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AMELIORATION OF SOYBEAN SEEDS VIABILITY BY NANO-PRIMING OF PHYTO-SYNTHESIZED NANOPARTICLES

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Abstract

Soybean is a legume as well as an oil seed crop worldwide. The Yield potential of the crop is being hampered by various factors. Poor germination is the leading cause of yield reduction. Seed priming is an important technique to boost the germination percentage. The present research work was executed to probe the seed priming potentials of some photosynthesized nanoparticles. The nanoparticles were prepared using plant extracts of *Azadirachta indica*, *Eucalyptus globulus* and *Syzygium cumini* and applied as seed priming agents to three soybean varieties; Ajmeri, Faisal, and Rawal-1 at different concentrations. Results of the germination percentage of soybean seeds primed with the nanoparticles revealed the germination ranged from 74-98%, while in the case of plant extracts, the maximum germination was ranged 70-86%. In case of seedling root length, maximum (9.63 cm) was recorded in soybean seeds of the ajmeri variety primed with 80 mg/L of *S. cumini* nanoparticles, while the lowest (6.50 cm) at 60 mg/L of the same plant extract nanoparticles. Plant extracts showed comparatively greater values than the nanoparticles. The value of root fresh weight in case of nano-priming were ranged 0.66-4.98 g comparatively superior over plant extracts i.e., 0.51-4.95 g. Maximum root dry weight (0.49 g) was recorded in case of $C_1 \times NP_1 \times V_3$ whereas lowest mean root dry weight (0.10 g) was recoded in case of $C_1 \times NP_1 \times V_1$. The values of other growth parameters were recorded as superior in the case of nano-priming than plant extracts seed priming. From the results, it can be concluded that nano-priming of soybean seed can boost up the germination potential, leading maximization of the crop yield.

Key words: Soybean, *Glycine max*, Oil seed, Seed priming, Photosynthesize, Nanoparticle.

Introduction

Soybean, *Glycine max* (L.) Merr. is a valuable and versatile field crop due to its rapid growth. Among the many names it has been given are "meat of the field," "meat without bones," "wonder crop," "golden bean," "agriculture's Cinderella," "queen of pulses," and "farmer's friend" (Surendra & Sharma, 2018). It tops the list of economically significant oilseed crops grown worldwide (Mishra *et al.*, 2024 and Fakhri *et al.,* 2024). This oilseed legume crop accounts for 80 percent of the area, equivalent to 68 percent of the world's total legume production (Naamala *et al.*, 2016). Soybean seed is a rich source of vitamins and minerals, comprised of 40-42% protein, 20-22% oil, and 20-30% carbohydrates (Akmalovna, 2022). It has become a significant agricultural crop and a remarkable export commodity in both temperate and tropical regions (Hamza *et al.,* 2024).

Pakistan has cultivated soybean as an oilseed crop since the 1960s, and commercial soybean cultivation is highly successful in KP, Punjab, and Sindh. But yet edible oil and oilseed-based foods and feedstocks are imported by Pakistan (Khurshid *et al.*, 2017) which may be due to a lack of adaptable soybean germplasm, area-specific production technology, extension service, and a consistent policy pest-resistant genotypes, poor seed germination, and marketing infrastructure despite favourable agro-ecological conditions (Asad *et al.*, 2020).

The Development and growth of plants are being profoundly impacted by a variety of environmental stresses, both biotic and abiotic (Safdar *et al.*, 2022). The critical stages during the growth of crops are the uniform seed germination, early seedling growth, and uniform plant stand. Low crop yield is attributed to uneven seed germination and seedling growth. Therefore, the quality of seed can be improved through priming in addition to the field management techniques for better seed germination (Rhaman *et al.*, 2020). Priming is an effective method that can increase the number of seeds that germinate and the overall growth of plants, which will ultimately lead to an increase in productivity despite changes in environmental conditions and stresses. The successful establishment of plants and the development of a robust root system are dependent on the germination rate of their seeds. Seed priming is commonly described as the deliberate moistening of seeds to a specific extent that permits early metabolic processes to occur without the emergence of the primary root.

