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Short Communication

Bio-synthesis of iron oxide nanoparticles using neem leaf cake extract and its influence in the agronomical traits of *vigna mungo* plant

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ABSTRACT

In this work reports the synthesis of iron oxide along with the complex formation from the neem cake using the biosynthesis and precipitation method. Ferrous sulphate ($FeSO_4$) and sodium hydroxide were used as the precursor precipitating agent, respectively. The resultant specimens were characterized using X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX), ultra-violet visible spectroscopy (UV-Vis), fourier-transform infrared spectroscopy (FT-IR), soil test, biochemical, and phytochemical analysis. To test the effect of the synthesized specimen as the nanofertilizer in the seed germination and the growth, the sample was incorporated in to the red soil and the agronomical traits including plant height. Number of leaves were studied over a survival period of 75 days of the selected plant species *vigna mungo* using POT analysis. The plant samples were harvested, and then the biochemical and phytochemical studies were carried out for alkaloids, glycosides, flavonoids, phenols, steroids, protein and total chlorophyll content. The results showed that the nanoparticles incorporation enhanced the plant growth and increased the concentration of the bioactive compounds in an appreciable level.

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Graphical Abstract

Introduction

Nanotechnology is a new and emerging technology with a great number of applications. It involves the synthesis and application of materials having one of the dimensions in the range of 1-100 nm [1, 2]. A wide variety of physico-chemical approaches are being used these days for the synthesis of nanoparticles (NPs). Biogenic reduction of metal precursors (for producing NPs) is so eco-friendly, low cost, free of chemical contaminants for medical and biological applications where purity of NPs is of major concern [3]. Biogenic reduction is a "*bottom up*" approach like chemical reduction where a reducing agent is replaced by extract of a natural products with inherent stabilizing, growth terminating and capping properties. Furthermore, the nature of biological entities in different concentrations in combination with reducing organic agents influences the size and shape of NPs [4].

The uptake efficiency of the plant species is one of the risky tasks in the field of plant cultivation. Majority of the plants didn't use the complete fertilizer and its effect in the growth due to some the internal and external factors including temperature, and soil nature [5]. Plant communities are directly related to geology and soil types that may occur in specific area morphology, penology and bio chemical constituents of the plant also various response to physico chemical characteristic of the soil [6]. Plants produce a large and diverse array of organic compounds that appear to have direct function in the growth and development. Nanomaterial complex materials which promote the plant growth through its activation of nano-sized fertilizer.

Experimental

The chemicals used for the experiment (sulphate and sodium hydroxide) were pure and of analytical grade. Oil cakes were obtained after oil extraction from various seeds. In this experiment neem oil cake were obtained from Tirupattur, Tamil Nadu, India. Neem cake is an organic material without toxicity. Neem cake is a perfect fertilizer for the agriculture, containing many micro and macronutrients that enhance the growth of the plant species. In this investigation, ferrous sulphate is a precursor and sodium hydroxide used as a precipitating agent. There are various methods available for synthesis of nanomaterials. Each method is unique, and it influences the size and morphologies of the material. Nowadays, bio-synthesis emerging is as a trend in the production of nanoparticles. In this study, we report a combination of bio synthesis and precipitation method. The main objective of adopting method is to get more yield and good quality of the sample. The extract was prepared and added to the ferrous sulphate solution. After continuous stirring precipitating agent, NaOH, was added. The specimen was transplanted to the tested red soil and moisturized for one week and the selected plant species seeds of v*igna mungo* have showed in to the soil and the agronomical traits were measured using POT studies. Pot studies were carried out using black polythene cover with the height is 15 cm and width is 17 cm.

