and their impact on seed germination, vigour and seed health was assessed [10]. Maize crop is more prone to biotic and abiotic factors when compared to other cereals and in addition it is one of the yield potential crops. The yield potential and nutrient status of maize is depleting due to severe biotic and abiotic stress resulting in reduction of chlorophyll and lower net photosynthesis. Iron deficiency usually causes chlorotic or yellowed interveinal areas and crop failure sometimes [11]. To overcome the above findings, a new iron delivery method, Fe_3O_4 NPs have been used in the present study. Hence the present study was carried out to study the Fe_3O_4 NPs uptake and accumulation of the same in the plant tissues when applied to seed as pretreatment sowing.

2. Materials and methods

The green method was used in the preparation of Fe_3O_4 NPs. The 0.2M of ferric chloride was dissolved in 100ml distilled water for 30 minutes. The 20 grams of fresh neem laves washed several times with tap water to remove adherent materials. The washed fresh neem leaves were boiled with 50ml distilled water at 80^0C for 40minutes. The supernatant was filtered with the help of whatman filter paper. The neem aqueous extract was added to above precursor solution and stirred for 4 hours. Then transfer to hotplate for biocombustion process.

2.1 Maize seeds procurement

The maize seeds (DHM: 117) were procured from Prof Jayashankar agriculture university rajendranagar, Hyderabad and were used without any modifications.

2.2 Seed treatment with Fe_3O_4 NPs:

Maize seeds of 20 g were treated with synthesized Fe_3O_4 NPs at different concentrations viz., 0.1 mg, 0.2mg, 0.3mg and 0.4 mg. The untreated seed was used as control. The treated seeds were then shade dried for further use. The pre treatment seeds were sown under field conditions in a randomized block design with three replications in plot size of 3X3 m with spacing of 60X20 cm. The experiment was carried out in kharif season with variety DHM 117 and followed the package of practices for the crop till harvest.

2.3 Germination and seedling vigour test:

The Fe_3O_4 NPs treated maize seeds were assessed for both tests after harvest and before harvest.

2.4 Germination and seedling vigour test:

The required Fe3O4 nano particles were synthesized using neem leaf extract and confirmed for seed treatment. The results from the table revealed that (Fig -8) the percentage of maize seed germination in 0.3 mg treated plot was 85% over control and maximum root length (11.47 cm) and seedling vigour index (2043) was also found to be maximum with 0.3 mg of Fe_3O_4 NPs over other concentrations of 0.1, 0.2 and 0.4 mg. The results might be attributed to the increase in intercellular spaces and chlorophyll content. The earlier study in maize treated with MgO NPs were in coordination with the present study [12] and nano silica in maize [13]. The SEM images of treated maize seeds evidenced the changes in the treated seed representing that the iron enriched particles in transverse section of treated roots compared to control. It is also observed that up rooted maize seedlings one month after sowing were also found to be well equipped with enriched iron content which was evidenced with the enhanced yield. However, the biological indicator of the toxicity or stress in plants such as reduction of chlorophyll pigment and inhibition of plant growth were not found during growth period with Fe_3O_4 NPs treatment even at different concentrations. Similar results of negative impact were not found in the plants with preharvest seeds [14].

2.5 Maize field experiment study

It is well understood that germination process improves the nutritional value and reduces anti nutritional factors of maize seeds compared with control whether Fe_3O_4 NPs pretreatment can enhance the quality and yield of maize is unknown. This study indicates that Fe_3O_4 NPs can enhance the yield and quality of maize. Results of this work may be useful for promoting the quality and maintenance of maize in practical at a concentration of 0.3mg.

2.6 Physiological parameters of maize influenced by Fe_3O_4 NPs:

The 30 days germinated seeds study examined the physiological parameters of maize influenced by Fe_3O_4 NPs. The total height plant increased for 0.3mg concentration when compared to control and remaining concentrations. Then after 90 days plant height and tassel height was increased for 0.3mg concentration when compared to the other concentrations and control. The maize leaf length, the average cob weight, cob rows, seed weight, total yield of maize seeds was enhanced at 0.3mg concentration when compared to the other concentrations and control.

