Effect of Green Synthesized Ferrous Oxide Nanoparticles on Seed Germination in Tomato (*Solanum lycopersicum*) and Eggplant (*Solanum melongena*)

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Abstract *– Seed treatments are effective in increasing seed germination. Nanotechnology has been developed as a seed-priming technology for smart agriculture. Fe is one of the essential micronutrients for plants. The aim of the present study is to investigate the effect of green synthesized Ferrous oxide (FeO) nanoparticles on seed germination. Salvinia molesta leaf extract was used to react with 0.1 M Ferric Chloride concentration. Synthesized nanoparticles were characterized using an Ultraviolet-visible spectrometer (UV-Vis) and Scanning Electron Microscopy (SEM). The average size of nanoparticles was 42.33 nm. UV-visible spectroscopy at 339 nm confirmed the formation of Ferrous Oxide nanoparticles. Seeds of tomato (Solanum lycopersicum) cultivar Tillina and eggplant (Solanum melongena) cultivar Lena Iri belonging to the family Solanaceae* were used in the seed germination test. *Surface sterilized seeds in Petri dishes were treated with different volumes; 2 ml (T1), 1 ml (T2), 0.5 ml (T3) of synthesized Ferrous Oxide nanoparticles. 2 ml Salvinia leaf extract (R1), and 2 ml distilled water (R2) are the two controls. Twenty seeds from each cultivar were placed on filter paper in a Petri dish and three replicates per treatment. Distilled water was added to each treatment at 2-day intervals to provide water requirement for germination. After seven days of each treatment, the effect on seed germination was evaluated by measuring shoot and root length and fresh weight. According to statistical analyses, Ferrous Oxide NPs 1 ml and 0.5 ml (T2 and T3) significantly promoted the % seed germination, root length, shoot height, and fresh weight. However, 2 ml strength (T1) of the NPs showed a negative effect for both root and shoot growth. The results confirmed that low strengths of Ferrous Oxide NPs promoted seed germination, shoot, and root growth rate. The higher strength of* Fe_2O_4 *metallic nanoparticles did not promote the germination rate.*

Keywords*: green nanotechnology, Ferrous Oxide Nanoparticles, Family- Solanaceae, Solanum lycopersicum*, *Solanum melongena*

1. INTRODUCTION

Seed treatments are effective in increasing seed germination, plant growth, and potential yield, which will lead to economic sustainability in commercial agriculture. More recently, nanotechnology has been developed as a seed-priming technology for smart agriculture. Due to their unique characteristics such as higher surface area to its mass higher active nature and advanced optical electrical, thermal, and physical properties, nanoparticles (NPs) can deliver the substances efficiently (Hamdy *et al.*, 2022). Because of the higher surfaces area, higher reactivity, and higher solubility, they can easily interact with cell membrane and other cellular components like proteins, lipids. Numerous reports indicate that NPs can increase crop production by enhancing various physiological processes including seed germination, plant growth, etc (Feng *et al*., 2022). Metallic nanoparticles and their carriers have the potential to act as a carrier agent in biological systems (Abid *et al*., 2022). Seed priming with nanoparticles is an arguable and most attractive topic in the current field of research. Literature is available on the performances of different nanoparticles on plants in various manners, CuO nanoparticles can inhibit seed germination while TiO NPs have rapid action on seed germination (Feng *et al*., 2022). Seed treatment with metallic NPs promote vigorous seedling growth, increase the seeding emergence speed. In addition, primary seed treatment induces enzymatic activity like protease, amylase, and the breakdown of the required macromolecules in the embryo that required for the emergence (Abid *et al*., 2022). Therefore, the cultivators can reduce the expenses for re-seedlings, time, and cost, and extra irrigation, fertilizer also.

The biological synthesis of NPs contributes to the development of both environmental and health. It has a simple protocol, low energy consumption, and low production cost compared to chemical and physical methods. Plants, microorganisms, algae, and yeast extracts are biological sources used as stabilizing and encapsulating agents in green NP synthesis. According to Demirezen *et al*., 2018, terpinols, polyphenols, and sugars are mainly responsible for determining the morphology of the biosynthesised nanoparticle. Interestingly, Ferrous Oxide NPs are biodegradable, biocompatible, nontoxic for human (Duffy *et al*., 2018). Ferrous is one of the essential micronutrients for plants which can regulate the number of growth process of the plant's chlorophyll biosynthesis, photosynthesis, chloroplast development, and dark respiration (Feng *et al*., 2022). Thus, iron deficiency causes structural and functional changes in the photosynthetic process. According to Feng *et al*., 2022, Fe_2O_4 nanoparticles improve the growth parameters and induce the germination of wheat grains.