Neem cake was well dried, and ground using a mixer, then sieved using a 150 µm mesh, dried in sunlight for 3 h until a constant weight was obtained and then the extract was prepared as per the procedure. It was stirred using a magnetic stirrer for 1 h as the color changed from light brown to dark brown. The prepared neem cake solution and ferrous sulphate solution mixed and stirred using a magnetic stirrer for 2 h. Then, 1 mole of the prepared sodium hydroxide solution was added drop wise, after, vigorous stirring for 0.5 h, the color of the solution changed tuned to black and it was kept air tight is a beaker at the room temperature for 1 week. After one week of fermentation, it was again mixed well using the magnetic stirrer and filtered. The specimen was centrifuged for 30 min and heated up to 230 °C in a microwave oven for 3 h. It was dried and ground in the mortar. Figure 1**a–f** reveals the synthesis of iron oxide nano-complex using neem cake extract. The synthesized iron oxide complex was incorporated in to the tested red soil by dissolving it in the tested water sample. The seeds were showed in to the plastic bags containing the red soil of equal amount. The nanoparticles were added twice after the first day of germination with the time interval of 15 days. From the day of germination, the plants of both the control and the nanoparticles treated sample were monitored and the agronomical traits including the plant height, shoot length, root length were measured for every three days of time interval.

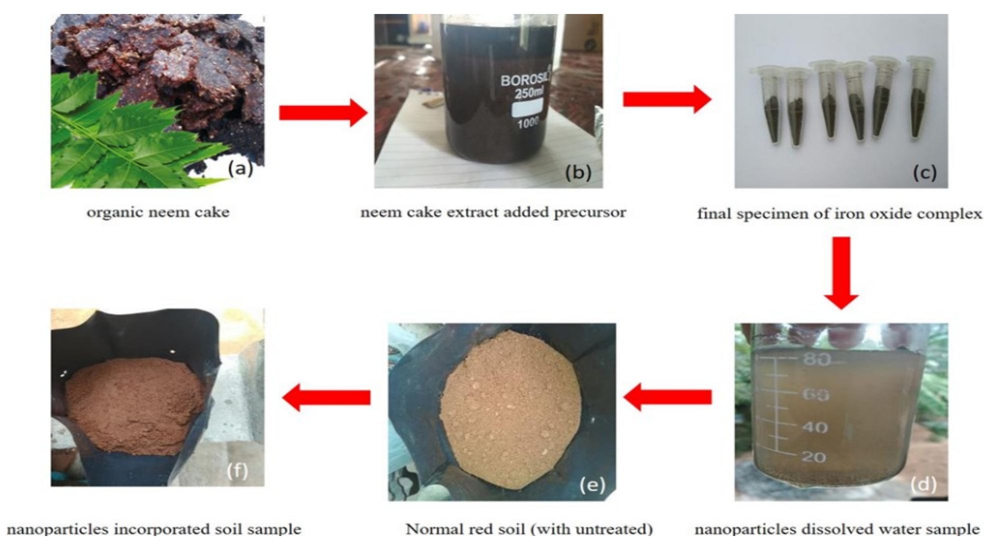


Figure 1. Schematic diagram of neem cake improves soil texture, water retaining capacity, and soil aeration for healthier root development

Results and Discussion

X-ray diffraction analysis

XRD is an easy tool to determine the shape of the unit cell of any material. Structure of the material is determined by the powder X-ray diffraction analysis. It was recorded using X-ray diffractometer with Cu-K_α radiations within the 2θ range of 10° - 80°. The recorded powder XRD pattern is shown in Figure 2. All the X-ray diffraction peaks are coincides very well with the JCPDS card number [89-7047], belonging to the cubic structure and indexed (hkl) values of (002), (121), (130), (004), (132), (114), (042) and (015).

