EFFECT OF SEED PRIMING OF GREEN SYNTHESIZED IRON OXIDE MAGNETIC NANOPARTICLES USING *Salvinia molesta* PLANT EXTRACT ON SEED GERMINATION AND SEEDLINGS GROWTH OF TOMATO (*Solanum lycopersicum*)

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ABSTRACT

An aqueous plant extract of Salvinia molesta was used as a bio-reductant, stabilizer, and capping agent to synthesize iron oxide nanoparticles. The formation of iron oxide nanoparticles was observed upon exposure of the aqueous plant extract to the ferric chloride solution, and the synthesized nanoparticles were characterized using Ultraviolet-Visible Spectrometry, 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 82 nm. The study investigated the effect of synthesized iron nanoparticles on seed germination and seedling growth by priming tomato (Solanum lycopersicum) seeds at different concentrations of 0, 5, 10, 50, 100, 500, and 1000 ppm.The experimental design was a completely randomized design (CRD) with 5 replicates. The results showed that the synthesized iron nanoparticles exhibited a biphasic effect on seed germination, root length, shoot length, and fresh weight of seedlings. According to statistical analysis by one-way ANOVA and Duncan's Multiple Range Test, 100 ppm (78.62381 ± 43.89327) showed significant ($p < 0.0001$, $F = 5.571$) seed vigor index compared to the control group of 0 ppm (54.68049 ± 36.93168).The possible mechanisms underlying the effects induced by iron nanoparticles for seed priming, including their role in promoting seed germination and influencing 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.

Keyword : - *green synthesis, iron oxide nanoparticles, seed priming, tomato, seed vigor index.*

1. INTRODUCTION

Nanotechnology utilizes particles at the nanoscale, with dimensions ranging from one to a hundred nanometers, it's also demonstrates significant potential in agriculture. Nanomaterials, owing to their size-dependent characteristics, high surface-to-volume ratio, and distinctive optical properties, offer promise in acting as plant protectors, enhancing nutrition, and mitigating both biotic and abiotic stress effects (Ghernaout, 2018). Two types of Iron oxide nanoparticles (FeO NPs), maghemite (γ-Fe_2O_3) and magnetite (formula Fe_3O_4) (Mazhar et al., 2022). FeO NPs are synthesized by various processes and exhibit significant utility in the domains of medicine, biological sciences, and biomedical engineering, showcasing a broad range of applications (Albrecht, Evans and Raston, 2006). Iron oxide nanoparticles are produced in different forms such as magnetite, maghemite, and hematite using chemical, physical, and biological methods (Khoirotin, Nuhaa Faaizatunnisa and Munasir Munasir, 2023). Fe_3O_4 nanoparticles can be synthesized by different approaches such as the top-down method, bottom-up method, sonochemical process, hydrodynamic cavitation, microemulsion process, radiolysis, microwave, laser ablation method, and biosynthetic methods (Latha and Gowri, 2014). Phytochemicals found in different plant parts such as roots, seeds, fruits, and leaves can synthesize magnetite nanoparticles (Khoirotin, Nuhaa Faaizatunnisa and Munasir Munasir, 2023). The green synthesis approach is considered as a better approach than others, as it does not use any chemical compounds and can reduce the emission of toxic substances into the environment (Aksu Demirezen et al., 2019). Several successful previous studies synthesized FeO NPs using plant extracts including *Couroupita guianensis* (Gnanasekar Sathishkumar et al., 2018), Citrus Sinensis peels extract (Eldeeb, El-Raheem and Elbeltagi, 2023), *Citrus aurantium* (Bassim et al., 2022), *Moringa olifera* (Altaf et al., 2021), and *Garcinia mangostana* (Yusefi et al., 2021) but no any evidences found on synthesizing FeO NPs using *Salvnia Molesta*. Phytochemicals in plants such as flavonoids, polyphenols, alkaloids, and terpenoids contribute as reducing, stabilizers, and capping agents in the synthesis of nanoparticles (Altaf et al., 2021)

S. molesta is a free-floating aquatic plant native to south-eastern Brazil has been spread widely throughout the world during the past 50 years and is invasive in a variety of aquatic habitats, including lakes, rivers and rice paddies and listed as world's most noxious aquatic weed only second to water hyacinth (Parys and Mikulyuk, 2022). In 1939, S molesta was introduced to Sri Lanka for research purposes. Investigations were conducted in Colombo, and unintentionally, the plant escaped into the natural environment. By 1952, *S. molesta* was classified as a scheduled weed under the Plant Protection Ordinance No. 10 of 1924. Subsequently, in 1954, the invasive species had spread across 8,907 hectares of paddy fields and 810 hectares of inland water in the Western province and was the most abundant aquatic invasive alien plant in the country (Dahanayake and Perera, 2016; Hemachandra, Nimalananda and Wasala1, 2022). According to the study conducted by Mithraja et al. (2011), they successfully identified that *S. molesta* contains different phytochemicals such as tannins, Saponins, quinones, terpenoids, steroids, flavonoids, phenol, and alkaloids by different extract solutions. Ace Baehaki (2020) was able to conclude that the highest content of bioactive components in *S. molesta* was a flavonoid level of 267 mg/ml by using ethanol solvents.