Tomato (*Solanum lycopersicum*) is a demanding and widely cultivated vegetable plant mainly grown by medium-scale commercial farmers in Sri Lanka. Tomato is consumed or utilized in miscellaneous ways including raw fruit, cooked food, as a drink, and a vegetable (Zhao *et al*., 2021). Abiotic factors and diseases are the main limiting factors for tomato production. It is susceptible to a number of diseases caused by microorganisms that include fungi, bacteria, viruses, and nematodes (Bala Raju *et al*., 2017). Eggplant (*Solanum melongena*) has a relatively low seed germination ability with the sought-after vegetables that have a health-promoting effect and can cure diseases such as disease exacerbations (Gonzales, 2015). In addition to various abiotic factors, seed genetic characteristics are accountable for seed germination and viability. The present study hypothesized that seed priming with plant-based nanoparticles would improve the germination rate/emergence rate, seedling vigour, and other growth parameters of tomato and eggplant. Currently, a few studies are reported on identifying the effect of green synthesized nanoparticles on the seed germination process. This study investigates the effects of green synthesized ferrous Oxide nanoparticles on tomato and eggplant seed germination and their shoot, root performances under different concentrations.

2. MATERIALS AND METHODOLOGY

2.1 Green Synthesis of Nanoparticles

FeO NPs synthesis using *Salvinia molesta* leaf extract *S. molesta* plants were collected from the Beddagana wetland park, Sri Jayawardhanapura, Kotte in June 2022. Plants were washed with tap water for three times and rinsed with de-ironized water for 30 minutes. The samples were then dried in an air dryer at 42^{o}C overnight. Dried leaf samples were grounded using a blender and 5 g of grounded sample was mixed with 80 ml of deionized water at 60°C of temperature. The extraction was filtered using Whitman's filter paper no 01 three times. Leaf extraction was stored in the refrigerator for future use.

Thirty ml (30 ml) of 0.1 M $FeCl_3$. $6H_2O$ was prepared and stirred for 10 minutes using a magnetic stirrer. Then leaf extract was added to $FeCl_3$. $6H_2O$ at 2:3 ratio. pH was adjusted to 8 by adding (1 M) NaOH. The reaction mixture was stored at room temperature for about 24 h and the mixture was centrifuged at 1400 rpm for 15 min. The supernatant was removed, and the pellet was washed using de-ionized water.

2.2 Nano Particle Characterization

2.2.1 SEM analysis and UV-Vis Spectroscopy

UV–Vis Spectrometry analysis was carried out by using CT-2600 UV-Vis Spectrometers (BioTek©) with a resolution of 1 nm between 200 and 700 nm. The resulting FeO NPs pellets were re-suspended in deionized water and used for characterization. SU6600 Scanning Electron Microscope, (HITACHI) was used for the morphology, size, and analysis of the particle distribution of Ferrous Oxide nanoparticles and microscopic structure was observed at 50.00 KV and 100 KV and under multiple (KX) magnifications.

2.3 Preparing Seeds

The prepared NPs pellet was diluted with 5 ml of deionized water and to avoid agitation and disperse the particles equally using (CLASSIC) Advanced vortex mixture (120 v) for 30 min. 0.1 M NPs were prepared in different volumes: 2 ml, 1 ml, and 0.5 ml.

S. lycopersicum and *S. melongena* seeds were purchased from Agri Acardy (SA/MTG/02642) (Purity Percentage, 98%, germination percentage 75 – 80 %). *Tillina* tomato cultivar and *Lena Iri* eggplant cultivar were used for the study. Seeds were sterilized in a 1:1:1 ratio of tap water, 2% sodium hypochlorite, and deionized water to ensure surface sterility. There were five treatments *ie*. T1 - 2 ml, T2 - 1 ml, T3 - 0,5 ml 0.1 M FeO NPs, Control 1 (R1) - 2 ml *Salvinia* leaf extract and Control 2 (R2) -2 ml distilled water. Seeds were soaked for about 4 hours in these treatment solutions separately. 20 seeds from each variety were placed in a petri dish on filter paper and there were three replicates per each treatment. The treatment solutions were added to separate Petri dishes with seeds and covered, sealed, and kept in the incubator. Distilled water was added to each treatment at 2-day intervals to provide water requirement for germination. After seven days, germination percentage was counted.

2.4 Determination of growth characteristics

2.4.1 Percentage Seed Germination

The number of germinated seeds was recorded after 7 days. Shoot emerged from half of the seed length is considered as the seed was germinated (Umar, 2016). % seed germination was calculated as a proportion to the total number of seeds (Feng *et al*., 2022).