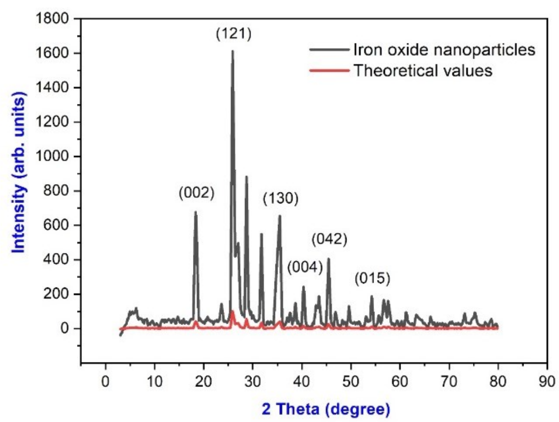


Figure 2. X-ray diffraction pattern of iron oxide nanoparticles

Fourier transform infrared

The synthesized iron oxide complex nanoparticles were showed absorption peaks at 3428.35 cm^{-1}, 3126.13 cm^{-1}, 2925.54 cm^{-1}, 2856.20 cm^{-1}, 2352.03 cm^{-1}, 1631.13 cm^{-1}, 1398.96 cm^{-1}, 1111.17 cm^{-1}, 808.19 cm^{-1}, 616.99 cm^{-1}, 525.03 cm^{-1} and 416.69 cm^{-1}. These FT-IR spectra were plotted wave number (cm^{-1}) versus transmittance (%) as shown in the Figure 3. The finger print region for iron oxide nanoparticles is 3428.35 cm^{-1} to 2352.03 cm^{-1}. The peak 3428.35 cm^{-1} corresponds to O–H stretching [7]. The peak 3126.13 cm^{-1} corresponds to the functional group of -OH stretching present in water. The peak 2925.54 cm^{-1} corresponds to -CH stretching in the functional group [8]. The peak 2856.20 cm^{-1} corresponds to C-H stretching. The peak value 2352.03 cm^{-1} corresponds to C–H bending. The absorption peak 1631.13 cm^{-1} corresponds to symmetric and asymmetric bending C=O. The peak value 1398.96 corresponds to C-H bending. The peak value 1111.17 corresponding to C=O stretching. The peak value 808.19 corresponds to the C–H bending [9]. The absorption bands from 523.03 cm^{-1} to 416.69 cm^{-1} corresponds to Fe–O stretching, which confirms the presence of iron oxide nanoparticles.

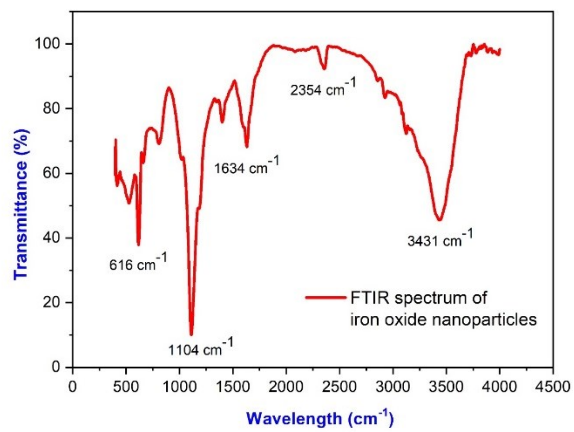


Figure 3. Fourier-transform infrared spectrum of iron oxide nanoparticles

UV-vis /near infra-red analysis

The UV-vis was recorded using Carry 300 UV Visible absorption spectrophotometer at the range of 200 nm to 2000 nm. Figure 4 demonstrates the optical absorption spectrum of the synthesized material. From the data and the percentage of absorption for the incident photon of energy the cut of wavelength of the iron oxide complex nanoparticles were observed at 294 nm [10]. The calculated bandgap energy E_g of the iron oxide complex nanoparticles is 1.2 eV using the relation of $E_g=hc/\lambda$ [11, 12].