(Abdalla *et al.*, 2022). Priming increases plant antioxidant activity and stress resistance, as well as plant growth and productivity. It also improves the viability of seeds and sprouting processes, which accelerates and synchronizes the germination (Johnson & Puthur, 2021). Different methods of seed priming; Osmo-priming, hydro-priming, hormonal priming, halo-priming and chemical-priming are being used. However, there are some challenges and limitations associated with seed priming, such as optimal priming duration, storage conditions, seed quality control and environmental variability (Rhaman *et al.*, 2020). Therefore, more research is needed to optimize seed priming protocols and explore new methods of seed priming for different crops. Nanotechnology has rapidly become an important area of research across all academic fields, including medicinal chemistry (Abdelghany *et al.*, 2018). Nanoparticles (NPs) are being synthesized in different ways. Chemical reduction is the process that is typically used to produce nanoparticles. Chemical

synthesis, on the other hand, requires the use of high pressure, toxic solvents, energy conversion, and extreme temperature. The green technique of nanoparticle synthesis is environmentally friendly, straightforward, and effective. This is made possible by the eco-friendliness of plant-based alternatives. Green nano priming is the name given to the process of priming seeds with green nanoparticles (Abbasi *et al.*, 2021).

Nano-priming has emerged as the most successful technique for priming seeds, principally due to its microscopic size and unique physicochemical features (Tripathi *et al.,* 2024).

Because of these physiological differences, the uptake of nanoparticles during the nano-priming process is different for each plant species, and as a result, so is the rate and manner in which they grow (Rhaman *et al.*, 2020). It is essential in the agricultural industry to have the capability of enhancing the germination performance of older or dormant seeds. (Vijayaram *et al.,* 2024)

This method can effectively stimulate the germination of seeds without causing the seedlings to become poisoned. As a result, green nano priming possesses a tremendous amount of untapped potential for boosting crop yield (Song & He, 2021).

Materials and Methods

Research site: The present research work was executed at the Department of Plant Breeding and Genetics (Seed Science and Technology), University of Agriculture, Faisalabad.

Collection of plant leaves: The leaves of *Azadirachta indica*, *Eucalyptus globulus,* and *Syzygium cumini* were collected from sites of the university. After being washed twice with distilled water, the leaves were allowed to dry at room temperature. After the leaves had been dried, an electric grinder was used to reduce them to a fine powder, which was kept at room temperature in an airtight container until further use (Fig. 1).

Extract in water: After stirring the mixture for six hours at a low temperature, ten grams of dried powder made from a variety of plant leaves were added to one hundred milliliters of distilled water. The contents were filtered through eight layers of muslin cloth every two hours, and then the filtrate was centrifuged at 5000 revolutions per minute for fifteen minutes. This process was carried out twice more, after which the supernatant was collected, and a rotary vacuum evaporator was used to concentrate it while the pressure was lowered. After being pasteurized, the concentrated extract was kept at a temperature of 4 degrees Celsius.

Extraction using a solvent: Using a rotator shaker set to 190-220 rpm, 10 grammes of leaf powder were extracted with 100 mL of ethanol, acetone, petroleum ether, and chloroform, in that order, over 24 hours. The contents were filtered through eight layers of muslin cloth every two hours, and then the filtrate was centrifuged at 5000 revolutions per minute for fifteen minutes. This process was carried out twice more, after which the supernatant was collected, and a rotary vacuum evaporator was used to concentrate it while the pressure was lowered. After being pasteurized, the concentrated extract was kept at a temperature of 4 degrees Celsius (Fig. 2).

Synthesis of NPs of zinc oxide: To synthesize zinc oxide nanoparticles, a 50 ml leaf extract of Eucalyptus globulus was freshly prepared and heated to 70-80°C. Subsequently, 4 gm of zinc nitrate hexahydrate was slowly introduced into the hot leaf extract, resulting in an immediate change in solution color to reddish-brown. The reaction mixture was then maintained at 70-80°C with magnetic stirring. Over time, the color gradually transitioned to pale yellow until finally turning reddish orange. Upon completion of the reaction, a paste formed, which was transferred to a crucible and heated at 400°C for 2 hours in an oven. The resultant end product was a pale white powder, utilized for further experimentation (Aminuzzaman *et al.*, 2018).

Characterization of nanoparticles: The plan for characterization analysis was done by the Government College University in Faisalabad, Pakistan. The experiment used a variety of spectroscopic techniques, including UV-visible spectroscopy, SEM, and XRD spectroscopy, to determine the characterization of the nanoparticles, such as their size, dimension, and so on (Fig. 4).

UV-Vis spectrophotometer: The diluted solution of synthesized zinc oxide nanoparticles was analyzed by UV-Vis spectrophotometer to check the maximum absorption of ZnONPs. The maximum peak with a wavelength of 370 nm was recorded (Fig. 3.).