3. Results and discussion

3.1 XRD analysis

The crystallinity of the Biogenic Fe_3O_4 NPs was determined with XRD using a Bruker D8 advance X-rays Diffractometer instrument with a Cu Kœ (k=1.54 A^o) source (voltage 40kV, current 30mA). The 0.1g of the calcined dried particles were deposited as a randomly oriented powder on the plexi glass sample container, and the XRD patterns were recorded at angle between 20^o to 70^o , with scan rate of 1/min. The XRD is an effective technique to identify the phase and to confirm the crystal structure of the biogenic synthesized Fe_3O_4 NPs [15-16] The XRD patterns obtained for the Fe_3O_4 NPs synthesized from using the neem leaves extract of *Azadaricta indica* is shown in figure8. The XRD patterns displayed six characteristic 2 Theta peaks at 34.6^o, 35.4^o, 50.9^o, 54.8 ^{o}, 63.6^o and 64.6^o marked with their indices (420), (216), (436), (034) (800) and (821) respectively. They are quite identical to the peaks of the Fe_3O_4 NPs with the orthorhombic structure. The results are in agreement with the standard XRD patterns of Fe_3O_4 NPs (JCPDS: 76-0958). The average particles size of the green synthesized Fe_3O_4 NPs was calculated using Debye-Scherrer's formula. The Fe_3O_4 NPs size was determined by taking the average of the sizes of the peaks and it was found to be 25.6nm shows in the (fig 1).

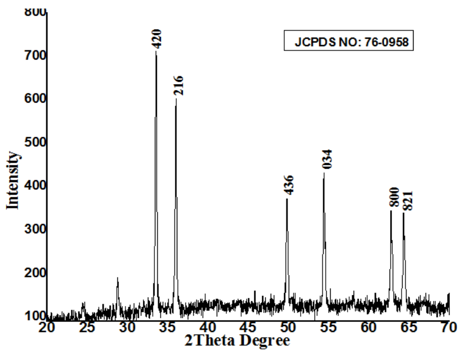


Fig. 1. XRD pattern of Fe_3O_4

3.2 FT-IR spectra analysis

FT-IR analysis was used to characterize the resulting biogenic Fe_3O_4 NPs shown (fig.2). Peaks at 3421.1cm-1 is assigned to the OH stretching of alcohol and phenol [17-18]. Signals at 2921.2 and 2851.5cm-1 indicate the presence of CH stretching groups. The absorption peak at 1743.3 cm-1 may be assigned to the amide band of proteins from carbonyl stretching in proteins. The peak at 1433.8cm-1 is attributed to the presence of carboylate ions (COO-), which is responsible for the formation of iron oxide NPs. The peaks at 1068.8 cm-1 indicate the presence of

CO groups. The strong broad peaks at about 864.9 cm-1 and 664.5cm-1 are due to the stretching vibrations of Fe-O bonds. Overall, the FT-IR spectra of synthesized Fe_3O_4 NPs showed that the bioactive molecule containing O-H, C=C, C-O groups presented in the plant extract, which act as capping agents in the synthesized biogenic Fe_3O_4 NPs.

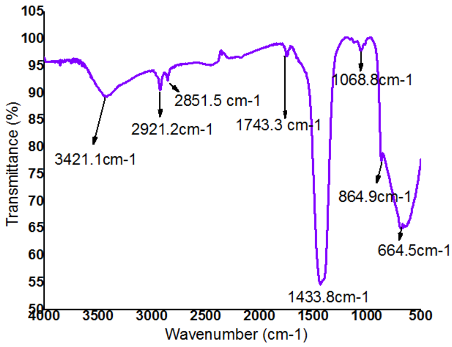
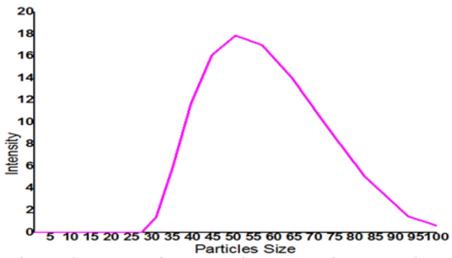


Fig. 2. FTIR pattern of Fe_3O_4

3.3 Particle size analysis

The average size of the particles, size distribution and polydispersity index (PDI) of the green synthesized Fe_3O_4 NPs were determined with Dynamic light scatter and the results are shown in (fig. 3) that revealed the particle size distribution of Fe_3O_4 NPs ranging approximately from 5 to 500nm with mean particle size of 40.4nm. The polydispersity index was found to be 0.340. The results show that the distribution of Fe_3O_4 NPs is more uniform with a narrow distribution range [19].