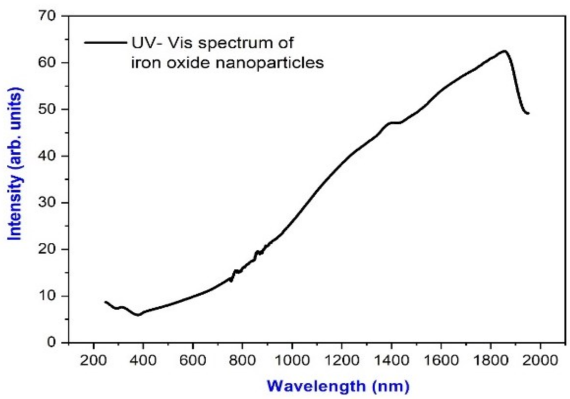
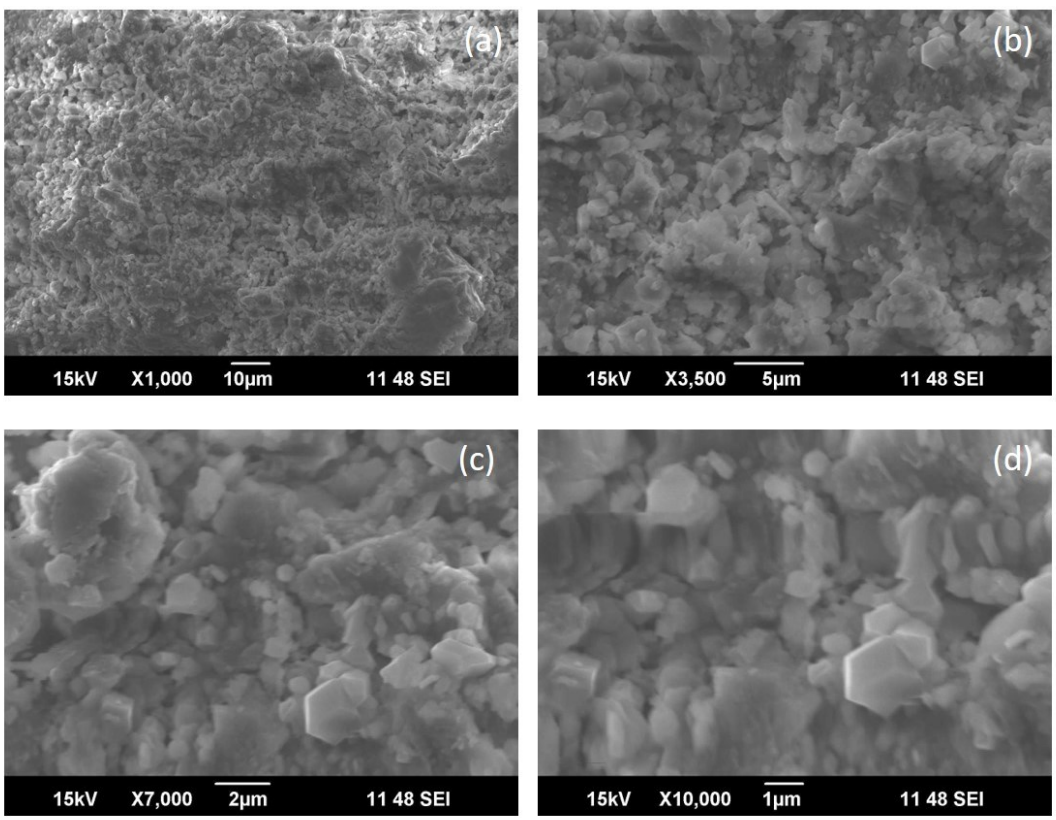


Figure 4. UV-Vis spectrum of iron oxide nanoparticles

Surface morphology and elemental analysis

The SEM image and the EDX of the specimen are shown in Figure 5**a–e**, which revealed the successful synthesis of the iron oxide complex nanoparticles using the neem cake extract [13, 14]. In this present study there are fewer aggregates in the bio synthesized sample as compared with other pure chemical processes [15–17]. The localized elemental composition of the sample was determined as exhibited in the figure, which contains intense peaks O and Fe, along with the presence of other peaks corresponds to Na, P, S, K signals are attributed to the macro as well as micro nutrients contained in the neem cake supplement [18]. The weight composition of synthesized nanoparticles percentage is 43.13%, 24.54% 13.99%, 7.81% and 7.59 % respectively along with fewer impurities [19].



