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Synthesis and Agricultural Applications of Iron Oxide Nanoparticles in Crop Enhancement

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***Abstract*—**Iron oxide nanoparticles (Fe_2O_3NPs) are widely used in different applications due to its ecofriendly nature and biocompatibility. Hence, in this investigation, biosynthesized Fe_2O_3NPs influence on flax (*Linum usitatissimum* L.) plant was examined. The biosynthesized nanoparticles were found to be cubic phase which is confirmed by XRD analysis. FTIR analysis confirmed the presence of functional groups corresponding to the iron oxide nanoparticle. The elemental analysis also confirmed that the obtained nanoparticle is iron oxide nanoparticle. The scanning electron microscopy and the transmission electron microscopy confirm that the average particle size was around 56 nm. The effect of Fe_2O_3NPs on seed germination followed by biochemical analysis was carried out using standard methods. The results obtained after four days and 11 days of seed vigor studies showed that the seedling length (cm), average number of seedling with leaves, increase in root length (cm) was found to be enhanced on treatment with iron oxide nanoparticles when compared to control. A positive correlation was noticed with the dose of the nanoparticle and plant growth, which may be due to changes in metabolic activity. Hence, to evaluate the change in metabolic activity, peroxidase and catalase activities were estimated. It was clear from the observation that higher concentration of iron oxide nanoparticles (Fe_2O_3NPs 1000 mg/L) has enhanced peroxidase and catalase activities and in turn plant growth. Thus, this study clearly showed that biosynthesized iron oxide nanoparticles will be an effective nano-nutrient for agriculture applications.

I. Introduction

CURRENTLY, science and technology is booming everywhere giving birth to new technologies [1] such as nanotechnology, one of the biggest achievements in science and technology. Nanotechnology covers almost every field of science and technology by its applications [2]. The recent survey has shown that nanotechnology has taken majority of contributions towards the leading world market [3]. Nanotechnology includes the synthesis and application of nanoparticles [4]. Nanoparticles are of different types such as metal based, metal oxides and composites [5]-[7]. The high surface to volume ratio has made the nanoparticles unique and efficient leading to their wide applications in fields like energy, food science, and electronics and biomedicine. However, the application of nanoparticles in agriculture is in elementary stage.

Recently, nanotechnology is focusing on the detection of plant diseases, plant disease management, and development of nano-nutrient for the enhancement of plant growth [8], [9]. In general, plant growth is promoted by applying commercially available fertilizers. However, most of the fertilizers are toxic to humans, animals, and ecosystem. In addition, application of fertilizer also faces problems like hydrolysis, decomposition, and leaching. Hence, the available fertilizers are less effective. Thus, an alternative to chemical fertilizer, which can overcome the above problems, is required now. Nanoparticlebased and nano-encapsulated fertilizers are found to be effective in enhancing plant growth by promoting precise release of nutrients to the plant [10], [11]. However, some limitations had led to its failure. In contrast, few researches have shown that some engineered nanoparticles boost the growth of plant by altering its metabolisms [12]-[16]. Recently, lot of researches are being focused on the evaluating the toxicity of nanoparticles towards microbial biomass [17], plant growth promoting bacteria [18], *Pseudomonas fluorescens* [19], cell lines [20], [21], *Chlorella pyrenoidosa* [22], maize seeds germination [23], [24], and plant phytochemicals [25]. To our surprise, only few reports are available on the beneficial effect of nanoparticles on plant growth. Hence, further research is essential to check the beneficial/toxic effects of nanoparticles on plant growth. With this insight, the present study was carried to develop a nanoparticle, which could improve the growth and yield of plant.