The germination percentage was calculated using sixty (60) seeds per treatment as per the equation given below.

$$\text{Germination (\%)} = {}^{\text{Number of germinated seed}}\!/_{\text{Number of incubated seeds}} \text{ X } 100$$

2.4.2 Shoot Length, Root Length and Fresh Weight of Seedlings

Seeds were wetted with 2 ml of de-ionized water in 2-day intervals. After 7 days seedlings were harvested and measured root length (RL= distance from the root base to the root tip), shoot length (SL= distance from the leaf base to the leaf tip), and fresh weight.

2.5 Data Analysis

For the analysis of the SAS statistical software was used. ANOVA, Least Significant Difference, T test was used to identify the significant in strength (2, 1, 0.5 mL) of the nanoparticles along with the two controls (R1 – seeds were treated with *Salvinia* leaf extract and R2 - seeds were treated with distilled water). A value of $p<0.0001$ was statistically significant, compared to the controls.

3. RESULTS AND DISCUSSION

Fig.1 shows the color change before and after the formation of nanoparticles in the reaction medium. *S. molesta* leaf extract color change confirmed the formation of FeO NPs. Color change (as shown in Fig. 1) of *Salvinia* plant extract is from greyish brown to brown color after adding $FeCl_3$. $6H_2O$ and it turned brownish black after adjusting the pH value to 8 in the mixture. This is due to the presence of a number of phytochemicals in the leaf extract, which play as a reducing agent of the metallic irons into its Nano form. These phytochemicals are bound with metallic nanoparticles and disperse in the aqueous liquid. Similar results have been observed in Ferrous Oxide Nanoparticles synthesised using *Pometia pinnata* leaf extract (Umar, 2016).

UV-Vis Spectroscopy of green synthesized FeO NPs of *S. lycopersicum* and *S. melongena.* confirmed by recording the absorbances of UV-Vis spectra in the range of 200- 800 nm (Fig. 02). In this study (SPR) the surface plasmon resonance at 339 nm was observed in FeO NPs extracted using *Salvinia* leaf extract. SEM image of the nanoparticles is a grain in shape and mean particle size 42.33 nm in diameter. The sizes, shape and particles distribution were characterized using the SEM (HITACHI) SU6600 Scanning Electron Microscope (Fig. 03). As shown in Fig.4, NPs are poly-dispersed without showing agglomeration. It demonstrates that nanoparticles are grain in shape and mean particle size 42.33 nm in diameter. The particle size distribution of green synthesized FeO NPs depicts that most particles are distributed in the range of 11 to 30 nm. The Ferrous oxide nanoparticle synthesis using *Trigonella* and tomato extracts in the range of 27.91 to 40.94 nm in sizes were previously reported (Abid *et al*., 2022).