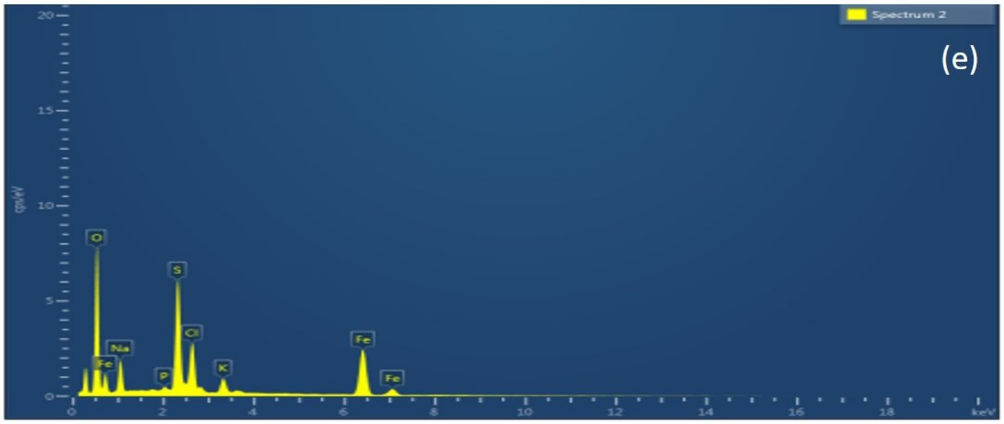


Figure 5. SEM and EDX images of iron oxide nanoparticles

Agronomical traits

The synthesized nanoparticles were dissolved in water, with the pH of 5.70 dsm^{-1}, and the salt content was 2.08 m.eq., /litre and other minerals like sodium, fluoride, chloride, sulphate and nitrates found to be nil [20]. Two plastic bags were used for the experimental study in which the tested red soil (~ 2 Kg) was filled and in one of the bags the nanoparticles incorporated water was poured and the other one was kept as control [21]. Each system was moisturized for one week and the seeds of *vigna mungo* have showed on simultaneous time [22]. The agronomical traits including the plant height, shoot length, root length and number of flowers were recorded for every 3 days of interval [23].

The following figures exhibit both the control and the nanoparticles treated plants. The recorded agronomical traits were plotted for the number of days versus plant height, number of leaves from the third day of plant germination to till the harvesting day (75 days) for both the control and the nanoparticles treated plant sample and it is shown in Figure 6**a–b**. The maximum height of the nano treated plant is 17.8 cm and control were 13.9 cm and the same trend is observed in the case of leaves also as 18 leaves in the control and 21 in the nanoparticles treated plant sample as shown in Figure 6**c–d**.