Iron is an essential element that is abundant in the soil for plant biological activities, such as nitrogen fixation, respiration, photosynthesis, hormone production, and DNA synthesis (Khan et al., 2023). According to the research conducted by Panakkal et al. (2021), it was observed that the presence of iron oxide nanoparticles led to an increase levels of chlorophyll, biomass, and plant height in peanut plants. Based on literature research, low concentrations of iron oxide nanoparticles support root growth, while elevated concentrations of these nanoparticles hinder the development of roots (Palchoudhury et al., 2018). This progress, however, is accompanied by unknown risks of phytotoxicity. Previous research on the effect of Fe_3O_4 nanoparticles on mung bean (*Vigna radiata*) concluded that there is no phytotoxicity of iron nanoparticles at a concentration of 1000 ppm (Sun et al., 2020). In a previous study on cellular responses to iron nanoparticles in *Capsicum annuum*, it was found that most FeO NPs were aggregated in the cell wall of peppers and transported through root apoptotic pathways, which might restrict iron transfer when the concentration of Fe NPs is high (Yuan et al., 2018).

Nanopriming is the utilization of environmentally friendly and commercially viable nanoparticles for seed preconditioning. This approach aims to provide seed protection, enhance plant growth, safeguard crops, and boost agricultural yields in the face of environmental stressors, such as drought (Rui et al., 2015) and is proving beneficial in minimizing the utilization of fertilizers and pesticides (Maswada, Djanaguiraman and Prasad, 2018). In the nano-priming of seeds, the utilized media consists of suspensions or nanoformulations, wherein the seeds may or may not absorb the nanoparticles (Acharya et al., 2019). Priming initiates certain metabolic processes during germination such as increasing enzyme activity and neutralizing the effects of seed aging without the emergence of a radicle (Kumar et al., 2018).

Plant cell plasma membrane has a phospholipid biliary with hydrophilic head groups while the tail part is hydrophobic and acts as a barrier for the transport of molecules (Nile et al., 2022). According to the Behzadi et al. (2017) nanoparticles can enter into the cells by three mechanisms. As described by Foroozandeh and Aziz (2018), the 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 of a process that allows cells to absorb substances from outside the cell by surrounding them with the cell membrane (Foroozandeh and Aziz, 2018). The third mechanism is through special proteins embedded in the cell membrane or designated channels that control nanoparticle entry (Li et al., 2020). The nanoparticle enters through roots or shoots and gets transported to the tissues either by an apoplastic or symplastic route (Pérez-de-Luque, 2017).

In the 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 germination and seedling growth were investigated using different concentrations of nanoparticle suspension, thereby identifying potential impacts.

2. MATERIALS AND METHODS

2.1 Materials

Fresh *Salvinia molesta* specimens were gathered in Uhana, Ampara, Sri Lanka, at coordinates 7°21'17.1"N and 81°39'17.7"E.Ferric chloride anhydrous analytical research grade ($FeCl_3$) was purchased from HiMedia Laboratories Pvt. Ltd. Tomato seeds (Solanum lycopersicum) of "Lanka sour" variety were bought from a nearby store in Nawala, Sri Lanka, with registration number SA/NIK/00011, ensuring a minimum germination rate of 75%, and an expiration date of 10 October 2024. The double-distilled water used was prepared by a laboratory within the Department of Agricultural & Plantation Engineering.

2.2 Preparation of the *Salvinia molesta* extract

The collected leaves of *S. molesta* were carefully cleaned with tap water and subsequently washed with double-distilled water to eliminate debris. Then the leaves were dehydrated at 42°C for 24 hours using a dehydrator, and all leaflets were ground with a kitchen blender, followed by sieving to eliminate larger particles. Five grams of powdered leaves were immersed in 80 ml of double-distilled water and stirred continuously with a magnetic stirrer for 1 h at a temperature of 60 °C. The extract was filtered thrice through Whatman No. 1, and the resulting filtrate was subsequently stored in the refrigerator for future applications.

2.3 Synthesis of FeO NPs

A 30 mL of 0.1 M ferric chloride (0.1 M $FeCl_3$) solution was created and stirred for 30 min at room temperature. Subsequently, 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 1 M sodium hydroxide. The reaction mixture was left undisturbed for 24 h to facilitate the synthesis of FeO NPs. Afterward, the mixture was subjected to centrifugation at 6000 rpm for 15 min, and the sediment was gathered. The synthesized FeO NPs were then washed trice with double-distilled water, followed by calcination at 450°C for 2 h. The resulting solid pellets were subsequently crushed using a mortar and pestle to get a fine powder-like structure. The same process outlined was repeated twice to confirm the accuracy.

2.4 Characterization techniques

To confirm the successful synthesis of FeO nanoparticles, a small quantity was suspended in 10% H_2SO_4 at room temperature, and UV-visible (UV-vis) spectroscopy analysis was performed (CT-2600 BioTek). The analysis covered a resolution of 1 nm within 200 nm to 600 nm, utilizing a quartz cuvette. The morphology of particles was analyzed using scanning electron microscopy (SEM) (ZEISS, ULTRA PLUS, Moratuwa University, Sri Lanka). SEM pictures were analyzed and particle size was measured using Image J software (Schneider et al., 2012).

2.5 Seed Preparation

The tomato seeds were washed under running tap water for 5 min, followed by immersion in a 5% (v/v) detergent solution for another 5 minutes. Then the seeds were disinfected in a 70% (v/v) ethanol solution for 1 minute. Further disinfection was done by immersing the seeds in a 1.5% (v/v) NaOCl solution for 10 minutes. Finally, the seeds were washed thrice with double distilled water (DDW) for 5 minutes each to eliminate any residual disinfectant.

2.6 Germination Experiment Parameter

Treatment at concentrations of 5, 10, 50, 100, 500, and 1000 ppm was prepared using DDW. Prepared treatments were sonicated for 45 min at room temperature. Tomato seeds were soaked for 4 h in the relevant concentration suspension of FeO NPs then sterilized tissue paper was placed in each petri dish and subsequently, 2 ml of FeO NPs suspension was immediately poured after thorough agitation of each treatment concentration using a sterile micropipette tip (Nichipet EX, 100-1000 µL) under Microbiological Safety Cabinets (Faster BH-EN 2003). 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 (25 0C) for seven days. 1 ml of DDW was added to each petri dish on the 3rd. day. The experiment was replicated five times and observations were made daily for seven consecutive days to count the number of germinated seeds on the 8th day root length, shoot length, and fresh weight were measured.