Determine the seed viability: The seed collection of three distinct genotypes; Ajmeri, Faisal, and Rawal-1 of soybean was done from a soybean lab at the CAS University of Agriculture, Faisalabad, Pakistan. Seeds were cleaned on the outside with 0.5% sodium hypochlorite for 10 minutes. Normal (T_0) seeds were exposed to hydro-priming for 24 hours. Sterilized seeds were exposed to green synthetic nano priming with 40 (T_1), 60 (T_2), and 80 (T_3) mg/L solutions for 24 hours at 27°C. During the first few hours, the seeds got oxygen from an aquarium pump. As required by the ratio of weight to volume. After that, the seeds were washed well with distilled water and then dried at 25°C so that they kept about the same weight as they had before. Seed-germination papers were rolled up twice and put in a plastic bag so that thirty seeds could be planted in each replication. These were kept in the growth chamber for 7 days at 25°C with 60% relative humidity. For T_0, T_1, T_2 and T_3 seed germination papers were moistened with water, making sure that the seeds were completely wet in the solution, and the papers were changed every two days. The following parameters were studied:

Germination percentage: It was calculated by dividing number of healthy seedlings by the entire number of seeds in the test and multiplies by 100 as described by Riis (1995):

$$\text{Germination percentage} = \frac{THS}{TNS} * 100$$

where: THS =Total number of seedlings, TNS = Total number of seedlings

Fig. 1. a & b, plant leaves; c & d, dry powders.

Fig. 2. Extraction process of plant extracts.

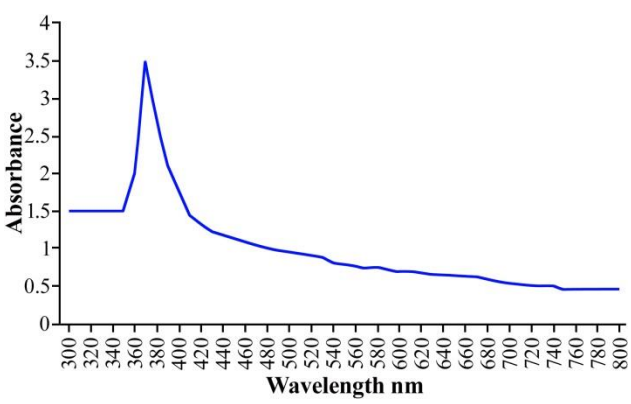

Fig. 3. UV-Visible spectroscopy of green-synthesized ZnONPs.

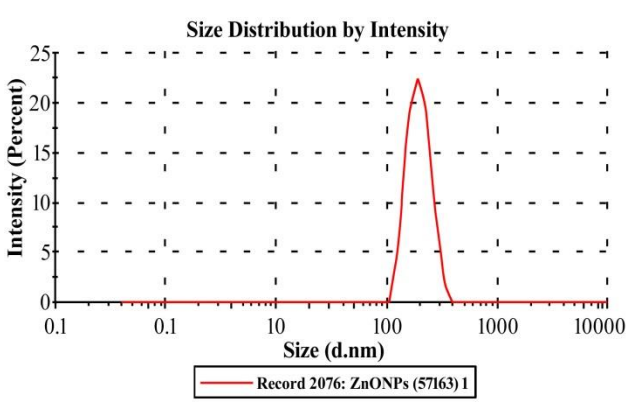

Fig. 4. Particle size analysis of green-synthesized ZnONPs by XRD spectroscopy.

Fig. 5. Seed germination of soybean: a, Plant extracts application; b, nanoparticles applied plot.

Seedling root and shoot length (cm): Three batches of twenty seeds each were employed for the germination process, all exposed to uniform testing conditions. The seeds were laid out longitudinally on germination paper, with their micropyles directed towards the paper's lower surface. These rolls were then enclosed in plastic bags and vertically positioned within a controlled germination chamber set at 25°C. The evaluation began 36 hours after sowing, with three batches of 10 randomly selected seedlings per treatment examined. Seedling lengths were gauged using a ruler marked in centimeters, and assessments were conducted at intervals up to 204 hours post-sowing. Each seedling was individually evaluated to track its unique growth pattern. The average results, expressed in centimeters per seedling, were documented for each treatment (Fig. 5).

Fresh and dry matter of the seedlings: Ten seedlings per treatment were randomly chosen for assessment of their fresh weights, including seedling, root, and shoot weights. An analytical balance with a precision of 0.001 g was employed for accurate weighing, and the results were averaged and expressed in milligrams per seedling. For dry weight determination, seedlings from each replication were separated into shoots and roots after the removal of cotyledons and then dried in an oven at 65°C for 72 hours. Subsequently, the ten seedlings were re-weighed using the analytical balance.

Statistical analyses: The collected data were subjected to analysis of variance (ANOVA). Treatments means were separated by the Tukey-HSD test at α=5%.

