SEED GERMINATION AND GROWTH PARAMETERS RESPONSE OF MUNGBEAN INFLUENCE BY BIOGENIC FE₃O₄ NANO PARTICLES

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ABSTRACT

Biotechnology and nanobiotechnology combined may result in rapid and significant development in the area of agricultural industry for their efficient delivery and production of copious nutritious food. Engineered iron oxide nanoparticles have been used extensively for environmental remediation. Plants are an important component of ecosystems and can be used for the evaluation of overall fate, transport and exposure pathways of iron oxide nanoparticles in the environment. In this work, the effect of engineered iron oxide NPs on the germination and growth of mungbean is studied. The influence of graded concentrations (0, 50, 100, 150 and 200 mg.L⁻¹) of nanosize iron oxide were studied on seed germination parameters including germination percentage (GP %), Fresh Biomass Weight (FBW), Fresh Shoot Weight (FSW), Fresh Root Weight (FRW), Dry Shoot Weight (DSW), Dry Root Weight (DRW) of mungbean plant. Results indicated that significant differences in examined traits were found among the plant species and also among the employed iron oxide concentrations. The highest GP (90%) was observed in 100 mg.L⁻¹ concentration, however, the lowest GP (80%) it was obtained at control. In conclusion, application of iron oxide stimulated the seed germination of mungbean plant. However, this response was dependent on the concentration of applied iron oxide and way of treatment.

Keywords: Green method, Iron oxide NPs, Mungbean seeds, growth parameters

Introduction:

Changes in agricultural technology have been a major factor shaping modern agriculture. Among the latest technological innovations, nanotechnology occupies a prominent situation in transforming agriculture and food production. Green nanotechnology is one of the most important research and advance topics in the modern era. Green synthesis of metal oxide nanoparticles from plant systems is an emerging as a new and recent development technique. The nanoparticles are of great interest due to their extremely small size and large surface to volume ratio, and they exhibited utterly novel characteristics compared to the large particles of bulk material. There is increasing in commercial demand for nanoparticles due to their wide applicability in various areas such as energy, electronics, catalysis, chemistry and medicine. Green synthesis of Fe₃O₄-NPs has been carried out because of its unique properties, such as being super paramagnetic [1] biocompatible, biodegradable, and expected to be non-toxic to humans [2-4]. These unique properties allow Fe₃O₄-NPs to be widely used in different areas of applications, such as catalysis [5-7] magnetic
storage media [8] biosensors [9] magnetic resonance imaging [10, 11] and targeted drug delivery. Recently, an extensive research has been focused on nano-structured magnetite because it posses unique magnetic and electric properties and its application in medical treatment and seed germination. Iron (Fe) is a necessary micronutrient for all organisms. It is involved in the transport of electrons in many ubiquitous metabolic processes such as respiration, photosynthesis and chlorophyll biosynthesis and is required as a co-factor of numerous enzymes. While highly abundant in the earth’s crust, the low solubility of Fe often limits plant growth. This is largely due to the high reactivity of Fe toward oxygen, in soils, the Fe tends to form highly insoluble ferric hydroxides, dramatically restricting the bioavailability of Fe. Undernourishment for Fe decreases productivity and yield, posing a major constraint for both agriculture and human health. Among the essential micronutrients, Fe is considered as the most deleterious when present in insufficient amounts. It is a major nutritional difficulty often limiting the growth and production in many crops, it induces low CO2 absorption rate, reduced yields, chlorosis development.

**Materials and methods:**

**Materials Used**

Ferric acetate, betel leaf extract are used as reducing agent and stabilizing agent.

**Green synthesis method**

In this green synthesis method Fe₃O₄ nanoparticles are prepared. It is simple, fast and environment Eco-friendly method. 0.01 M of Ferric acetate were used as the precursor material and mixed with 100 ml distilled water under vigorous stir for 40 minutes. For this solution 25 ml of prepared betel leaf extract used as a reducing agent and was added under stirring for 30 min. Then the mixed solution was placed on hot plate for 6 hours at 150°C then collected sample crushed into powder using mortar and pestle. The dried precursor then was calcinated at 400°C.