2.7 Data Analysis

Germination percentage (GP%), Mean germination time (MGT), and seed vigor index were calculated according to the equation mentioned below.

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

MGT quantifies the average duration for seeds to germinate and is calculated by taking the inverse of the germination rate. It is determined by observing the emergence of a 2 mm radicle at regular intervals throughout the germination period. For each treatment group, MGT was calculated using the daily counts based on the formula provided below. (Mavi et al., 2010).

$MGT = \sum f.x / \sum f$

Where f is the number of seeds newly germinated, x is the number of experimental days. The germination rate index (GRI) is the parameter that reflects the percentage of germination of seeds during each day that best describes the germination percentage/speed relationship (Esechi, 1994). GRI was calculated using the daily counts based on the formula provided below.

GRI=G1/1 + G2/2+· · ·+ Gx/x

Where G1=Germination percentage × 100 on the first day after sowing, G2=Germination percentage × 100 on the second day after sowing. After 8 days, the seedlings were harvested and their biometric data, including shoot length (cm), root length (cm), and fresh weight seedlings (mg), were measured, and averaged. The vigor index was calculated using the following equation (Krushangi Maisuria and Patel, 2009).

Vigor index = {Root length (mm) + Shoot length (mm)} x Seed germination %

Data was analyzed to identify the significance of each treatment along with the control group (5, 10, 50, 100, 500, and 1000 ppm). All the data of each parameter in all experiments were subjected to statistical analysis using a completely randomized design of the one-way ANOVA test performed by SAS Studio statistical software. The value of the p-value of less than 0.0001 was considered statistically significant compared with the control group. Duncan's Multiple Range test (DMRT) was conducted to measure the least significant difference (LSD) between pairs of means.

3. RESULTS AND DISCUSSION

3.1 Formation of FeO NPs

A B C D

Fig -1: (A) Precursor salt and plant extract, (B) Mixture of precursor salt and plant extract after adjusting pH to 10, (C) Mixture after 24 hours incubation, (D) Magnetic FeO NPs after calcination.

Salvinia molesta plant extracts possess naturally occurring phytochemicals with dual functionality which act as green reducing and capping agents. These phytochemicals facilitate 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, the process involves the interconversion of their enol into the 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. This process as explained by Singh et al. (2018), offers a sustainable and environmentally friendly approach for nanoparticle synthesis. By utilizing readily available plant extracts, this method avoids the use of harsh chemicals and reduces environmental impact. As illustrated in Fig-1, the rapid observed alteration in the pale red precursor salt solution's color upon the addition of the brown plant extract, transitioning to a consistent hue reported by Dhavale et al. (2023), serves as a visual confirmation of the reaction and successful synthesis of FeO NPs. 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 color change, with the mixture transitioning to a brownish-black color. This observation is consistent with the established notion that a higher pH than 8 promotes magnetite (Fe_3O_4) formation, as reported by Kürsteiner et al. (2022), thus validating the study conducted.

3.2 Characterization of FeO NPs

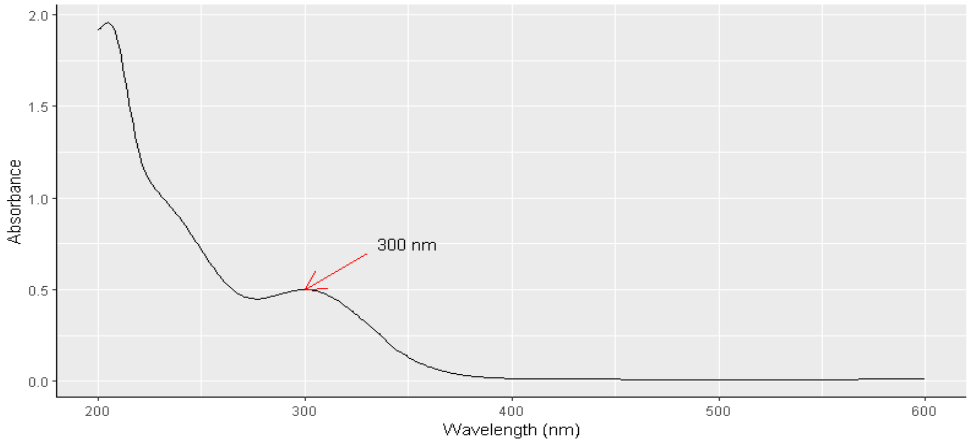

Chart -1: UV-vis spectroscopy analysis of FeO NPs.

UV-vis spectrometry has revealed the characteristic formation of green synthesized nanoparticles during color change based on the absorption spectra using surface plasmon resonance. Chart -1 illustrates scanning wavelength measurement from 200 to 600 nm was executed to reveal a peak value at 300 nm which indicated the formation of nanoparticles. A characteristic peak at 300 nm confirmed the formation of Fe_3O_4 Nanoparticles. From the spectrum, it can be seen the absorption of FeO NPs has stronger absorption near the UV band compared to the visible band of the electromagnetic spectrum. The previous studies align with this study's findings having a peak value of SPR at nearly 300 nm (Abderrhmane et al., 2021; Muthusamy, James and Sahoo, 2022).