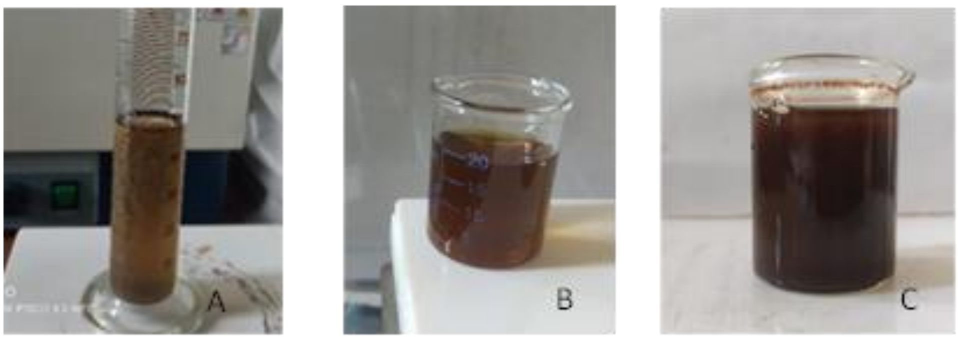
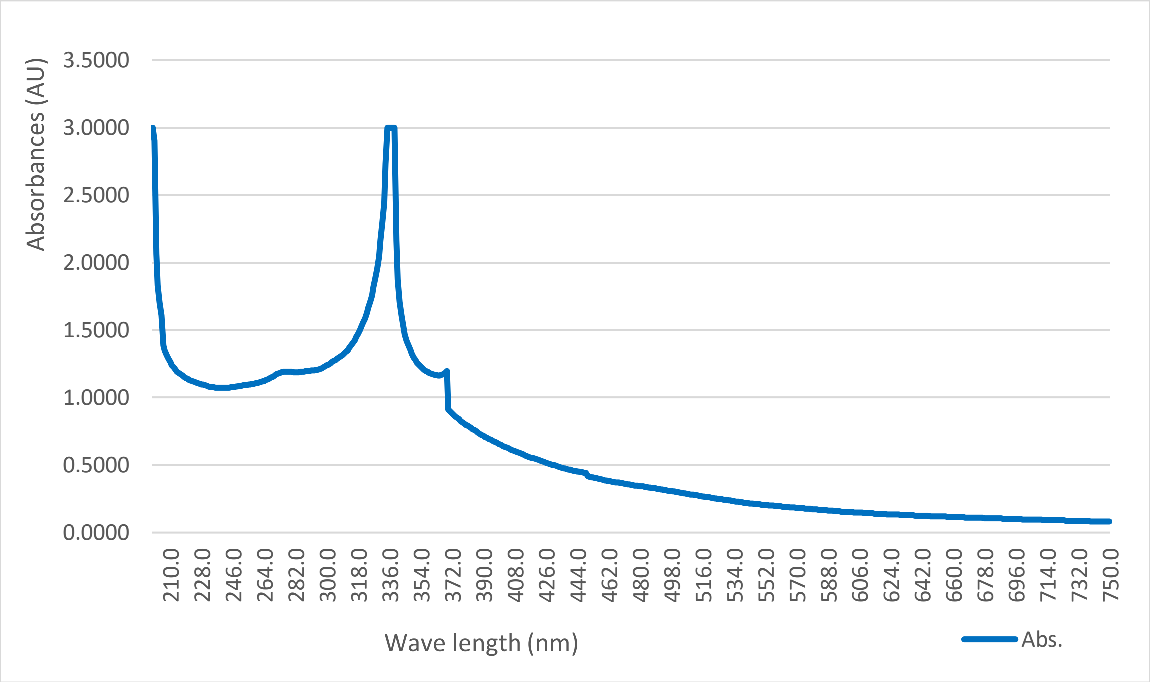
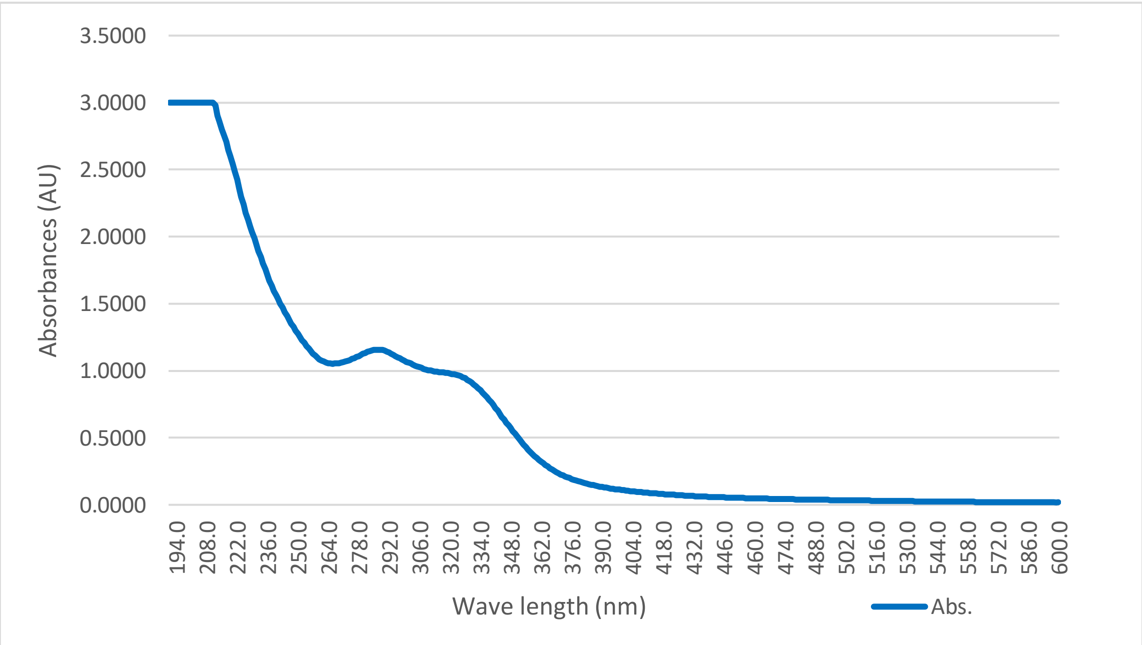


Fig. 1. (A): Colour of the S. molesta leaf extract, (B): Reaction mixture, (C): Colour of the reaction mixture after 24 hours