A wide variety of nanoparticles such as calcium, magnesium, cobalt, nickel and iron oxide are available at present, among which iron oxide nanoparticles are most commonly and widely used. It is being used in different applications such as catalyst, drug delivery, environmental protection and sensors, because of its high activity and less

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toxic to the environment [26]-[28]. Iron oxide nanoparticles increased the root growth of soybean plant [29], as well as enhanced seed germination in mung bean and watermelon plants [30], [31]. However, iron oxide was found to be toxic to algae such as *Isochrysis sp*. and *Nannochloropsis sp*. [32]. Hence, the toxic effect of iron nanoparticles is not yet clearly understood. Hence, in this study, we have synthesized iron oxide nanoparticle in controlled condition, and its influence on the plant growth was determined using standard protocols.

II. Materials and Turning Tests

The precursor solution was prepared by using 0.2 M iron nitrate Fe $(NO_3)_2.6H_2O$, of AR grade with water as solvent, (Milli-Q Water, Millipore, Germany).

Flowers of *Hydrangea paniculata* were collected from the nearby Gorky Park, Russia. The flower petals were plucked and washed using sterile water to remove the adhered dust particles. The excess water was allowed to drain out at room temperature. Petals (10 g) were crushed well with mortar and pestle, and the extract was collected using Whatman No.1 filter paper. The process of filtration continued until a clear homogeneous extract is obtained.

Green synthesis was performed according to our previous study on nickel, magnesium and silver nanoparticles synthesis [33], [34]. To 50 ml of 0.2 M iron nitrate (Fe $(NO_3)_2.6H_2O$) solution on a magnetic stirrer, the flower extract was added in a drop-wise manner until the color of the solution changes. The addition of flower extract was continued until viscous colloids were obtained. The colloidal solution was centrifuged for 25 mins at 4300 rpm. The process of centrifugation was continued until a clear supernatant was obtained. This process was carried out to remove the excess of the extract, nonreactive and other unwanted residues present in the nanoparticles suspension. Once the supernatant becomes clear, the pellet was carefully collected from the bottom of the centrifuge tube followed by drying under 40-50 ^oC to remove the excess moisture present in it. The dried pellet was crushed into a fine powder with the help of sterile mortar and pestle.

The fine powder was subjected to various analyses, in order to confirm the formation of the targeted nanoparticle. To investigate the crystalline nature of the powder, X-ray diffraction (Difray, Saint Petersburg) technique was used using chromium (λ = 2.2909 Å) as X-ray source. To explore the different functional groups present in the nanoparticles, Fourier transform (FTIR) infrared spectrophotometer (Nicolet 380, USA) technique was used. The presence of elements in the nanoparticles was confirmed by energy dispersive (EDS) spectrum analysis (EDX SSD, JAPAN). The structural characterisation of the synthesized nanoparticles was done by scanning electron microscope (SEM-VEGA3 TESCAN, Brno, Czech Republic) analysis.

To assess the influence of the synthesized nanoparticle on the growth and development of plants *Linum usitatissimum L.*, viability of seeds and morphometric parameters were checked. For the plants grown in laboratories, germinating ability and vigour of germination in seeds, length of roots and vegetating parts were analyzed. To study germination, the seeds were placed in humid chamber consisting moistened filter paper with the nanoparticle solution (1 mg/L, 100 mg/L and 1000 mg/L) in a Petri dish. Each variant of the experiment was produced in four replicas. Each replica consisted of 100 seeds. Distilled water was used as control for comparison. Germination rate was assessed by the method suggested by McGuire. The procedure was followed by accounting the number of days required for completing germination studies [35]. The results were processed with Student's T-criterion at 5% significance level.

Activity of antioxidant system enzymes, such as peroxidase and catalase was analyzed in seedlings. The activity of peroxidase was determined spectrophotometrically by measuring the oxidation of benzidine (4,4'-diaminobiphenyl) in the presence of hydrogen peroxide and peroxidase [36]. The activity of catalase was determined by measuring the decomposition of hydrogen peroxide with catalase spectrophotometrically [37].