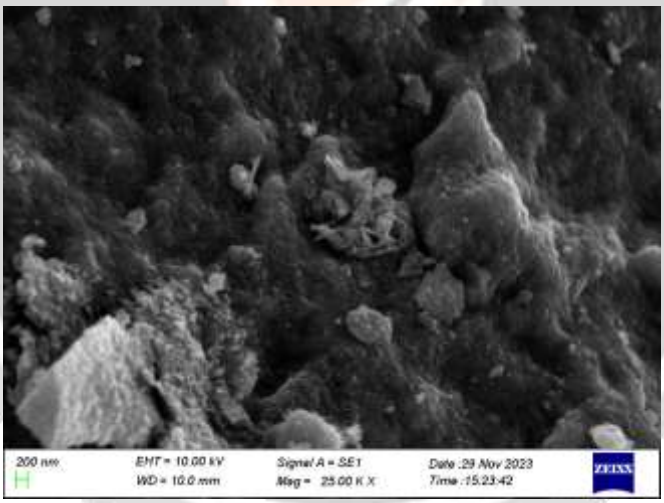

Fig -2: SEM images of FeO NPs at a scale of 200 nm.

The shapes and sizes of the FeO nanoparticles depicted in Fig-2 were observed through SEM images. The nanoparticles have a variety of shapes and sizes within the nanoscale range, with an average size of around 82 nm (ranging from 41 to 140 nm). They also tend to clump together and have multiple forms. These SEM results agree with previous observations by V.G. and A. Prem (2018). The aggregation of nanoparticles could be due to several reasons such as higher calcination temperatures and longer processing times can cause nanoparticles to develop uneven crystal structures and increase in size, both of which make them stick together more. Calcination is a thermal treatment that eliminates impurities of the synthesized nanoparticle (Parra and Haque, 2014) while the same treatment could affect the degradation of active agents, such as polyphenols, which are present on the surface of the nanoparticles and lead to aggregation of nanoparticles (Bhebhe et al., 2015).

3.3 Effect of FeO NPs on Germination

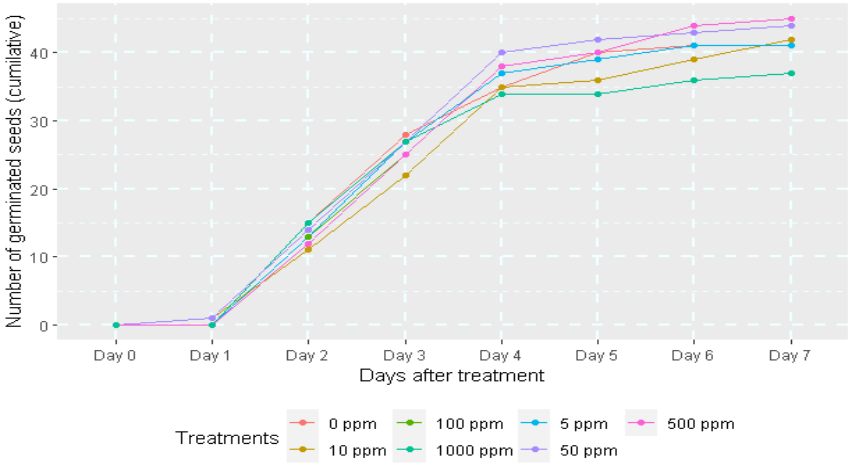

Chart -2: The cumulative daily germination count of tomato seeds.

Chart -2 illustrates the number of germinated seeds from day zero to day seven. By the end of the seven days, the maximum number of seeds germinated at 100 ppm and 500 ppm, with 45 out of 50 seeds germinating. The lowest germination occurred at 1000 ppm, where only 37 out of 50 seeds germinated. From day one, all treatment seeds exhibited rapid germination, peaking on day four and reaching the maximum number of germinated seeds by day seven. On day three, the control treatment (0 ppm) showed the highest number of germinated seeds, while 10 ppm had the least germinated seeds on the same day.

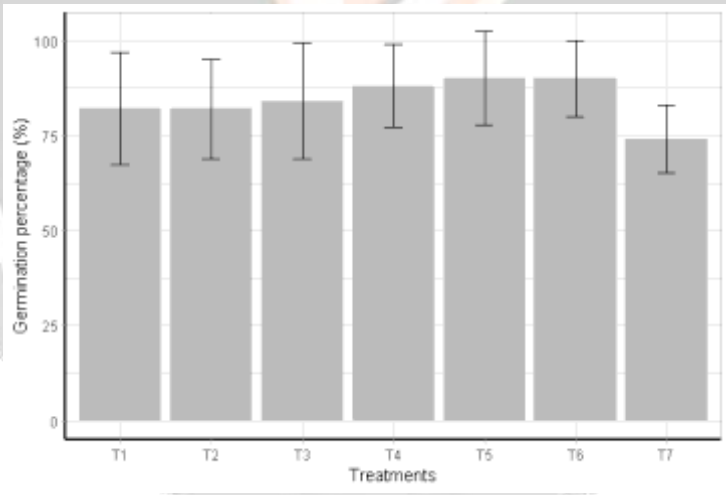

Chart -3: Effect of different concentrations of FeO NPs on the germination percentage of tomato seeds after 7 days of incubation. Bars illustrate ± standard error. T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