(a)



(b)

Fig. 2. UV-Vis Spectroscopy of green synthesised FeO NPs of (a) ***S. lycopersicum*** **and (b)** ***S. melongena***

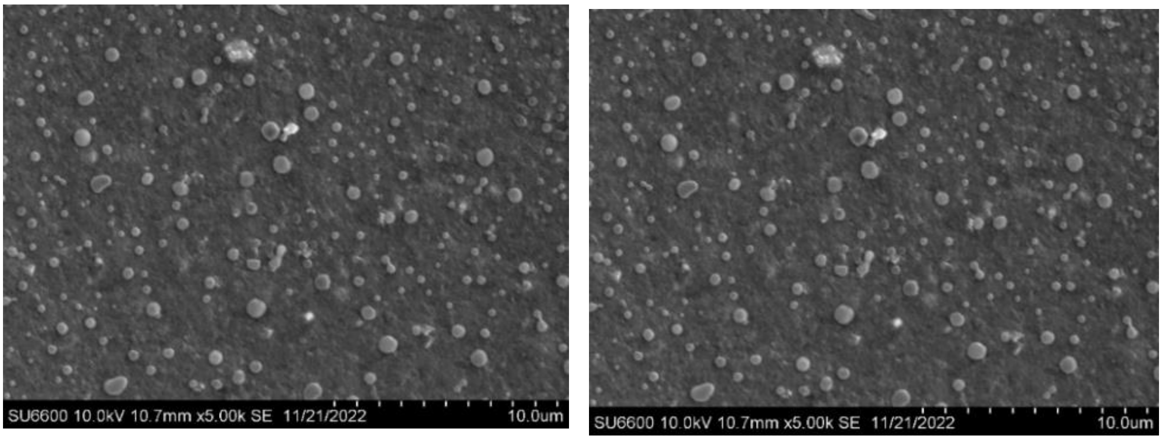


Fig.3. SEM image on green synthesised FeO NPs

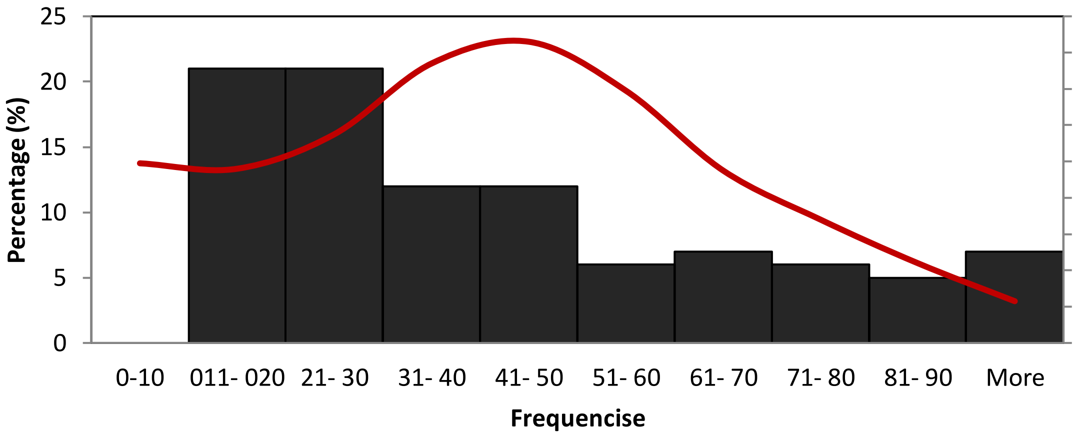


Fig.4. Partical sizes distribution of green synthesisied FeO NPs.