Peak No.	S.P.Area Ratio	Mean	S.O	Mode
1	1.00	52.4 nm	181.7 nm	9.7 nm
Total	1.00	52.4 nm	181.7nm	9.7 nm

Fig. 3. PSA pattern of Fe_3O_4

3.4 Zeta potential analysis

The surface zeta potential measurement was investigating the surface charge of the synthesized nanoparticles. The zeta potential of the Fe_3O_4 NPs was determined in ethanol as dispersant. Shown in the (fig.4) the zeta potential was found to be -42.6mV the particles contain good stability.

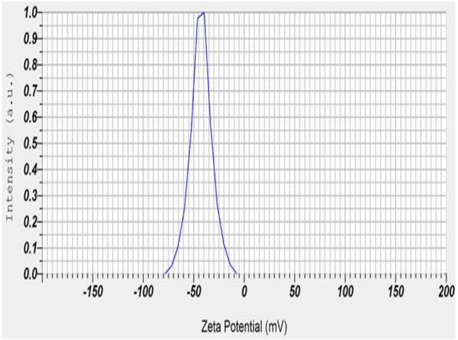


Fig. 4. Zeta potential of Fe_3O_4

3.5 SEM, EDX and TEM analysis

The SEM image (Fig. 6) showed that synthesized Fe_3O_4 NPs were spherical and highly uniform in size. The Fe_3O_4 NPs were capped with the bioactive compounds present in the neem leaves extract, that indicating the stabilization of Fe_3O_4 NPs. Therefore the biogenic Fe_3O_4 NPs were successfully synthesized by green method using neem leaves extract as a reducing agent and stabilizer for Fe_3O_4 NPs. The energy X- ray analysis (EDX) (Fig. 5) revealed the binding energies of the iron and confirmed the formation of Fe_3O_4 NPs [20-22]. TEM (Fig. 7) images of Fe_3O_4 NPs were approximately spherical with a mean size of 50nm and showed the Fe_3O_4 NPs at high magnifications.