A statistical analysis using a one-way ANOVA test for the completely random design of independent groups, as illustrated in Chart -3, was conducted. The obtained P-value, greater than 0.0001, confirms that there is no significance of FeO NPs on seed germination, despite observed differences in each treatment. Once the seeds are treated with FeO NPs, the nanoparticles are presumed to diffuse through nano-holes on seed coats, thereby affecting the biological processes in the germinating seeds and causing different results for each treatment (Shankramma et al., 2015). Still, the higher concentration of FeO NPs (1000 ppm) has negatively affected seed germination compared to the control treatment (0 ppm). This effect could be attributed to the toxicity level of FeO NPs, leading to a retardation of cell development at higher concentrations. A previous study conducted by Vázquez-Núñez and Jessica Denisse Valle-García (2017) on FeO NPs and their impact on the percentage of germination and root elongation of carrot (Daucus carota), tomato (Solanum lycopersicum), broccoli (Brassica oleracea), and alfalfa (Medicago sativa) identified that the higher concentration (1000 ppm) resulted in the least germination percentage after 14 days of treatment. The same study conducted by Sundaria et al. (2018)

concluded that a concentration of 600 ppm suppressed seed germination, whereas concentrations lower than 200 ppm can promote germination and enhance the uptake of nanoparticles by the seeds. Iron nanostructures containing Fe0 and Fe(II) could directly reduce molecular oxygen dissolved in the aqueous solution inside the cell during seed germination, leading to the generation of Reactive Oxygen Species (ROS). This process induces oxidative stress, causing the excessive formation of hydroxyl radicals through the Fenton reaction, which damages the plant DNA (Wu et al., 2014). 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(Li et al., 2015). The gibberellins synthesized in the embryo then regulate the production of the enzyme α-amylases in aleurone layers, which is essential for seed germination(Raju, Mehta and Sashidhar Rao Beedu, 2016). High levels of α-amylases facilitate the hydrolysis of endosperm starch, providing energy for the germinating seed to develop roots and shoots (Kaneko et al., 2002).

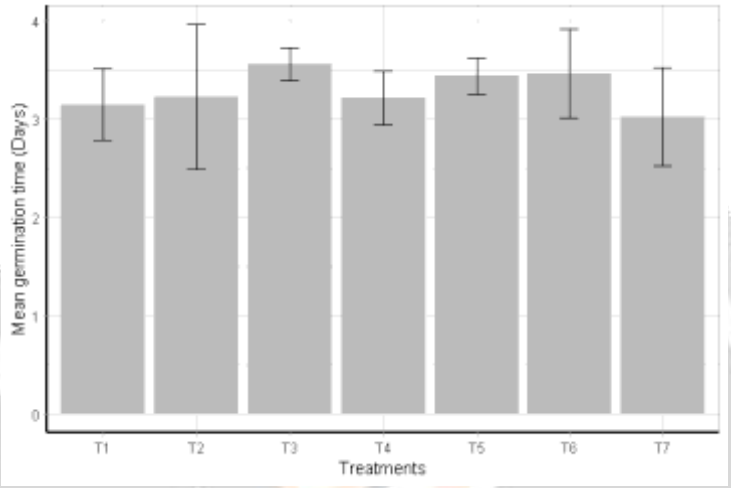

Chart -4: Effect of FeO NPs on the mean germination time Results illustrated the means of population and the bars illustrate ± standard error. T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

The mean germination time is illustrated in Chart -4. MGT can be defined as the duration of the lag phase, extending from the initiation of imbibition to the emergence of the radicle (Mavi, Demir and Matthews, 2010). No significant difference had been observed after the statistical analysis of ANOVA. A lower MGT was preferred as the population of seeds had germinated faster, and a higher MGT meant the seeds took a longer time for germination as the population's germination was slower. The lowest MGT was observed at 1000 ppm, measuring 3.023 days, while the highest MGT was observed in the 10 ppm treatment.

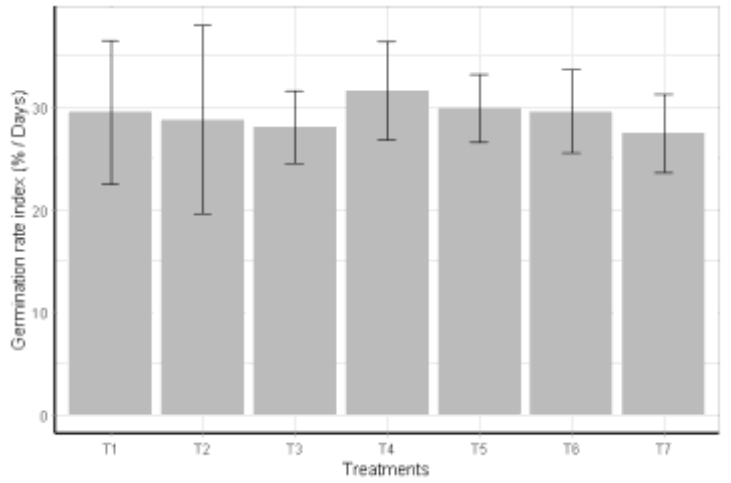

Chart -5: Effect of FeO NPs on the germination rate index. Results illustrated the means of population and the bars illustrate ± standard error. T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6

(500 ppm), T7 (1000 ppm).

The germination rate index of the study is illustrated in Chart -5. GRI calculations indicate the daily percentage of germination. Therefore, a higher percentage coupled with a shorter duration results in a higher GRI, and vice versa. After conducting statistical analysis using one-way ANOVA, no significant difference was observed for GRI. In the study, the highest value was obtained at 50 ppm, reaching 31.586% per day, while the lowest was 27.452% per day. Several studies indicate minimal phytotoxic effects of biogenically synthesized metallic nanoparticles on various plant species (Anwar et al., 2021). They also witnessed a decline in the germination index, root and shoot lengths, as well as the levels of photosynthetic pigments and total proteins in Vigna radiata seedlings when subjected to biogenically produced silver nanoparticles.In a study assessing the impact of biogenically synthesized iron nanoparticles on corn germination, positive outcomes were observed, including a higher germination index and increased seedling root/shoot length (Tovar et al., 2020)