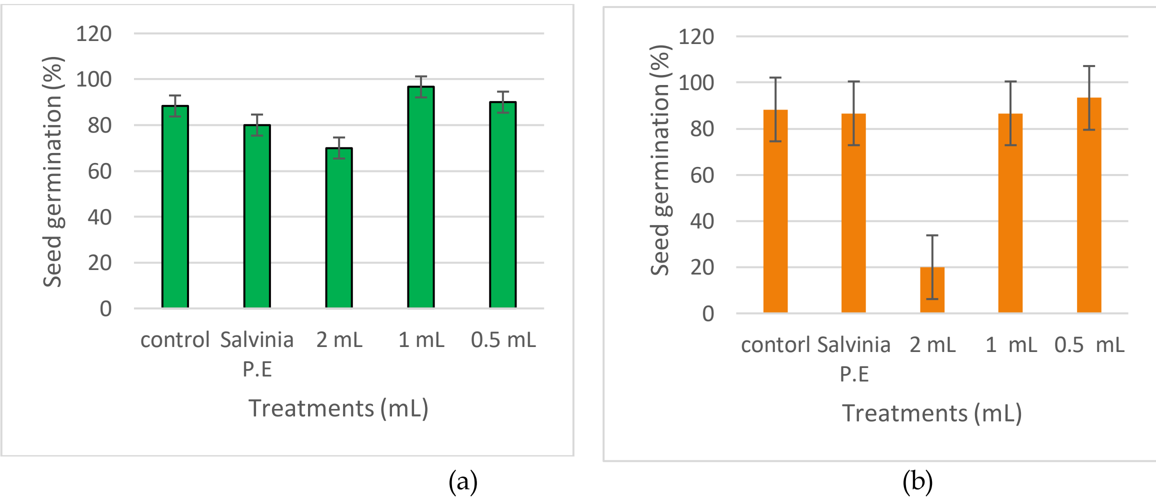
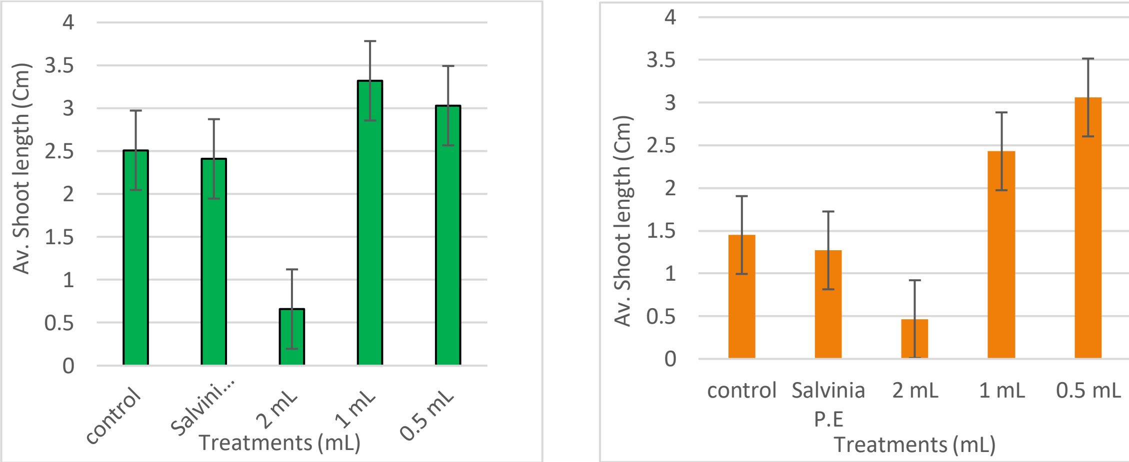


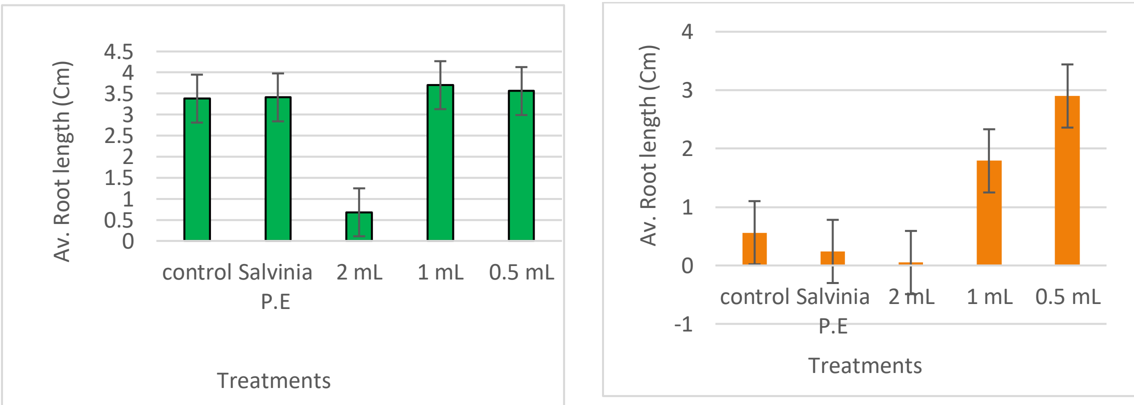
Fig. 5. Effect of FeO NPs on (a) *S. lycopersicum* and (b) *S. melongena* seed germination



(a) (b)