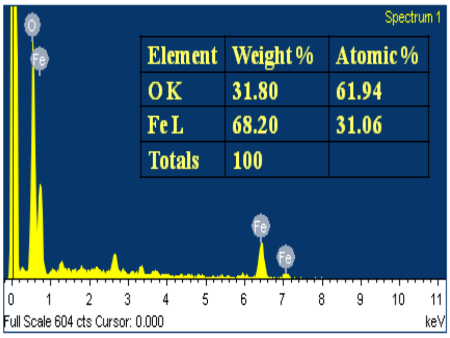


Fig. 5. EDAX of Fe_3O_4

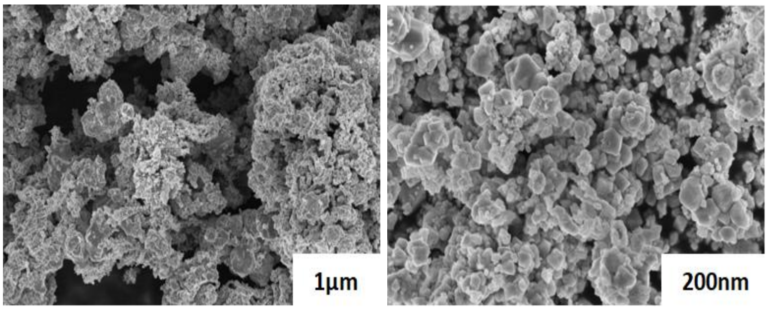


Fig. 6. FESEM images of Fe_3O_4

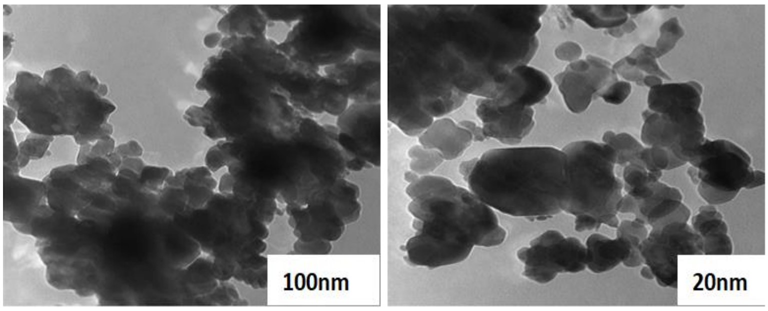


Fig. 7. TEM images of Fe_3O_4

3.6 Yield of maize as influenced by Fe_3O_4 NPs

The present study indicated that Fe_3O_4 NPs had focused impact on growth and yield of maize. The maximum yield was found with the application of Fe_3O_4 NPs at 0.3 mg which was superior when compared to control (15%). Per cent increase over control was not yielded much with other concentrations. However, they are found to be superior over control. The same concentration was also found to enhance the number of rows (16.9) per one cob in the treatment 0.3 mg which was superior over control (14.6). The average length of the cob ranged from 6.8 to 8.7 cm and the average cob length of the untreated plot was found to be minimum (6.8 cm) as against 0.3 mg (8.7 cm). The maize cob weight 0.208 g in 0.3 mg treated seed as against 0.168 in control. Song Z et al. [23] reported the yield increase of wheat with TiO_2 representing the positive effects of the TiO_2 NPs even under water deficit conditions. Srivastava G et al [24] reported that treatment effect might be attributed to attractive increase in the amount of chlorophyll content in the leaves of maize crop. There by increasing the photosynthetic efficiency of plants resulting in maximum yields [25-26]. Like the average cobs per plant the cob weight was comparatively lesser in other treatments. The average cob weight was recorded maximum in the treatment with 0.3 mg and the increase more pronounced effect than the weight of the cob in control and other treatments. Like the average cobs per plant the cob weight was comparatively lesser in other treatments. Likewise, the number of grains per one row was 38.4 with 0.3 mg of Fe_3O_4 NPs and higher that the control [27-28] and other treatments. The decline in the number of grains per cob was noticed with the treatment Fe_3O_4 NPs at 0.3 mg (38.4) followed by 0.4 concentration (37.4) and the same treatments recorded maximum test weight of 35.2 g over 0.2 and 0.4 mg. The percent increase in the valuable treatment for the cob rows, cob seed rows and average cob length over control was 15.7, 23.8 and 27.9, respectively. Similar results of increase in cob length, cob weight, length of the cob and grains per cob were reported by their studies on the effect of Silica NPs in maize.