**Mungbean seeds procurement**

Mungbean seeds were procured from Acharya N.G.Ranga agriculture University rajendranagar, Hyderabad and were used without any modifications.

**Results and discussion:**

**XRD analysis**

XRD is an effective technique to identify the phase and to confirm the crystal structure of the synthesized iron oxide nanoparticles. The X-ray diffraction patterns obtained for the iron oxide nanoparticles synthesized using the betel leaf extract is shown in Figure 1. The XRD pattern displayed five characteristic 2θ peaks at 33.7°, 36.0°, 50.4°, 54.4°, 62.4° and 64.9° marked by their indices (420), (216), (436), (034), (800) and (821) respectively. They are quite identical to the peaks of Fe3O4 crystal with the orthorhombic structure. The results are in agreement with the standard XRD pattern of Fe3O4 (JCPDS 76-0958). Hence, from the XRD result, it was clear that iron oxide nanoparticles formed using betel leaf extract were crystalline in nature.[12]
The average particle size of the synthesized iron oxide nanoparticles was calculated using Debye-Scherrer formula as given in Equation 1,
\[ D = \frac{K\lambda}{\beta \cos \theta} \] (1)

Where \( D \) is the mean diameter of nanoparticles, \( \beta \) represents the full width at half maximum value of XRD diffraction lines, \( \lambda \) represent the wavelength of X-ray radiation source 0.15405 nm, \( \theta \) is the half diffraction angle (Bragg angle) and \( K \) represent the Scherrer constant with value from 0.9 to 1. The particle size was determined by taking the average of the sizes at the peaks and it was found to be 24.5 nm.

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\text{Fig. 1. XRD patterns of as Green synthesized Fe3O4 NPs using neem leaf extract}
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**Chemical compound analysis:**

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\text{Fig. 2. FTIR spectra of Fe3O4 NPs}
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The FT-IR spectra of neem leaf extract is given in Figure 3. A broad band between 3420.4 and 2924.1 cm\(^{-1}\) centered at 2854.6 cm\(^{-1}\) are due to the OH stretching and bending vibration of amine group NH\(_2\) and O-H the overlapping of the stretching vibration of attributed for water and neem leaf extract compounds. The shoulder peak at 1607.8 cm\(^{-1}\) is assigned for C=O stretching mode indicating the
presence of –COOH group in the neem leaf extract. The peaks at 1362.3 and 1110.4 cm\(^{-1}\) can be assigned to the C-O group of polyols. FT-IR spectroscopy confirmed that the neem leaf extract has the ability to act as reducing agent and stabilizer for iron oxide nanoparticles. Nanoparticles can be seen by two strong absorption bands at around 775.7 and 624.9 cm\(^{-1}\) which, corresponding to the Fe-O stretching band of (Fe\(_3\)O\(_4\)).

**PSA analysis:**

![PSA analysis of Fe3O4 NPs synthesized using neem leaf extract](image)

In this measurement, 1 ml of particle suspensions was employed and placed in a 10 mm × 10 mm quartz cuvette. The iron oxide MNP used in this study was synthesized by a green method. The sample concentration for optimal measurements is highly dependent on the sample material and their size. If the sample is too dilute, there may be not sufficient scattering events to make a proper measurement. On the other hand, if the sample is too concentrated, then multiple scattering can occur. Furthermore at high concentration, the particle might not be freely mobile with its spatial displacement driven solely by Brownian motion but with the strong influences of particle interactions [13]. This scenario is especially true for the case of Fe\(_3\)O\(_4\)NPs with inter particle magnetic dipole-dipole interactions. Finally the average size of iron oxide nanoparticles is found to be 32 nm.