Results

Results revealed that maximum germination (98.00%) was recorded in the case of each of the $C_1 \times NP_2 \times V_1$ and $C_2 \times NP_3 \times V_1$. Similarly, $C_1 \times NP_3 \times V_1$ and $C_3 \times NP_1 \times V_3$ triggered equal germination percentage i.e. 95.00%. $C_2 \times NP_1 \times V_3$ and $C_3 \times NP_2 \times V_3$ showed equal effects regarding germination (%) of the three soybean varieties. $C_3NP_3 \times V_3$ caused 90.00% germination, while the lowest germination (71.00%) was recorded in the case of $C_1 \times NP_1 \times V_1$ (Fig. 6).

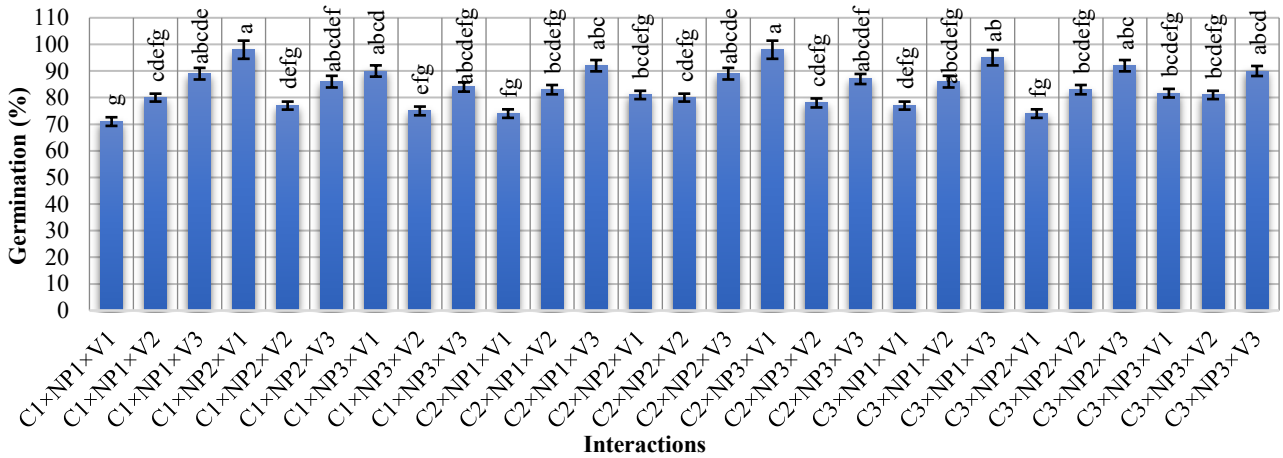

Fig. 6. Interactive effect of the different, green-synthesized nanoparticles and concentrations for seed germination percentage of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. NP_1 = Green-synthesized nanoparticles of *Eucalyptus globulus* extract, NP_2 = Green-synthesized nanoparticles of *Azadirachta indica* extract, C = concentration, NP_3 = Green-synthesized nanoparticles of *Syzygium cumini* extract, V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean.

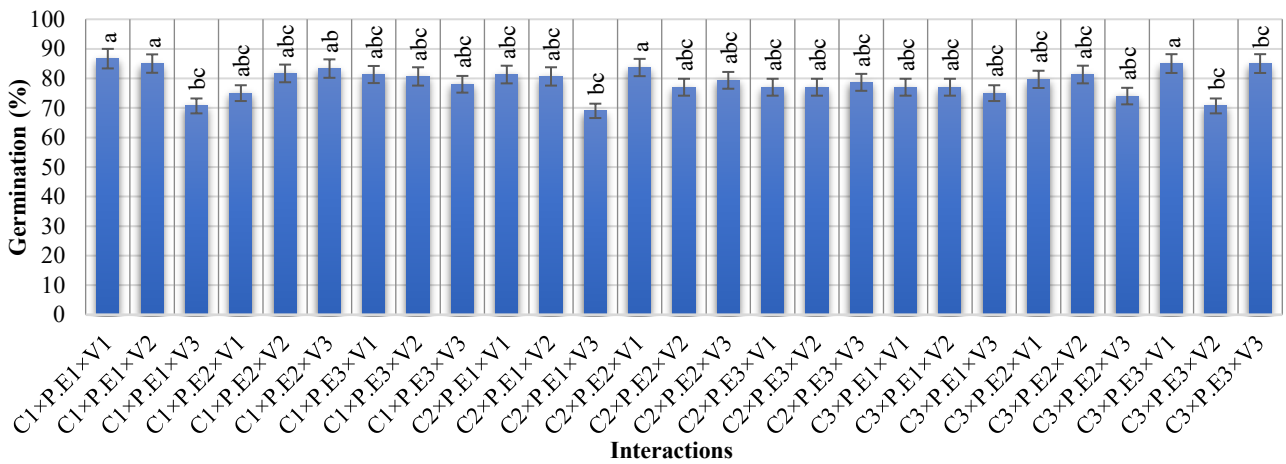

Fig. 7. Interactive effect of the different plant extracts and concentrations for seed germination percentage of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. P.E = Plant extract, $P.E_1$ = *Eucalyptus globulus* extract, $P.E_2$ = *Azadirachta indica* extract, C=concentration, $P.E_3$ = *Syzygium cumini* extract V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean.