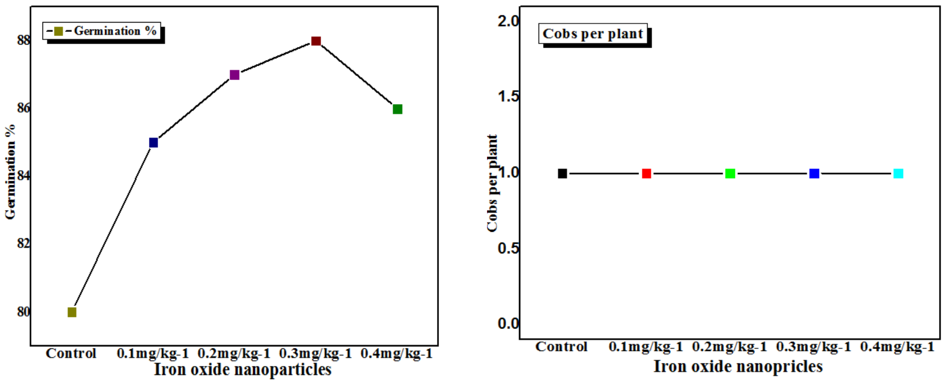


Fig. 8. Fe_3O_4 NPs treated Maize seed germination, cobs per plant

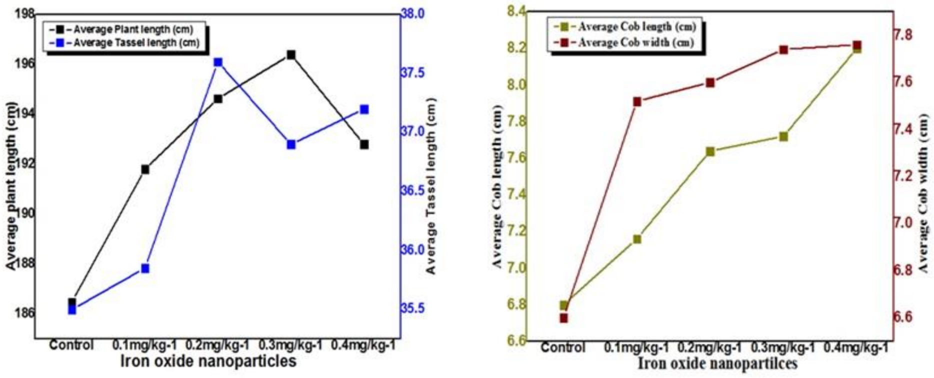


Fig. 9. Fe_3O_4 NPs treated Maize plant Average Plant length, Tassel length, cob length, cob width

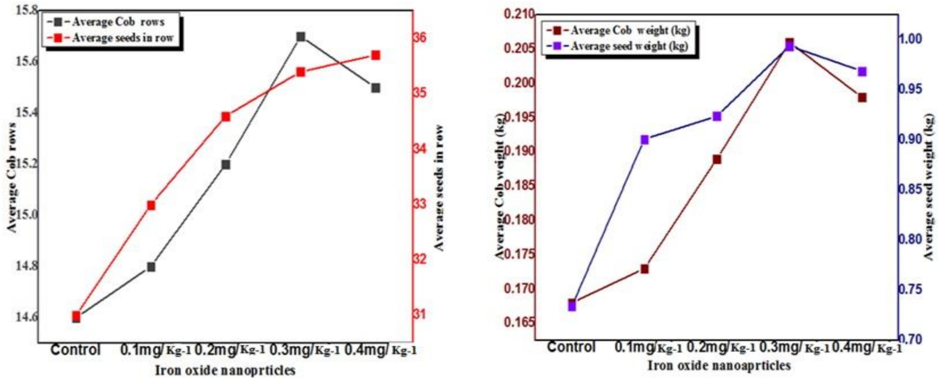


Fig. 10. Fe_3O_4 NPs treated Maize plant Average cob rows, seeds in rows, cob weight seed weight

3.7 Depositions of Fe on root surface of maize plant

As seen in the (fig.12) the Fe_3O_4 NPs content in the roots exposed to 0.1mg, 0.2mg, 0.3mg and 0.4mg of Fe_3O_4 NPs and along with control group. However, at the treatment of 0.3mg and 0.4mg Fe_3O_4 NPs, Fe content on roots surface uniformly deposited. 0.1mg and 0.2mg of Fe_3O_4 NPs the Fe content deposited insufficient amount on the surface of maize root plant shown in (fig.11). The sizes of aggregated Fe_3O_4 NPs adsorbed on the root surface were larger than the size of porous space in the cell wall, only a few individual ones could move into the endodermis and cortex of maize root and were available for the upward transport. These studies indicate the Fe_3O_4 NPs are able to penetrate inside seedling and drastically influence their biological activity by enhancing the amount of water uptake at germination stage.

