EFFECT OF GREEN SYNTHESIZED IRON OXIDE NANOPARTICLES ON SEED GERMINATION OF TOMATO (*Solanum lycopersicum*)

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The study investigates the impact of seed priming with green synthesized iron oxide nanoparticles (FeO NPs), utilizing the extract of the *Salvinia molesta*, on the seed vigor index of tomato (*Solanum lycopersicum*). The formation of FeO NPs was observed upon exposure of the plant extract to the ferric chloride solution, and the synthesized nanoparticles were characterized using UV-visible spectroscopy, showing a sharp peak at 300 nm due to the surface plasmon resonance. Scanning Electron Microscopy confirmed the formation of nanoparticles with an average size of 81 nm. The study investigated the effect of FeO NPs on seed vigor index of tomato (*Solanum lycopersicum*) at different concentrations; 0, 5, 10, 50, 100, 500, and 1000 ppm. Completely Randomized Design (CRD) was applied with 5 replicates. The synthesized FeO NPs exhibited a biphasic effect on the seed vigor index. According to statistical analysis by one-way ANOVA and Duncan's Multiple Range Test, 100 ppm (78.62381 ± 43.89327) showed a significant ($p < 0.0001$, $F = 5.571$) seed vigor index compared to the control of 0 ppm (54.68049 ± 36.93168). The possible mechanisms underlying the effects induced by FeO NPs for seed priming, including their role in promoting seed germination and influence on primary and secondary metabolic processes, were thoroughly examined. These mechanisms encompass various biological phenomena such as programmed cell death, the generation of reactive oxygen species, DNA damage, reduced transpiration rates, enhanced lipid peroxidation, protein carbonylation, and enzyme activity loss. It is recommended to conduct future studies to focus on investigating the effects of FeO NPs within the concentration range of 50 ppm to 500 ppm. This range appears to be particularly promising based on the observed biphasic effect on the seed vigor index in the current study.

Keywords: Green synthesis, iron oxide nanoparticles, seed priming, tomato, seed vigor index

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INTRODUCTION

Nanotechnology utilizes particles at the nanoscale, with dimensions ranging from one to a hundred nanometers. Nanomaterials, owing to their size-dependent characteristics, show a high surface-to-volume ratio, enhancing nutrition, and mitigating both biotic and abiotic stress effects. Iron oxide nanoparticles are produced in different forms such as magnetite, maghemite and hematite using chemical, physical and biological methods. Phytochemicals in plants such as flavonoids, polyphenols, alkaloids and terpenoids contribute as reducing, stabilizers and capping agents in the synthesis of iron nanoparticles. Previous studies successfully identified that *Salvinea molesta* contains different phytochemicals such as tannins, Saponins, quinones, terpenoids, steroids, flavonoids, phenol and alkaloids by different extract solutions (Mithraja et al., 2011).

Iron is an essential element that is abundant in soil for plant biological activities, such as nitrogen fixation, respiration, photosynthesis, hormone production and DNA synthesis. Previous research observed that the presence of iron oxide nanoparticles led to increased levels of chlorophyll, biomass and plant height in peanut plants. Based on literature reviews, low concentrations of iron oxide nanoparticles support root growth while elevated concentrations of these nanoparticles hinder the development of roots (Palchoudhury et al., 2018).

In the nano-priming of seeds, the utilized media consists of suspensions or nano-formulations, wherein the seeds may or may not absorb the nanoparticles. Priming initiates certain metabolic processes during germination such as increasing enzyme activity and neutralizing the effects of seed ageing without the emergence of a radicle (Kumar et al., 2018).

Nanoparticles can easily pass across the plasma membrane by direct diffusion process as the particle size is small. The second way of transport is by active transport into the cell by endocytosis which is a process that allows cells to absorb substances from outside the cell by surrounding them with the cell membrane. The third mechanism is through special proteins embedded in the cell membrane or designated channels that control nanoparticle entry (Li et al., 2020).

In the present study, FeO nanoparticles were green synthesized using the plant extract of *Salvinia molesta*, and the synthesized nanoparticles were characterized. The potential effects of the synthesized FeO nanoparticles on seed vigor index were investigated using different concentrations of nanoparticle suspension, thereby identifying potential impacts.