**SEM morphological study of Fe\(_3\)O\(_4\) NPs:**

![SEM image of the Fe\(_3\)O\(_4\) NPs synthesized using neem leaf extract](image)
A Scanning Electron Micrograph (SEM) of Fe3O4 NPs synthesized by using neem leaf extract sample as shown in Fig.4 shows 10μm and 200 nm magnificance images which are relatively uniform, smooth spherical shape, high density and agglomerated NPs, attributed to the mild bio-mediated process. When Ferric acetate mixed with prepared leaf extract, which is Fe+2 ions are distribute uniformly and exhibits a synergistic effect to get the complex structures. The resultant performs the oxidation and reduction of Fe+2 ions, keep the molecules together, and form spherical shape structures with leaf extract and undergo slow decomposition when subjected to calcinations.

**Effect of iron oxide nanoparticles on seed germination and growth parameters:**

![Graphs showing Effect of Fe3O4 NPs on mungbean growth parameters](image)

The biosynthesized Fe3O4 nanoparticles have enhanced the seed germination compared to the control. Seed germination enhancement have been speculated as dispersed nanoparticles can penetrate through the seed coat and create “nano-holes” on seed coats, resulting in improved germination conditions, slow and minimal release of Fe+2 ions could be one of the reasons for Fe3O4 to have no major effect on germination of mungbean seeds. The highest percentage of seed germination was 94% at 50 mg/L of Fe3O4 nanoparticles. Since results revealed treating seeds with Fe3O4 nanoparticles did not reduce germination, so possible to use this treatment in
agricultural practices. This may explain that seed germination in this study was not greatly altered by nanoparticles. Fe$_3$O$_4$ nanoparticles can protect seeds against fungi as well as the conventional fungicide. Results of seed protection test indicate that Fe$_3$O$_4$ nanoparticles may be an alternative to conventional fungicides for protecting seeds against fungi. Thus, nano treated seeds can be used to lower the environmental impacts of fungicides and reduce the cost of agricultural production. Engineered nanoparticles could also adhere to plant roots and exert physical or chemical toxicity in plants. Radicals, after penetrating the seed coats, could contact the nanoparticles directly. The root elongation of sensitive plant species would have a dose-dependent response. Since roots are the first target tissue to confront with excess concentrations of pollutants, toxic symptoms seem to appear more in roots rather than in shoots. The affect of Fe$_3$O$_4$ nanoparticles on seed germination, root length, shoot length, fresh biomass, dry shoot, root weight and dry biomass weight of mungbean given in Fig.5. Higher the concentration of Fe$_3$O$_4$ nanoparticles decreased the root, shoot and total seedling length. It was observed that higher concentration of nanoparticles had adverse effect on plant species. Inhibition of seed germination and root elongation has been found to be highly dependent on both plant type and nanoparticle properties.

**Conclusion:**

This synthetic method for Fe$_3$O$_4$ nanoparticles was green, simple and eco-friendly benign because it does not require any extra reductant or surfactant. Based on the XRD analysis studied, purity crystalline of Fe$_3$O$_4$ NPs was prepared. FTIR spectroscopy showed the involvement biomolecules present in the extract of betel leaf which were verified in the synthesizing process of Fe$_3$O$_4$ NPs. The formation of Fe$_3$O$_4$ NPs was confirmed due to the noticeable absorption peaks at 775.7 and 624.9 cm$^{-1}$. Results clearly demonstrate the greater improvement in seed germination, shoot-root growth, fresh biomass and dry weight; it may be also affect on chlorophyll (photosynthetic pigment by the application of biosynthesized Fe$_3$O$_4$ nanoparticle at 50mg/L concentration on one week old plants. However invention opens new door for fertilizer industries to produce “Bionanofertilizer” for plant nutrition. The industries can bank upon this product in order to formulate Fe$_3$O$_4$ bionanofertilizer in very near future.

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