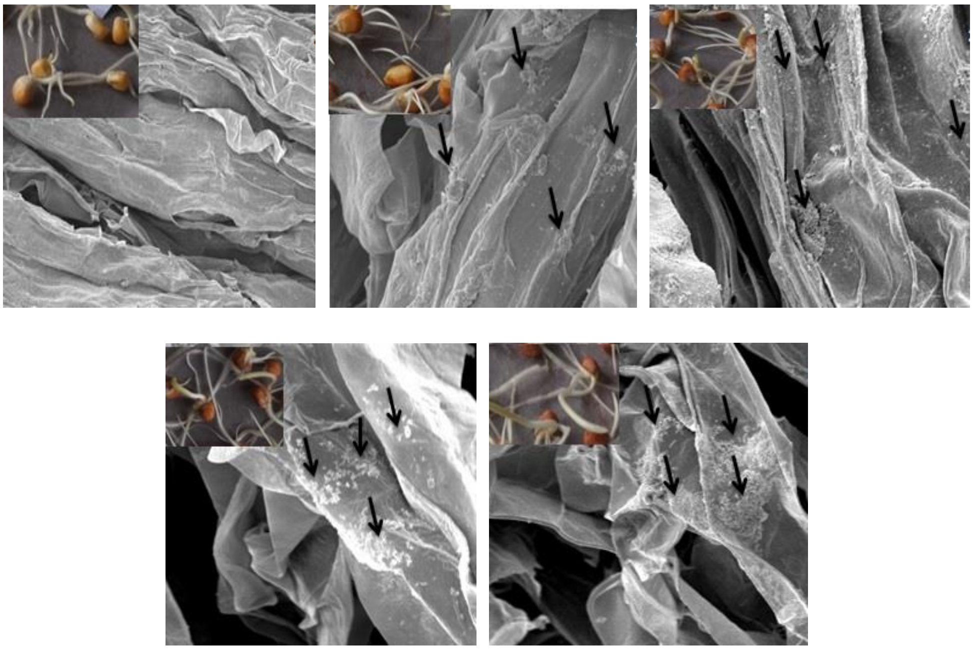


Fig. 11. Fe_3O_4 NPs treated maize root surface SEM analysis of a) control b) 0.1mg c) 0.2mg d) 0.3mg e) 0.4mg

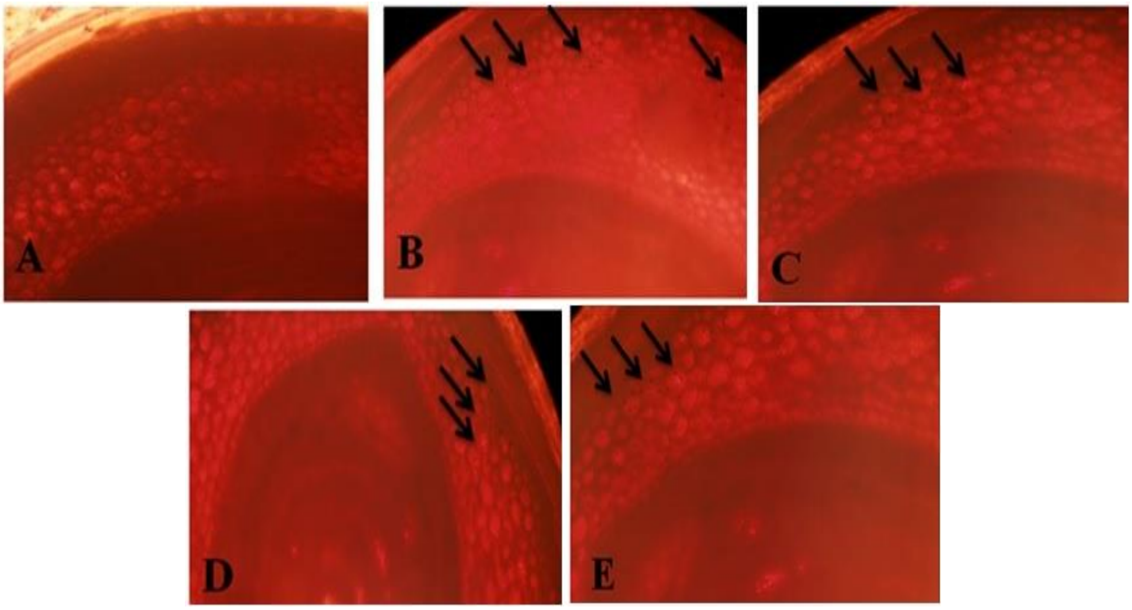


Fig. 12. Fluorescent microscopic images of Fe_3O_4 NPs treated maize plant cross section of a) control b) 0.1mg b) 0.2mg d) 0.3mg e) 0.4mg