METHODOLOGY

Preparation of the *Salvinia molesta* extract and synthesis of FeO NPs

Thoroughly washed *S. molesta* was dehydrated at 42°C for 24 hours. All leaflets were then ground and sieved to eliminate larger particles. Five grams (5g) of powdered leaves were immersed in 80ml of double-distilled water (DDW) and stirred continuously for 1h at a temperature of 60°C. The extract was filtered three times through Whatman No. 1. leaf extract was introduced into the pre-prepared
0.1 M $FeCl_3$ solution at a ratio of 2:3. The pH of the mixture was then raised to 10 by incrementally adding 1M sodium hydroxide. The reaction mixture was left undisturbed for 24h to facilitate the synthesis of FeO NPs. Afterward, the mixture was subjected to centrifugation at 6000 rpm for 15min, and the sediment was gathered. The synthesized FeO NPs were then washed twice with double-distilled water, followed by calcination at 450°C for 2h. The resulting solid pellets were subsequently crushed using a mortar and pestle to get a fine powder-like structure.

Characterization techniques
A small quantity of synthesized FeO nanoparticles was suspended in 10% H_2SO_4 at room temperature, and UV-visible spectroscopy analysis was performed in the range of 200 nm to 600 nm utilizing a quartz cuvette. The morphology of particles was analyzed using scanning electron microscopy (SEM).

Germination experiment parameter
Treatment at concentrations of FeO NPs 5, 10, 50, 100, 500 and 1000ppm was prepared using DDW. Prepared treatments were sonicated for 45mins at room temperature. Surface sterilized tomato seeds were soaked for 4h in the relevant concentration. Sterilized tissue paper was placed in each petri dish and subsequently, 2ml of FeO NPs suspension was immediately poured after thorough agitation of each treatment concentration. Ten numbers of pre-soaked seeds were placed in each petri dish covered by parafilm and allowed to germinate in vitro in an incubator at room temperature for seven days. The experiment was replicated five times and observations were made daily for seven consecutive days.

Data collection
Germination percentage (GP%), and seed vigor index were calculated according to the equation mentioned below. After 8 days, the seedlings were harvested, and shoot length (mm) and root length (mm) were measured.

GP%= (number of germinated seeds/numbers of incubated seeds) × 100

Seed vigor index = {Root length (mm) + Shoot length (mm)} x GP %

RESULTS AND DISCUSSION

Formation of FeO NPs
These phytochemicals in the *Salvinia molesta* plant extracts facilitated the reduction of $FeCl_3$ ions to their atomic state (Fe^0), followed by their subsequent attachment by capping agents to the phytochemical surface. This capping process stabilizes the synthesized nanoparticles, preventing aggregation and ensuring their suspension at the nanoscale. The stabilization lies in the tautomeric transformation of flavonoids, a process that involves the interconversion of their enol into keto forms, facilitated by the presence of reactive hydrogen atoms. These hydrogen atoms are readily donated during the reduction of metal ions, leading to the formation of stable metal nanoparticles. The initial acidic pH of 1.8, measured after combining the precursor salt and plant extract was elevated to 10 using 1 M NaOH. This pH alteration resulted in a notable colour change with the mixture transitioning to a brownish-black colour, confirming the formation of FeO NPs.

Characterization of FeO NPs

Synthesized nanoparticles were analyzed by UV-Visible spectroscopy and SEM analysis.

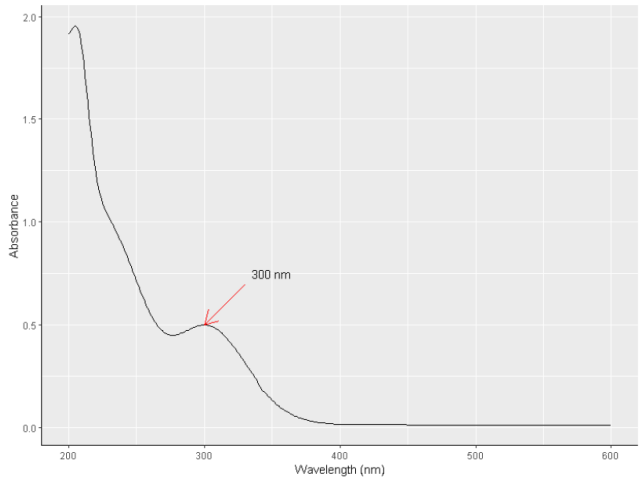
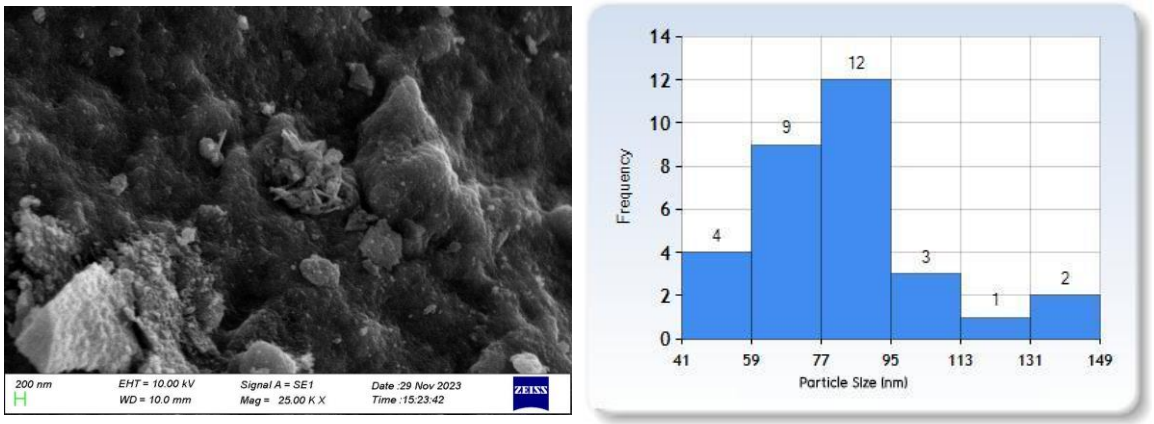