3.4 Effect of FeO NPs on seedling fresh weight

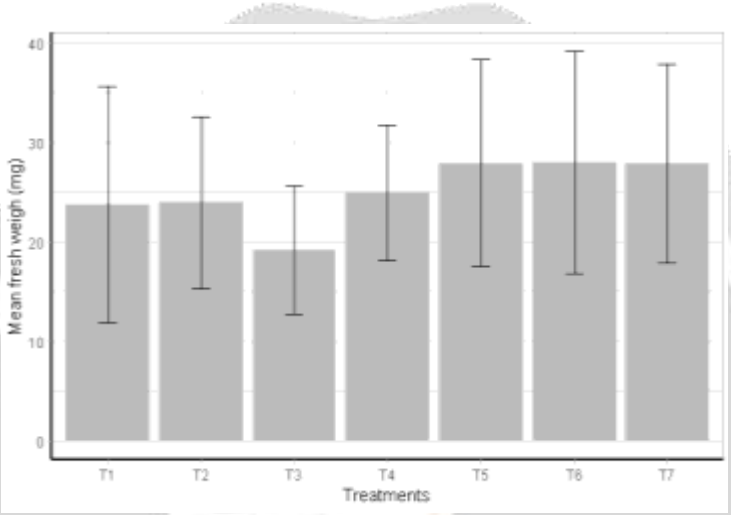

Chart -6: Effect on different concentrations of FeO NPs on fresh weight (mg). Results illustrated the means of population and the bar illustrates ± standard error.T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

The fresh weight of seedlings was analyzed using one-way ANOVA, and no significance was found in each treatment for the mean value. However, as illustrated in Chart -6, the highest mean fresh weight, 29.971 ± 9.99 mg, was obtained in the 1000 ppm treatment, while the lowest, 19.160 ± 6.646 mg, occurred in the 10 ppm treatment. The control treatment yielded a mean fresh weight of 23.736 ± 11.84 mg. Notably, all other treatments, except for 10 ppm other treatmetns of 5 ppm, 50 ppm, 100 ppm, 500 ppm, and 1000 ppm resulted in higher mean fresh weights(23.961 ± 8.64 mg, 24.902 ± 6.82 mg, 27.878 ± 10.39 mg, 27.925 ± 11.16 mg, 2.971 ± 9.99 mg respectively) demonstrating the promoting effect of FeO NPs on the fresh weight of tomato seedlings. This suggests that FeO NPs have a positive effect on enhancing the fresh weight of tomato seedlings, which is beneficial. The research conducted on the effect of iron nanoparticles on seed germination and seedling growth in rice identified that iron nanoparticles promoted the fresh weight of rice seedlings (Hao et al., 2016). Similar results were obtained in the research conducted by linseed priming with iron oxide nanoparticles, showing enhanced fresh weights compared to their corresponding control levels (Muhammad Waqas Mazhar et al., 2022). According to the study conducted by Dipanjoli Baral Dola et al. (2022) on the effects of foliar application of Fe_3O_4 nanoparticles on the growth, yield, and seed quality of soybeans under drought-stressed conditions, it was concluded that applying 200 ppm nano-Fe_3O_4 boosted fresh and dry plant weight in both drought and well-watered conditions, compared to untreated plants. The same conclusion was drawn from a study on green-synthesized nanoparticles that influenced seed germination and seedling growth in tomatoes. The study stated that iron nanoparticle-treated tomato seedlings exhibited increased parameters such as seed vigor, shoot length, and fresh and dry weight (Abusalem et al., 2019). In another study on the effect of iron nanoparticles on soybeans, it was discovered that the application of iron nanoparticles at concentrations of 50, 100, 250, 500, 1000, and 2000 ppm resulted in root length increases of 6, 8, 19, 27, 37, and 40%, respectively, compared to the control group (Alidoust and Isoda, 2013). A study conducted on the influence of iron nanoparticles (Fe_3O_4 and Fe_2O_3) on the growth of wheat plants (Triticum aestivum) identified that even though there was no significant difference in the fresh weight between the treated and control groups, the dry weight of the treated plants showed an increase compared to the untreated control plants (Kreslavski et al., 2022).

3.5 Effect of FeO NPs on seedling root length

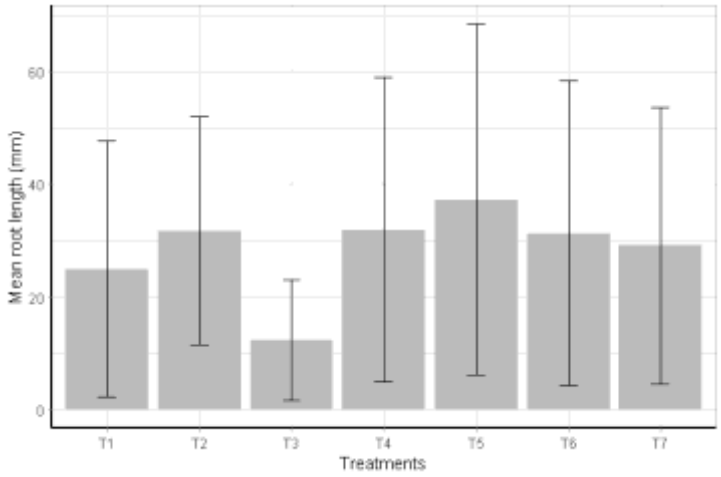

Chart -7: Effect on different concentrations of FeO NPs on root length (mm). Results illustrated the means of population and the bar illustrates ± standard error.T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