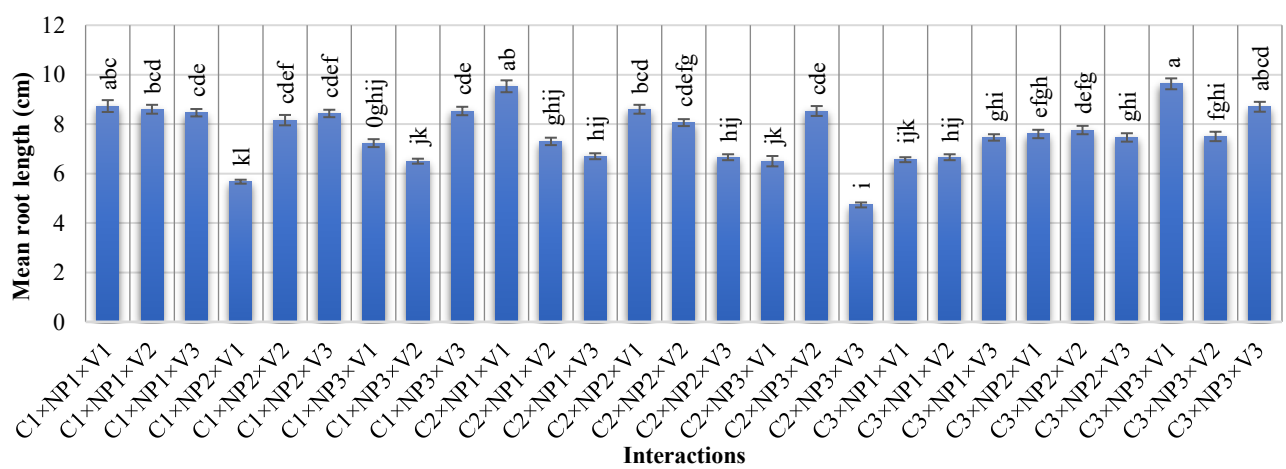

Fig. 8. Interactive effect of the different, green-synthesized nanoparticles and concentrations for mean root length of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. NP_1 = Green-synthesized nanoparticles of *Eucalyptus globulus* extract, NP_2 = Green-synthesized nanoparticles of *Azadirachta indica* extract, C = concentration, NP_3 = Green-synthesized nanoparticles of *Syzygium cumini* extract V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean.

Table 1. Interaction effects of different concentrations, nanoparticles and soybean varieties on root dry weight.

Concentrations × Nanoparticles × Varieties	Root dry weight ± S.E.
$C_1 \times NP_1 \times V_1$	0.10 ± 0.02j
$C_1 \times NP_1 \times V_2$	0.28±0.02 abcdefghij
$C_1 \times NP_1 \times V_3$	0.49 ± 0.09a
$C_1 \times NP_2 \times V_1$	0.24 ± 0.01cdefghij
$C_1 \times NP_2 \times V_2$	0.42 ± 0.04abc
$C_1 \times NP_2 \times V_3$	0.19 ± 0.01fghij
$C_1 \times NP_3 \times V_1$	0.38 ± 0.02abcde
$C_1 \times NP_3 \times V_2$	0.15 ± 0.01hij
$C_1 \times NP_3 \times V_3$	0.33 ± 0.05abcdefgh
$C_2 \times NP_1 \times V_1$	0.16 ± 0.01ghij
$C_2 \times NP_1 \times V_2$	0.34 ± 0.01abcdefg
$C_2 \times NP_1 \times V_3$	0.12 ± 0.01ij
$C_2 \times NP_2 \times V_1$	0.30 ± 0.02abcdefghi
$C_2 \times NP_2 \times V_2$	0.32 ± 0.01abcdefgh
$C_2 \times NP_2 \times V_3$	0.24 ± 0.01cdefghij
$C_2 \times NP_3 \times V_1$	0.44 ± 0.01ab
$C_2 \times NP_3 \times V_2$	0.21 ± 0.01efghij
$C_2 \times NP_3 \times V_3$	0.39 ± 0.01abcde
$C_3 \times NP_1 \times V_1$	0.23 ± 0.02defghij
$C_3 \times NP_1 \times V_2$	0.41 ± 0.03abcd
$C_3 \times NP_1 \times V_3$	0.18 ± 0.02fghij
$C_3 \times NP_2 \times V_1$	0.33 ± 0.04abcdefgh
$C_3 \times NP_2 \times V_2$	0.13 ± 0.02 ij
$C_3 \times NP_2 \times V_3$	0.34 ± 0.01abcdefg
$C_3 \times NP_3 \times V_1$	0.36 ± 0.03abcdef
$C_3 \times NP_3 \times V_2$	0.27 ± 0.06 abcdefghij
$C_3 \times NP_3 \times V_3$	0.45 ± 0.06ab

Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. NP1 = Green-synthesized nanoparticles of *Eucalyptus globulus* extract, NP2 = Green-synthesized nanoparticles of *Azadirachta indica* extract, C = concentration, NP3 = Green-synthesized nanoparticles of *Syzygium cumini* extract V = variety, V1 = Ajmeri variety of soybean, V2 = Faisal variety of soybean, V3 = Rawal-1 variety of soybean

Table 2. Interaction effects of different concentrations, plants extracts and soybean on root dry weight.

Concentrations × Nanoparticles × Varieties	Root dry weight ±S.E.
$C_1 \times PE_1 \times V_1$	0.134 ± 0.01efghijklmn
$C_1 \times PE_1 \times V_2$	0.11 ± 0.02mn
$C_1 \times PE_1 \times V_3$	0.119 ± 0.02jklmn
$C_1 \times PE_2 \times V_1$	0.128 ± 0.06ghijklmn
$C_1 \times PE_2 \times V_2$	0.137 ± 0.09defghijklmn
$C_1 \times PE_2 \times V_3$	0.146 ± 0.04 abcdefghijkl
$C_1 \times PE_3 \times V_1$	0.155 ± 0.04abcdefghi
$C_1 \times PE_3 \times V_2$	0.164 ± 0.04abcdef
$C_1 \times PE_3 \times V_3$	0.173 ± 0.03abc
$C_2 \times PE_1 \times V_1$	0.160 ± 0.05abcdefg
$C_2 \times PE_1 \times V_2$	0.113 ± 0.03lmn
$C_2 \times PE_1 \times V_3$	0.122 ± 0.04ijklmn
$C_2 \times PE_2 \times V_1$	0.130 ± 0.01fghijklmn
$C_2 \times PE_2 \times V_2$	0.140 ± 0.06 cdefghijklmn
$C_2 \times PE_2 \times V_3$	0.149 ± 0.08 abcdefghijk
$C_2 \times PE_3 \times V_1$	0.158 ± 0.06 abcdefgh
$C_2 \times PE_3 \times V_2$	0.167 ± 0.06 abcde
$C_2 \times PE_3 \times V_3$	0.176 ± 0.06ab
$C_3 \times PE_1 \times V_1$	0.10 ± 0.06n
$C_3 \times PE_1 \times V_2$	0.116 ± 0.08klmn
$C_3 \times PE_1 \times V_3$	0.125 ± 0.05hijklmn
$C_3 \times PE_2 \times V_1$	0.134 ± 0.09 efghijklmn
$C_3 \times PE_2 \times V_2$	0.143 ± 0.01bcdefghijklm
$C_3 \times PE_2 \times V_3$	0.152 ± 0.02 abcdefghij
$C_3 \times PE_3 \times V_1$	0.161 ± 0.03abcdefg
$C_3 \times PE_3 \times V_2$	0.170 ± 0.02abcd
$C_3 \times PE_3 \times V_3$	0.179 ± 0.18a

Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. P.E = Plant extract, $P.E_1$ = *Eucalyptus globulus* extract, $P.E_2$ = *Azadirachta indica* extract, C = concentration, $P.E_3$ = *Syzygium cumini* extract, V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean

Results (Fig. 7) revealed that maximum germination (86.67%) was noted in the case of $C_1 \times PE_1 \times V_1$ which was not statistically different from $C_1 \times PE_1 \times V_2$, $C_3 \times PE_3 \times V_3$, $C_3 \times PE_3 \times V_1$ and $C_2 \times PE_2 \times V_1$. 83.31% germination was recorded in the case of $C_1 \times PE_2 \times V_3$. The Lowest germination (69.00%) was recorded in case of $C_2 \times PE_1 \times V_3$ but statistically not different from $C_3 \times PE_3 \times V_2$. The effects of the rest–of the interactions were statistically non-significant.