Figure 01: *UV-vis spectroscopy analysis of FeO NPs*



A **B**

Figure 02: *SEM images of FeO NPs at a scale of 200 nm (A), Particle Size Distribution (B)*

As illustrated by Figure 01, UV-Vis spectrometry peak value at 300nm which indicated the formation of nanoparticles. A characteristic peak at 300nm confirmed the formation of Fe_3O_4 nanoparticles according to the literature. As illustrated by Figure 02, SEM images show that nanoparticles have a variety of shapes and sizes within the nanoscale range, with an average size of around 82nm (ranging from 41 to 140nm). They also tend to clump together and have multiple forms.

Seed vigor index

Figure 03 displays the effects of different concentrations of FeO NPs on the seed vigor index. The seedling vigor index was statistically analyzed using one-way ANOVA revealing a significant effect ($p < 0.0001$, $F = 5.571$).

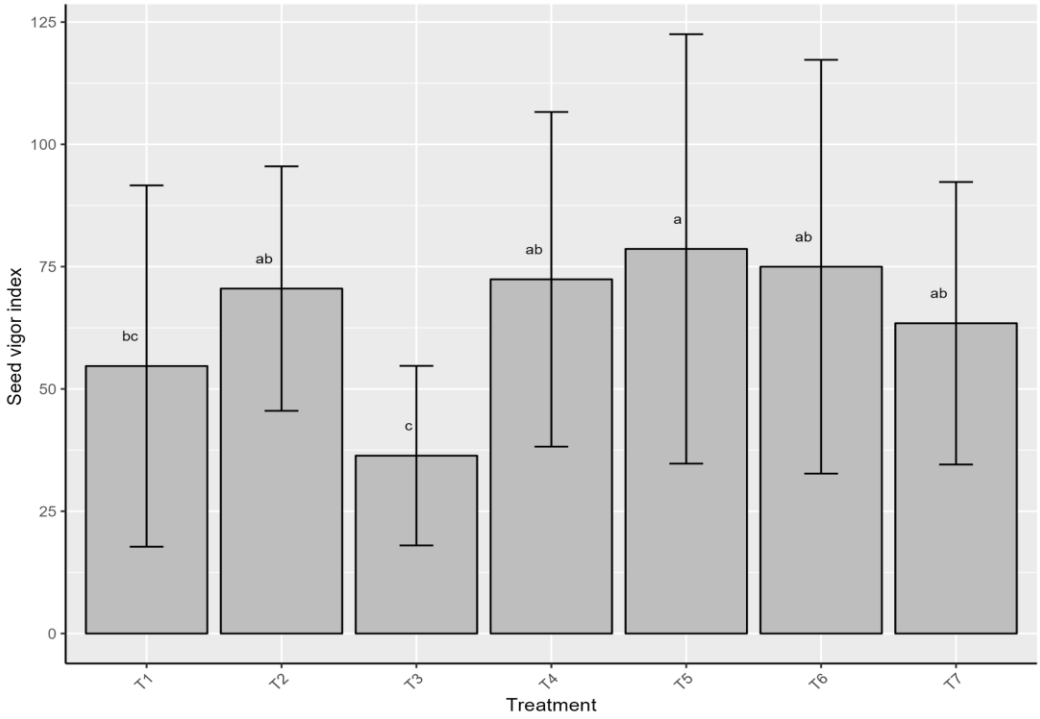


Figure 03*: Effect of different concentrations of FeO NPs on seed vigor index. Results illustrated the means of population, and the bar illustrates ± standard error. Different letters above the bars indicate significant differences at p < 0.0001 per DMRT analysis. T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm)*