The Fe_3O_4 NPs were observed in the maize shoots in this study by fluorescent tracking shown in (fig.12). Even though there is substantial evidence for upward translocation of Fe_3O_4 NPs in shoots due to the methodological limitations, the Fe_3O_4 NPs entering to the aerial parts of maize was very low amount. It would be expected that some Fe_3O_4 NPs enter plant roots and they will be liable to undergo biotransformation to form of Fe+2 upward transport pathways, an assertion that was supported by the observation that Fe existed mainly as Fe in the plant tissues. Such biotransformation will, as a result, prevent the upward translocation of Fe_3O_4 NPs inside maize. The possibility of entry and translocation of Fe_3O_4 NPs in maize stems was studied by fluorescent microscopic technique. (Fig.11) shows the cross section of maize stems treated with Fe_3O_4 NPs and control. In these micrographs, the pink color represents the fluorescent microscopic image of plant tissue, while the black color dots indicated the aggregates of Fe_3O_4 NPs. (Fig.12) shows the cross section of maize stem of the control, the micrographs show the tissue of maize stem in pink, and the absence of black dots. In contrast, for maize seedling treated with Fe_3O_4 NPs, the cross section stems shown the deposition of the nanoparticles was observed inside the endodermis and vascular bundles. These black particles were assigned to clusters of Fe_3O_4 NPs, are agglomerated. Thus, the micrographs reveal that they have been translocate into maize stems treated with Fe_3O_4 NPs.

These Fe_3O_4 NPs are located in xylem and phloem vessels and in the cell wall of xylem vessels. Similar results have been reported for the uptake and translocation. It means the Fe_3O_4 NPs are taken by maize roots, travel through the stem and are accumulated in leaves [29-30]. These results suggest the Fe_3O_4 NPs were absorbed by root tip cells, subsequently, the Fe_3O_4 NPs were internalized into cell walls, embedded by new cells later, and they enter into the vascular system, and are accumulated in maize leaves. This transportation system could be the way that Fe_3O_4 NPs aggregates take into the maize seedling. Iron constitutes an essential element for the optimal growth of plants, as well as their biomass and fruit production [31]. Iron plays an important role for the structure and or function of the photosynthetic electron transfer chain. Additionally, the photosynthetic efficiency, the structure and function of the photosynthetic apparatus are heavily iron dependent, directly or indirectly via the porphyrin biosynthesis pathway. The schematic diagram shown in figure 13.

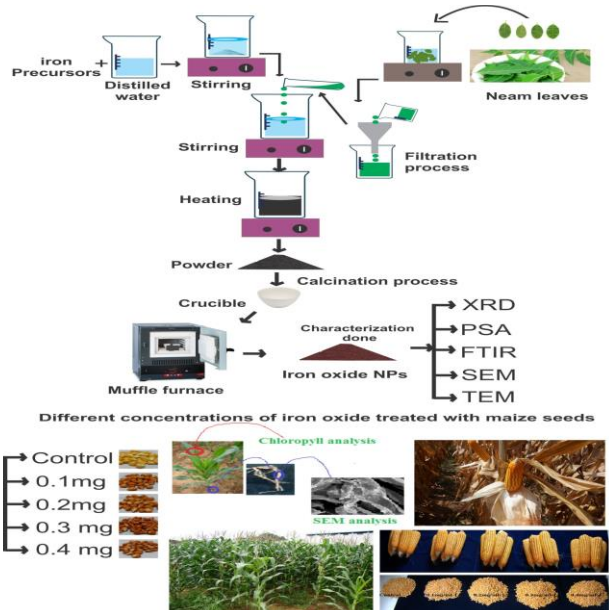


Fig. 13. Schematic diagram of Fe3O4 NPs green synthesized, different concentrations of NPs treated with maize seeds and field test

4. Conclusions

The biological indicators of toxicity or stress in maize seedlings were not observed in these treatments with Fe_3O_4 NPs. In contrast, the Fe_3O_4 NPs treatment increased the growth of maize and yield. Similar results have been observed in the previous works using IO-NPs such as magnetite and maghemite NPs. On the other hand, the deposition of Fe_3O_4 NPs into maize root surface was shown by scanning electron microscopic technique. The clusters of Fe_3O_4 were found in endodermis of maize root. This study highlights the importance of Fe_3O_4 NPs on germination and seedling growth of maize and yield. In the present investigation, it was clear that the nanoparticles delivery of Fe_3O_4 NPs could be effective and was beneficial in order to improve the growth yield and yield attributes of maize. In the controlled conditions even at the high concentrations of Fe_3O_4 NPs showed promontory effects, whereas, at field scale conditions, even at the 0.3mg concentrations of Fe_3O_4 NPs increase in the yield and uptake of Fe. Therefore, it's evident that NPs delivery of nutrient into the plant system can be effective and micronutrient deficiencies could be corrected using nanomaterials through agronomic biofortification which reflect in human health.

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