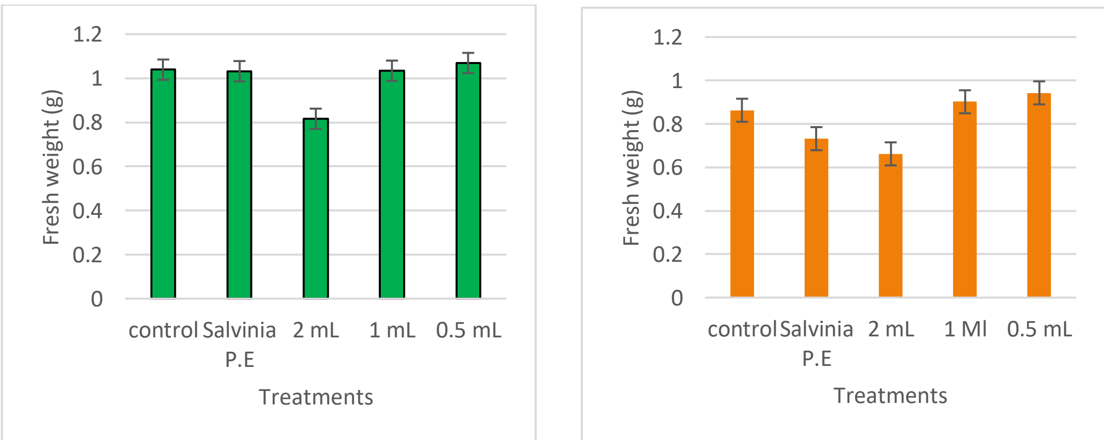
Fig.6. Effect of FeO NPs on (a) *S. lycopersicum* and (b) *S. melongena* shoot length.

The % seed germination was calculated 7 days after each seed treatment. It was around 75 - 80% in both cultivars. According to Fig.5 (a) and (b), % seed germination is considerably high in T3 - 0.5 ml metallic NPs in *S. lycopersicum* and *S. melongena* compared to Control 1 (R1), Control 2 (R2) and other treatments. There was a considerable reduction in the % seed germination with T1-2 ml metallic NPs treated seeds.



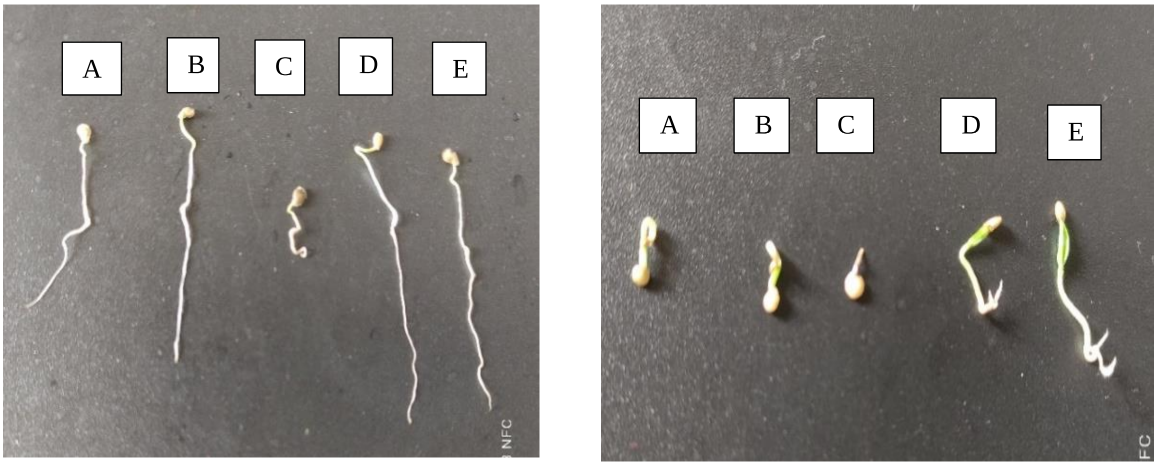
(a) (b)

Fig. 7. Effect of FeO NPs on (a) *S. lycopersicum* and (b) *S. melongena* root length



(a) (b)

Fig.8. Effect of FeO NPs on (a) *S. lycopersicum* and (b) *S. melongena* fresh weight



(a) (b)

Fig.9. Growth of seedlings of (a) *S. lycopersicum* and (b) *S. melongena* after applying treatments (A - Control 1 - 2 mL Distilled water, B - Control 2 - 2 mL *Salvinia* leaf extract, C-T1-2 mL FeO NPs, D-T2-1 mL FeO NPs, E-T3-0.5 mL FeO NPs)