Outcomes (Fig. 8) showed that the highest root length (9.63 cm) was noted in the case of $C_3 \times NP_3 \times V_1$, $C_2 \times NP_1 \times V_1$ was the next effective one (9.53 cm) while the lowest mean root length (4.73 cm) was measured in case of $C_2 \times NP_3 \times V_3$. Effects of $C_1 \times NP_1 \times V_3$ and $C_1 \times NP_3 \times V_3$ were statistically not different. $C_3 \times NP_1 \times V_3$ and $C_3 \times NP_2 \times V_3$ proved equally effective for root length. Similarly, $C_2 \times NP_2 \times V_3$ and $C_3 \times NP_1 \times V_2$ resulted in equal root length, i.e. 6.66 cm. The values were 8.73, 8.70, 8.56, 8.43, 7.76, 7.60, 7.50, 6.56 and 5.66 cm in case of $C_1 \times NP_1 \times V_1$, $C_3 \times NP_3 \times V_3$, $C_2 \times NP_3 \times V_1$, $C_1 \times NP_2 \times V_3$, $C_3 \times NP_2 \times V_2$, $C_3 \times NP_2 \times V_1$, $C_3 \times NP_3 \times V_2$, $C_3 \times NP_1 \times V_1$ and $C_1 \times NP_2 \times V_1$, respectively.

Outcomes (Fig. 9) exhibited that highest root length (14.66 cm) was noted in the case of $C_1 \times PE_2 \times V_3$ followed by $C_3 \times PE_3 \times V_1$ (14.16 cm) while the lowest length (5.63 cm) was recorded in the case of $C_1 \times PE_1 \times V_1$**.** The values were not statistically different from each other in case of $C_2 \times PE_3 \times V_3$ and $C_2 \times PE_1 \times V_2$. $C_1 \times PE_2 \times V_1$, $C_3 \times PE_2 \times V_2$ and $C_2 \times PE_3 \times V_1$ resulted in 13.16, 12.75 and 12.16 mean root value. The value of mean root lengths was statistically not different from each other in case of $C_3 \times PE_2 \times V_3$, $C_3 \times PE_1 \times V3$, $C_3 \times PE_1 \times V_1$, $C_2 \times PE_3 \times V_1$, $C_2 \times PE_2 \times V_2$ $C_2 \times PE_2 \times V_1$ and $C_1 \times PE_3 \times V_1$.

Outcomes (Fig. 10) revealed that root fresh weight was recorded to range from 0.66-4.98 g. Maximum fresh weight (4.70 g) recorded in case of $C_1 \times NP_3 \times V_1$ and $C_3 \times NP_1 \times V_3$ while the minimum (0.66 g) was recorded in the case of $C_3 \times NP_2 \times V_3$. The values 4.63, 4.60, 4.56,4.46, 4.43, 3.73, 3.67,3.63 and 3.60 g $C_3 \times NP_3 \times V_3$, $C_3 \times NP_1 \times V_2$, $C_3 \times NP_1 \times V_1$, $C_3 \times NP_3 \times V_2$, $C_1 \times NP_2 \times V_3$, $C_2 \times NP_1 \times V_1$, $C_1 \times NP_3 \times V_3$, $C_1 \times NP_2 \times V_1$, $C_2 \times NP_1 \times V_2$, respectively. The values were equal i.e. 3.56 g in case of $C_1 \times NP_2 \times V_2$ and $C_2 \times NP_2 \times V_3$. Similarly, $C_1 \times NP_1 \times V_2$ and $C_2 \times NP_1 \times V_3$ proved equally effective. Interactions; $C_3 \times NP_2 \times V_2$, $C_2 \times NP_3 \times V_3$ and $C_3 \times NP_3 \times V_1$ resulted in 2.66, 2.60 and 2.53 g fresh root values, respectively.

Results (Fig. 11) revealed that variation in mean values of root fresh weight of soybean varieties was statistically non-significant and ranged from 0.51-4.95 g. $C_3{\times}P.E_3{\times}V_3$ resulted in the highest fresh weight (4.95 g) followed $C_2{\times}P.E_2{\times}V_3$ which was equally effective as $C_2{\times}P.E_3{\times}V_3$ while the lowest weight (0.51 g) was recorded in the case of $C_1{\times}P.E_1{\times}V_2$. The values were 4.65 4.50. 4.35. 4.20 and 4.05 g in case of $C_1{\times}P.E_3{\times}V_3$, $C_3{\times}P.E_3{\times}V_2$, $C_2{\times}P.E_3{\times}V_2$, $C_1{\times}P.E_3{\times}V_2$ and $C_3{\times}P.E_3{\times}V_1$, respectively.