Seedling root length was measured and analyzed using one-way ANOVA, revealing no significance with a P-value greater than 0.0001. As illustrated in Chart -7, the highest mean root length was observed at 100 ppm, reaching 37.238 ± 31.15 mm, while the control seedlings (0 ppm) had a mean root length of 24.951 ± 22.83 mm. All treatments, except for 10 ppm (mean root length 12.333 ± 10.74 mm), exhibited higher root lengths than the control treatment. Concentrations of 5, 50, 500, and 1000 ppm resulted in mean root lengths of 31.730 ± 20.35 mm, 31.951 ± 27.11 mm, 31.300 ± 27.12 mm, and 29.114 ± 24.63 mm, respectively. After reaching the highest mean root length at 100 ppm, a decline in mean root length can be observed with increasing concentrations. The study conducted on iron oxide nanoparticles' impact on *Vigna mungo* seed germination, shoot, and root development identified that iron oxide nanoparticles affected root length at different concentrations. Specifically, at 0 ppm, 100 ppm, 200 ppm, 300 ppm, and 400 ppm, the mean root lengths were found to be 1.9 ± 0.8 mm, 4.1 ± 0.5 mm, 6.8 ± 0.2 mm, 1.7 ± 0.7 mm, and 1.1 ± 0.5 mm, respectively (Rajiv et al., 2017). The study's findings suggest that 200 ppm resulted in the highest mean root length, and increasing iron nanoparticle concentration after that led to the inhibition of root length. A similar study conducted on the effect of iron nanoparticles on wheat seedlings identified varying concentrations. The root lengths for 0, 1.0, 1.5, 2.0, and 2.5 ppm of iron nanoparticles were found to be 3.7 cm, 5.1 cm, 7.02 cm, 7.96 cm, and 2.3 cm, respectively (Toufiq Iqbal, Jahangir Alam, and Sultana, 2015). Their findings also suggest that at some point, the maximum root length is achieved, and concentrations of iron nanoparticles beyond that point inhibit root growth. The difference in mean root length could be attributed to the biphasic effect of nanoparticles. Nanoparticles involve two distinct phases or stages in plant growth and development (Tripathi et al., 2017). The majority of nanoparticles induce toxicity at certain concentrations, impacting crop productivity by modifying morpho-anatomical, physiological, biochemical, and genetic compositions. The toxicity thresholds of iron oxide nanoparticles depend on factors such as the concentration of the nanoparticles, exposure media, and plant species (Zuverza-Mena et al., 2017). 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, and Komatsu, 2015). 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 (Konate et al., 2018). In light of this study, it is suggested that FeO NPs have the potential to be utilized as a seed priming agent, influencing the elongation of tomato seedling roots.

3.6 Effect of FeO NPs on seedling shoot length

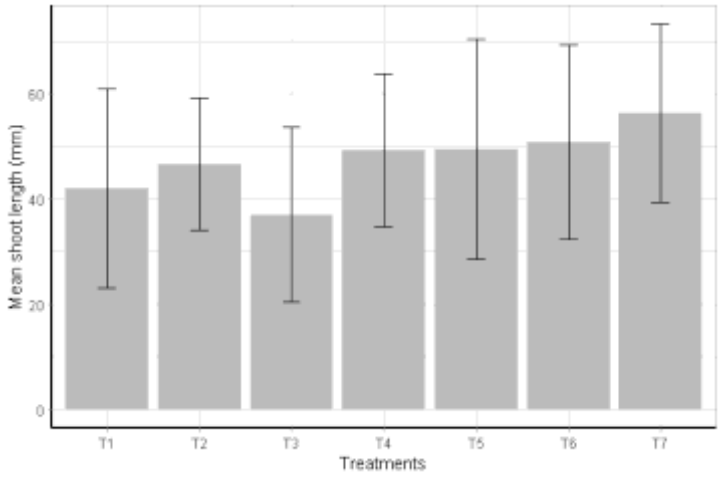

Chart -8: Effect on different concentrations of FeO NPs on shoot length (mm). Results illustrated the means of population and the bar illustrates ± standard error.T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

In the study, seedling shoots were measured, and statistical analysis using one-way ANOVA was conducted, revealing no significant difference. As illustrated in Chart -8, the highest mean shoot length occurred at 1000 ppm, measuring 56.257 ± 16.89 mm. For the control (0 ppm), 5 ppm, 50 ppm, 100 ppm, 500 ppm, and 1000 ppm, the mean shoot lengths were 41.923 ± 18.91 mm, 46.615 ± 12.57 mm, 49.170 ± 14.49 mm, 49.476 ± 21.01 mm, 50.800 ± 18.42 mm, and 56.257 ± 16.89 mm, respectively. The lowest mean shoot length was recorded at 10 ppm, measuring 37.000 ± 16.56 mm. Except for the mean shoot length at 10 ppm, all other treatments exhibited a positive effect of FeO NPs on shoot length, demonstrating a gradual increase. In the study, the seedlings were cultivated in a petri dish, and a significant portion of the shoot developed on the surface of the petri dish, indicating that nanoparticles can be absorbed into the growing shoot. Similar results were obtained in a research study on the influence of iron oxide quantum dots on black gram (Vigna mungo) seeds treated with a wide spectrum of concentrations ranging from 0 to 1000 ppm. The highest shoot length was observed at 500 ppm, measuring 25.4 cm. There was a gradual increase in shoot length from 0 ppm to 500 ppm (Mugundha, Pon and Sk, 2022). In another study on the bioaccumulation of transition metal oxide nanoparticles and their influence on the early growth stages of Vigna unguiculata seeds, different concentrations of iron nanoparticles (0 ppm, 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm) were tested. The study identified that the highest shoot length was achieved at 1000 ppm (Suriyaprabha et al., 2018). A study conducted on the effects of Fe_3O_4 nanoparticle stress on the growth and development of rocket (Eruca sativa), with seedlings exposed to different concentrations of Fe_3O_4 nanoparticles (1 mg/L, 2 mg/L, and 4 mg/L), identified that the highest shoot length occurred at 4 mg/L (Plaksenkova et al., 2019). 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 1000 ppm (Rui et al., 2016). Tawfik et al. (2021) conducted a study on the effect of Fe_3O_4 nanoparticle treatments on the growth parameters of Moringa oleifera L by applying different concentrations of Fe_3O_4 (0, 20, 40, and 60 ppm) as a foliar application. The study identified that the maximum plant length occurred in the 60 ppm treatment, and the 40 ppm treatment had the highest values for the content of crude protein, crude fiber, and ash in plant leaves. They also identified that foliar application of 40 ppm Fe_3O_4 recorded the highest values for N, P, and K, as well as the K/Na ratio.As per the findings of Tripathi et al. (2017), the interplay between plants and various nanoparticles is intricately intricate, relying on diverse factors including nanomaterial characteristics such as shape, size, surface attributes, crystal chemistry, dosage, etc., as well as plant-related variables such as genotype, age, the supportive soil or growth medium, illumination intensity, exposure route (bottom-up or top-down), etc. The varying factors associated with both plants and nanoparticles contribute to differences in root and shoot development. Further investigation is required to identify the underlying causes and processes.