According to Fig.6 (a) and (b) as well as Fig. 7 (a) and (b), the average shoot and root length of S. *lycopersicum* is considerably high with T2 - 1 mL metallic NPs and in *S. melongena* it is high with T3 - 0.5 ml metallic NPs. Fig. 8 (a) and (b) explains the variation in fresh weight of *S. lycopersicum* and *S. melongena.* T3- 0.5 ml showed the highest fresh weight in *S. lycopersicum* as well as *S. melongena* seedlings. Tables 1 and 2 showed significant differences in the growth rate of seedlings compared to the two controls R1 and R2. Shoot and root growth of both *S. melongena* and *S. lycopersicum* seedlings showed significant differences with the control samples. Since iron is an essential micronutrient for plant growth, under 1.0 ml, and 0.5 ml of FeO NPs it showed significant growth in both shoot and root of seedling. In the study of (Hao *et al*., 2016; Devi and Singh, 2016). demonstrated the bio-effect of Fe_2O_3 nanoparticles on germination of rice seed, root and shoot length, and fresh weight. The effect of NPs depends on particle sizes, morphology, and surface response, and exposure status of NPs (Verma *et al*., 2020), it directly affects the uptake and translocation of the particles.

The average shoot and root length of *S. lycopersicum* and *S. melongena* have significantly reduced with T1-2 ml metallic NPs. Thus, there was no clear trend observed in the root growth of *S. lycopersicum* seedlings comparative to the controls (Table 1). According to Fig. 8 (a) and (b) and 9 (a) and (b), all treatments except T1-2 ml of metallic NPs have a more or less similar impact on the fresh weight of S. *lycopersicum* and *S. melongena* seedlings. These results provide evidence (Fig.9 (a) and (b)) that metallic NPs could affect the seed germination, root length, shoot length and fresh weight of seedlings depending on the strength of NPs.

Table 1. ANOVA, LSD, T test shoot output

T grouping	Mean	Treatments
A	3.0600	T3 - 0.5 ml
B	2.4300	T2 - 1 ml
C	1.4500	R1 control 1
C	1.2700	R2 control 2
D	0.4650	T1- 2 ml

Table 2. ANOVA LSD, T test Root output

T grouping	Mean	Treatments
A	2.90	T3 - 0.5 ml
B	1.79	T2 - 1 ml
C	0.56	R1 control 1
C	0.24	R2 control 2
D	0.05	T1 - 2 ml

Overall, all the treatments show significant influence ($p<0.0001$) on the shoot and root of *S. lycopersicum* and *S. melongena* plants with different strengths of green synthesised Ferrous Oxide NPs (Fig.6 and 7, Table 1 and 2). 0.5 ml (T3) of green synthesized Ferrous Oxide NPs gave significantly highest shoot and root length in seedling of *S. lycopersicum* and *S. melongena* than all the other treatments. 1 ml (T2) green synthesized Ferrous Oxide NPs showed next significant effect on shoot and root length of *S. lycopersicum* and *S. melongena* seedlings than the control treatments, R1 and R2 and T1 treatment. R1, R2 (Control treatments) showed significantly lower shoot and root length in seedlings of *S. lycopersicum* and *S. melongena* than the two treatments T3 (0. 5 ml) and T2 (1 ml) green synthesised FeO NPs. However, within R1 and R2 treatments, there was no significant differences between each other. T1 (2 ml) green synthesised FeO NPs treatment recorded the lowest significant effect on shoot and root length in seedlings of *S. lycopersicum* and *S. melongena*.

According to (Feng et al., 2022) this positive effect is due to the higher solubility of the Iron Oxide particles and the effect on the seed radicals. In addition to the particle concentration, the shape of the synthesised nanoparticles also depends on the performance of the seeds. According to (Sandeep et al, 2019; Montanha et al., 2020), germination, root length, shoot length, seedling dry weight and seedling vigour index were significantly increased in Soybean with Ag and Fe NPs and (Zhao et al., 2021) reported that $Fe2O3$ enhances the seed germination ratio of Spanish plant during the hydroponics.

5. CONCLUSIONS

The present study investigated the impact of Ferrous Oxide NPs synthesized using *Salvinia molesta* leaf extract on seed germination, root, shoot length, and fresh weight of *S. lycopersicum* and *S. melongena* seedlings. Nanoparticles at lower strengths (0.5 ml and 1 ml) can promote % seed germination, root length, shoot height, and fresh weight. According to statistical analyses, 0.5 ml of Ferrous Oxide NPs was identified as the highly performing treatment for shoot and root length of *S. lycopersicum* and *S. melongena* seedlings. This could be due to requirement of Fe as a trace metallic element for plant growth and other biological activities. The findings could be used as an alternative methodology to promote seed germination and other related growth factors of seedlings.

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