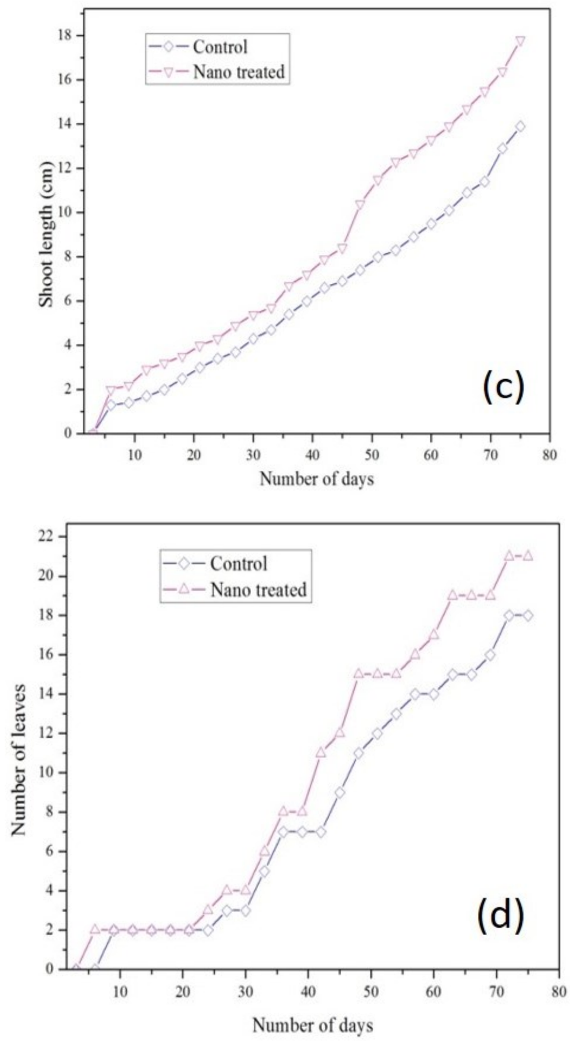


Figure 6. a) Control and b) iron oxide nanoparticles treated *vigna mungo* plant. c) No. of Days Vs Plant height d) No. of Days Vs No. of Leaves

Phytochemical screening and biochemical analysis

After 75 days of complete growth of the plant, the leaves were harvested and dried in shadow for a week and its ground well and the phytochemical analysis of *vigna mungo* plant were analyzed for nano treated plant and control plant as shown in Figure 7**a–b** [24, 25]. The phytochemical tests are carried out that is alkaloids, glycosides, flavonoids, phenols, steroids by water extract method according to the prescribed procedure for the phytochemical screening [26–29]. Based on the reaction occurred for the estimation of phytochemical the levels of the concentration the inference of the observation is given below in the Table 1 [30]. The chlorophyll reports were studied which is chlorophyll A, chlorophyll B and chlorophyll A+B. The chlorophyll study report was tabulated, in which the total chlorophyll content of the nanoparticles treated plant sample was appreciably higher than the normal as shown in Table 2 [31, 32].

Table 1. Phytochemical screening of the control and the nanoparticles treated plant sample

Sample/Test	Alkaloids	Glycosides	Flavonoids	Phenols	Steroids
A (control)	++	-	++	-	++
B(Nanoparticle treated plant sample)	+++	+	+++	++	+++

Interference
+++ Highly positive
++ Medium concentration
+ Sparingly positive
- Negative

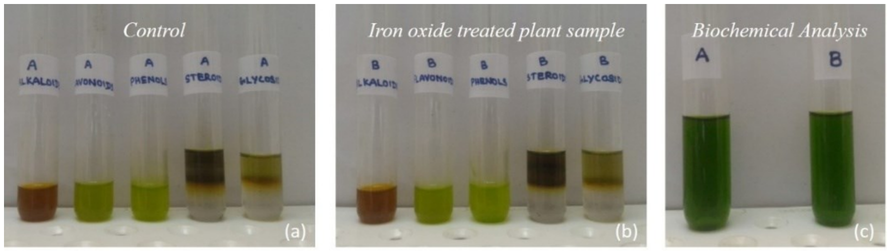


Figure 7. a) Control and b) iron oxide nanoparticles treated *vigna mungo* plant c) Total chlorophyll content of the control and the nanoparticles treated sample

Table 2. Biochemical test and screening analysis

Sample	Chlorophyll A (Ca)	Chlorophyll B (Cb)	Total chlorophyll (Ca + Cb)
Control - A	5.95114	12.0514	18.002254
Nano treated - B	6.99927	14.1093	21.10857

Conclusion

The iron oxide nano-complex was synthesized via the combined method of biosynthesis and precipitation method using the neem cake extract which gives good yield. The synthesized iron oxide complex nanoparticles were characterized using FT-IR, XRD, UV-vis-NIR, SEM, EDX, phytochemical and biochemical studies. The specimen was incorporated to the red soil to check its effect in the seed germination and the physiological growth of the plant species *Vigna Mungo* and the pot studies revealed that the addition of nano-powder enhanced the growth factor which was due to the interaction of nanomaterial along with the soil nutrients that modified the levels of phytochemical and biochemical compounds in an appreciable amount.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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