III. Results and Discussion

The phase of the green synthesized nanoparticles was analyzed by using XRD technique and is shown in Fig. 1. The obtained 2θ values were at ~27.7°, ~36.1°, ~39.4°, ~45.9°, ~54.3°, ~66.7°, ~69°, ~77°, ~84.6°, ~91.1°, ~101.8°, ~120.6°, ~128.4°, and ~131.2°. These values matched well with the ICDD standard data files (JCPDS: 96-900-5817) for iron oxide (Fe_2O_3), thus confirming that the synthesized Fe_2O_3 nanoparticles possessed face centered cubic crystal system. The functional groups present in the synthesized nanoparticle were determined by FTIR analysis. The FTIR spectrum as shown in Fig. 2 revealed different peaks at 3139, 1652, 1511, 1348, 890, and 792 cm^{-1}.

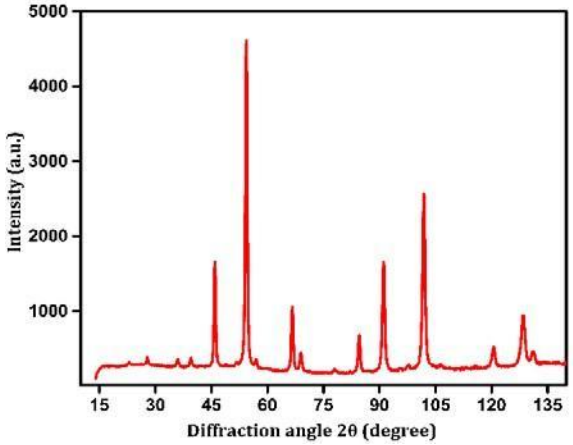

Fig. 1 XRD pattern of Iron oxide nanoparticles

Peak at 3139 indicated the presence of O-H group, which may be due to the organic molecules like esters, organic acids and phenols that participates in nanoparticles synthesis. Peaks at 1652, 1511, and 1348 cm^{-1} indicated –CH, C-H and C=O groups, whereas peaks at 890 and 792 cm^{-1} confirmed the presence of iron and its corresponding forms [38]. The organic molecules that were present in the plant extract might have attached to the nanoparticles and hence, reflected in FTIR analysis.

Fig. 4 Elemental analysis (EDS) of Iron oxide nanoparticles

The surface topology of the synthesized nanoparticles was analyzed by scanning electron microscopy and is given in Fig. 3. The observed result revealed the spherical shape of the nanoparticles. The elemental compositions of the synthesized nanoparticles were evaluated by EDS analysis (Fig. 4). The result indicated that the nanoparticles contained about 59.2% of iron and 40.8% of oxygen. TEM analysis (Fig. 5) revealed the size of the nanoparticles to be in the ranges 41 to 71 nm, however the average particle size was found to be 56 nm.

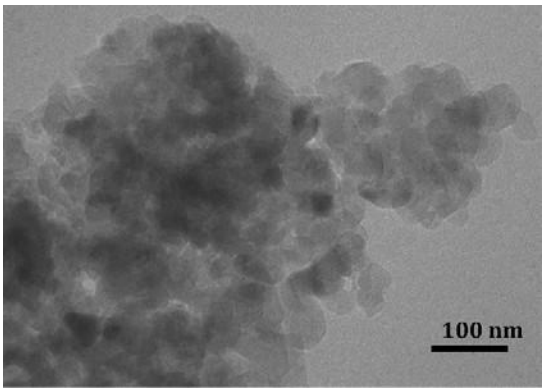

Fig. 5 Transmission electron microscopy image of Iron oxide nanoparticles

The influence of iron oxide nanoparticles on seed germination and level of antioxidant enzymes was studied employing standard protocols using different concentrations (1 mg/L, 100 mg/L and 1000 mg/L). The results obtained are shown in Tables I-III. The seedling morphometric was performed after 11 days of treatment, and the seed vigor was analyzed after four days of treatment. The seedling morphometric was found to vary with respect to nanoparticle concentration. It was noticed that the seedling length (cm) increased with an increase in the concentration of the iron oxide nanoparticles when compared to control.