3.7 Effect of FeO NPs on seed vigor index

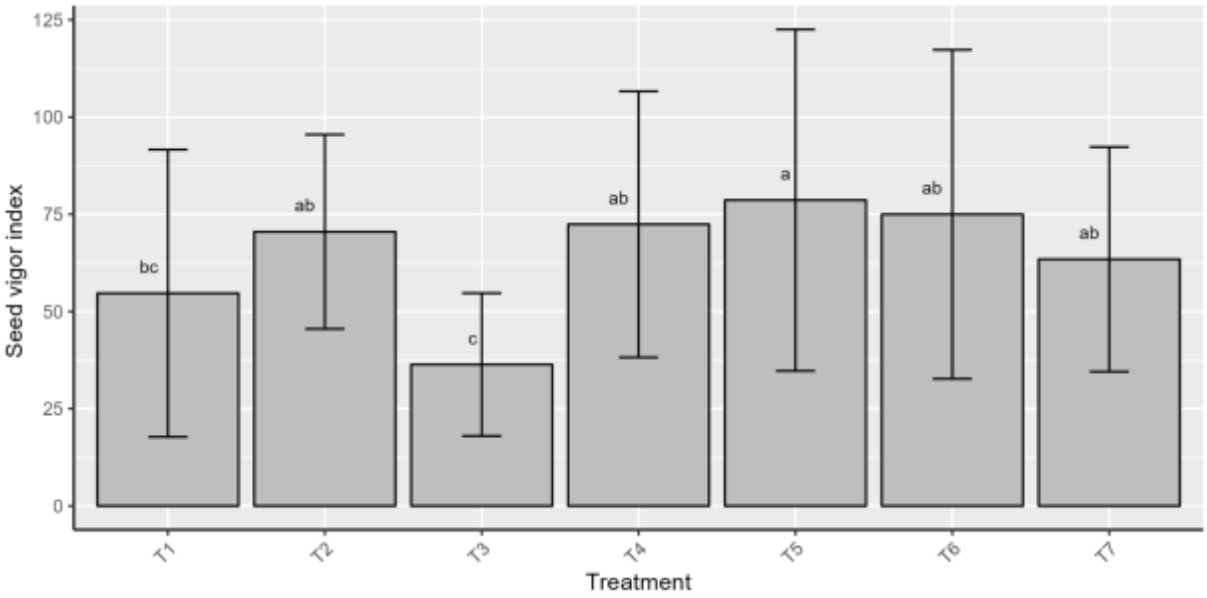

Chart -9: Effect on different concentrations of FeO NPs on seed vigor index. Results illustrated the means of population and the bar illustrates ± standard error. Different letters above the bars indicate significant differences at $p < 0.0001$ per DMRT analysis. T1 (0 ppm), T2 (5 ppm), T3 (10 ppm), T4 (50 ppm), T5 (100 ppm), T6 (500 ppm), T7 (1000 ppm).

Seed vigor is a crucial aspect of agricultural research. Chart -9 displays the effects of different concentrations of FeO NPs on the seed vigor index.. The seed vigor index was statistically analyzed using one-way ANOVA, revealing a significant effect, and the significance was observed ($p < 0.0001$, $F = 5.571$). Duncan's multiple range test revealed the highest seed vigor at a concentration of 100 ppm, reaching 78.62381 ± 43.89, while the control group (0 ppm) exhibited a seed vigor of 54.68049 ± 36.93. According to the Dunnett's test having a 95% family-wise confidence level all treatments except for 10 ppm (seed vigor of 36.35926 ± 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 70.51154 ± 24.99, 72.40976 ± 34.20, 74.98500 ± 42.29, and 63.42857 ± 28.87, 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 (HU et al., 2012), the production of ROS (Bailly, 2004), protein carbonylation (Rajjou et al., 2008), and the loss of enzyme activity (Lehner et al., 2008). 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 ROS that can cause severe damage to cells. Superoxide (O_2^-), the precursor of many other ROS, is formed through the reduction of molecular oxygen (O_2) (Turrens, 2003). 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, including IONPs, 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). However, the study is limited and requires further study on identifying and understanding of the FeO NPs on seed germination and seedling growth.

4. CONCLUSIONS

FeO NPs were successfully synthesized using *Salvinia molesta* plant extract and characterized through UV-visible spectroscopy and SEM analysis. Green-synthesized nanoparticles exhibit a biphasic effect on seedling germination and growth. The impact of different concentrations can both promote and inhibit germination simultaneously, while also affecting plant height in various ways. The study recommends a concentration range of 50 ppm to 500 ppm for treating seedling germination. Further research is needed to elaborate on how

effective FeO NPs on germination and seedling growth in a wide range of nanoparticle concentrations and greater population size.

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