Table 01: Experimental data on seed vigor with DMRT results

Level of Concentration	Seed Vigor Mean ± Std Dev
0 ppm	54.68 ± 36.93 ^{bc}
5 ppm	70.51 ± 24.99 ^{ab}
10 ppm	36.36 ± 18.35 ^{c}
50 ppm	72.41 ± 34.21 ^{ab}
100 ppm	78.62 ± 43.89 ^{a}
500 ppm	74.99 ± 42.29 ^{ab}
1000 ppm	63.43 ± 28.88 ^{ab}

Duncan's multiple range test revealed, as illustrated in table 01, the highest seed vigor at a concentration of 100ppm, reaching 78.62 ± 43.89, while the control group (0 ppm) exhibited a seed vigor of 54.68 ± 36.93. According to Duncan's test having a 95 % family-wise confidence level, all treatments except for 10 ppm (seed vigor of 36.36 ± 18.35) demonstrated a higher mean seed vigor index compared to the control group. At treatment concentrations of 5, 50, 500, and 1000 ppm, the mean seed vigor was recorded as 70.51 ± 24.99, 72.41 ± 34.21, 74.99 ± 42.29, and 63.43 ± 28.88, respectively.

Since seed vigor is a composite measure influenced by factors such as germination percentage, shoot length and root length, various parameters contribute to the final seed vigor. The reduction in seed vigor can be caused by several reasons, such as the process of programmed cell death, the production of reactive oxygen species (ROS), protein carbonylation and the loss of enzyme

activity. Once nanoparticles are taken up by plants, they are translocated to various parts such as shoots/fruits and accumulate in different plant tissues. Upon accumulation, they participate in processes that could degrade the quality of plants, resulting in a lower germination rate and biomass, as well as affecting root and shoot length, photosynthesis, damaging DNA, reducing the rate of transpiration, enhancing lipid peroxidation and up-and down-regulating various stress-related genes. Ultimately, this may lead to apoptosis (Hossain, Mustafa, & Komatsu, 2015). The seed's capacity for germination is significantly impacted by a complex interplay of biological events occurring before seed imbibition, heavily dependent on the metabolism of ROS (Bailly, 2022). Iron oxide nanoparticles induce oxidative stress, generating reactive oxygen species that can cause severe damage to cells. Superoxide (O_2^-), the precursor of many other reactive oxygen species is formed through the reduction of molecular oxygen (O_2). As per Ratajczak et al. (2015), ROS can be generated in various sections of seed cells, with a higher concentration observed in the embryo, particularly in the embryonic axis. This correlation may be closely associated with the reduction in seed vigor. The interaction between metal oxide nanoparticles and biological cells has resulted in the identification of various forms of DNA damage. These include chromosomal aberrations, breakage of DNA strands, oxidative damage to DNA and mutations (Koedrith et al., 2014).

According to a previous study on the comparative effects of nano and bulk-Fe_3O_4 on the growth of cucumber (*Cucumis sativus*), it was identified that Fe_3O_4 nanoparticles can interact with plants and produce OH-free radicals. Subsequently, the produced OH^- free radicals could stimulate the degradation of pectin, which is a vital polysaccharide found in the cell walls. This process ultimately eases the root cell wall and promotes root elongation. The effect of different concentrations of FeO NPs on the shoot length of peanuts (*Arachis hypogaea*) was studied using varying concentrations of Fe_2O_3 (2, 10, 50, 250, and 1000 ppm) and a study found that the highest shoot length was observed at 1000ppm. FeO NPs (Fe_3O_4) regulate gibberellin and cytokinin, which are directly involved in cell division and elongation, while also reducing ethylene production, thereby enhancing plant growth. The gibberellins synthesized in the embryo then regulate the production of the enzyme α-amylases in aleurone layers, which is essential for seed germination. High levels of α-amylases facilitate the hydrolysis of endosperm starch, providing energy for the germinating seed to develop roots and shoots which ultimately affect the seed vigor index.

CONCLUSIONS/RECOMMENDATIONS

FeO NPs were successfully synthesized using *Salvinia molesta* plant extract and characterized through UV-visible spectroscopy and SEM analysis. Green synthesized nanoparticles exhibited a biphasic effect on the seed vigor index. The impact of different concentrations can both promote and inhibit seed vigor index simultaneously. The study recommends a concentration range of 100 to 500ppm for seed nano-priming for further investigation of the seed vigor index.

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