The results obtained after four days of seed vigor studies showed that the average number of seedling with leaves was found to increase with iron oxide nanoparticles treatment when compared to control. The root morphometric study was performed after 11 days of the treatment. The observed difference in the root morphometric was based on the difference in the concentration of the nanoparticles used for the analysis. The higher is the concentration of the nanoparticles, the greater is the root length (cm), when compared to control.

The total length of lateral roots also increased on treatment with iron oxide nanoparticles. Plants respond to stress and injury by changing their metabolic activity (such as stress hormone, jasmonic acid, and auxin), which in turn leads to a change in their morphology and growth rate. Such a phenomenon is known as thigmomorphogenesis. Hence, to evaluate the change in metabolic activity due to nanoparticle treatment, peroxidase and catalase activities were estimated [39].

It was clear from the observed results that the order of peroxidase and catalase activity was found to be Fe_2O_3NPs 1000 mg/L > Fe_2O_3NPs 100 mg/L > Fe_2O_3NPs 1 mg/L. This clearly showed that nanoparticles have decreased oxidative stress in plant by triggering the enzyme activity, and hence reduced the formation of reactive oxygen species (ROS) [40]. The reduction of ROS has promoted the plant growth as reflected from seedling and root lengths. Thus, iron oxide nanoparticles could efficiently trigger plant growth and hence, could be used instead of harmful fertilizers for agriculture applications.

TABLE I
SEEDLINGS MORPHOMETRIC (11 DAYS) AND SEED VIGOR (4 DAYS) STUDIES

Treated Sample names	eedlings length (cm)	Average Number of seedlings with leaves (n)	Average Number of seedlings with roots (n)
Control	5.1	4	6
Fe_2O_3NPs 1 mg/L	5.9	4	10
Fe_2O_3NPs 100 mg/L	6.2	3	10
Fe_2O_3NPs 1000 mg/L	6.6	5	23

TABLE II
ROOTS MORPHOMETRIC STUDIES (11 DAYS)

Treated Sample names	Length of main root (cm)	Total length of lateral roots (cm)	Number of lateral roots, pieces
Control	4.1	3.5	3.1
Fe_2O_3NPs 1 mg/L	4.8	3.0	3.1
Fe_2O_3NPs 100 mg/L	5.1	3.1	3.3
Fe_2O_3NPs 1000 mg/L	5.7	3.8	4.0

TABLE III
PEROXIDASE AND CATALASE ACTIVITY IN SEEDLINGS

Treated Sample names	Peroxidase activity, mU/mg	Catalase activity, mU/mg
Control	4.568	24.1
Fe_2O_3NPs 1 mg/L	5.981	28.32
Fe_2O_3NPs 100 mg/L	6.551	29.67
Fe_2O_3NPs 1000 mg/L	6.751	31.58

IV. CONCLUSION

In this study, iron oxide nanoparticles were synthesized by biosynthesis method and its influence on the growth and yield of Flax (*Linum usitatissimum* L.) plant was analyzed by using standard protocols. The synthesized iron oxide nanoparticles were characterized by different characterization techniques to confirm its physicochemical properties. The obtained result revealed that the nanoparticle exhibited cubic phase and possessed spherical shaped with size ranged between 41 to 71 nm. The average particle size was 56 nm. The influence of different nanoparticles on seed germination and antioxidant enzymes (peroxidase and catalase) was analyzed using standard protocols. It was observed that the seedling length (cm), average number of seedling with leaves and root length (cm) were increased with an increase in the concentration of the nanoparticles when compared to control. Hence, to evaluate the change in metabolic activity due to nanoparticle treatment, peroxidase and catalase activities were measured. The result showed that higher concentration of iron oxide nanoparticles (Fe_2O_3NPs 1000 mg/L) has enhanced the activity of both the enzymes indicating the inhibition of ROS generation and hence promoted plant growth. Thus, it is concluded that iron oxide nanoparticles could be efficiently applied in agriculture for the better growth of plants in place of harmful chemical fertilizers.

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