Outcomes showed that the highest root dry weight (0.49 g) was noted in the case of $C_1{\times}NP_1{\times}V_3$ whereas the lowest mean root dry weight (0.10 g) in case of $C_1{\times}NP_1{\times}V_1$. Effects of $C_3{\times}NP_3{\times}V_3$ and $C_2{\times}NP_3{\times}V_1$ were not significantly different from each other. Similarly, $C_2{\times}NP_3{\times}V_3$ and $C_1{\times}NP_3{\times}V_1$ were not significantly different from each other. Likewise, impacts of $C_3{\times}NP_2{\times}V_3$ and $C_2{\times}NP_1{\times}V_2$ were statistically non-significant. The values of root dry weight were 0.42, 0.41, 0.36, 0.30, 0.23 and 0.21 g in case of $C_1{\times}NP_2{\times}V_2$, $C_3{\times}NP_1{\times}V_2$, $C_3{\times}NP_3{\times}V_1$, $C_1{\times}NP_2{\times}V_1$, $C_3{\times}NP_1{\times}V_1$ and $C_2{\times}NP_3{\times}V_2$, respectively as shown in Table 1.

Data reflected that highest root dry weight (0.179 g) was recorded in the case of $C_3{\times}PE_3{\times}V_3$ followed by $C_2{\times}PE_3{\times}V_3$ (0.176 g), $C_1{\times}PE_3{\times}V_3$ (0.173 g), $C_3{\times}PE_3{\times}V_2$ (0.170 g) while the lowest root dry weight (0.10 g) was recorded in the case of $C_3{\times}PE_1{\times}V_1$**.** The values were not statistically different from each other in the case of $C_2{\times}PE_1{\times}V_1$, $C_2{\times}PE_3{\times}V_1$ and $C_3{\times}PE_3{\times}V_1$. Similarly, effects of $C_3{\times}PE_3{\times}V_1$ and $C_2{\times}PE_1{\times}V_1$ were not statistically different from each other. $C_3{\times}PE_2{\times}V_1$ and $C_1{\times}PE_1{\times}V_1$ proved statistically non-significant as indicated in Table 2.

Results showed that the effects of plant extracts for germination (G), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) was significantly and positively correlated with plant extracts while were negatively correlated with root length (RL) and seedling fresh weight (SLFW) as shown in Fig. 12.

Outcomes displayed that impacts of Phyto-synthesized nanoparticles for germination, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight were significantly and positively correlated with plant extracts while were negatively correlated with root length, shoot fresh weight, shoot dry weight and seedling length as shown in Fig. 13.

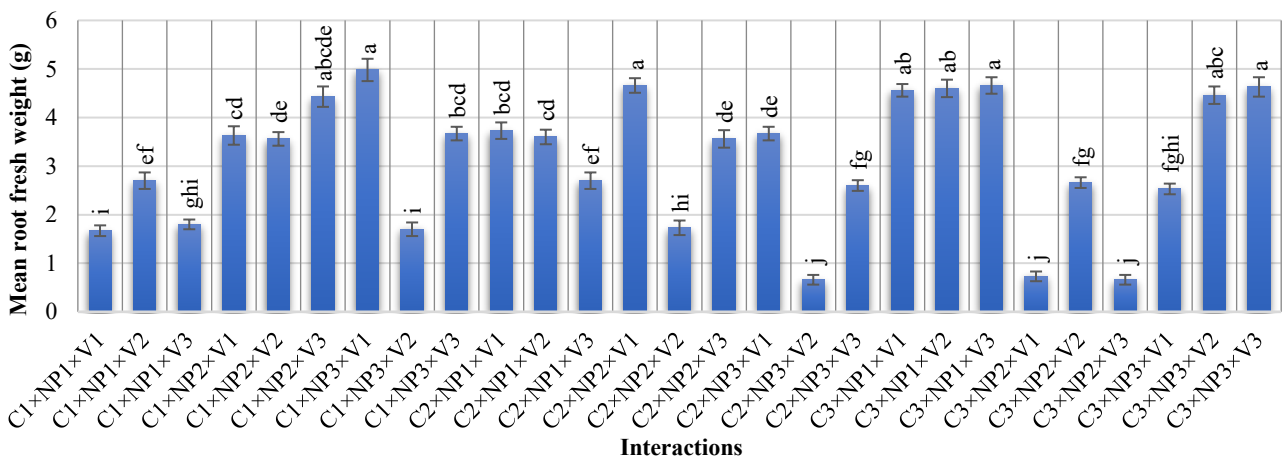

Fig. 9. Interactive effect of the different plant extracts and concentrations for mean root length of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. P.E = Plant extract, $P.E_1$ = *Eucalyptus globulus* extract, $P.E_2$ = *Azadirachta indica* extract, C = concentration, $P.E_3$ = *Syzygium cumini* extract V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean.

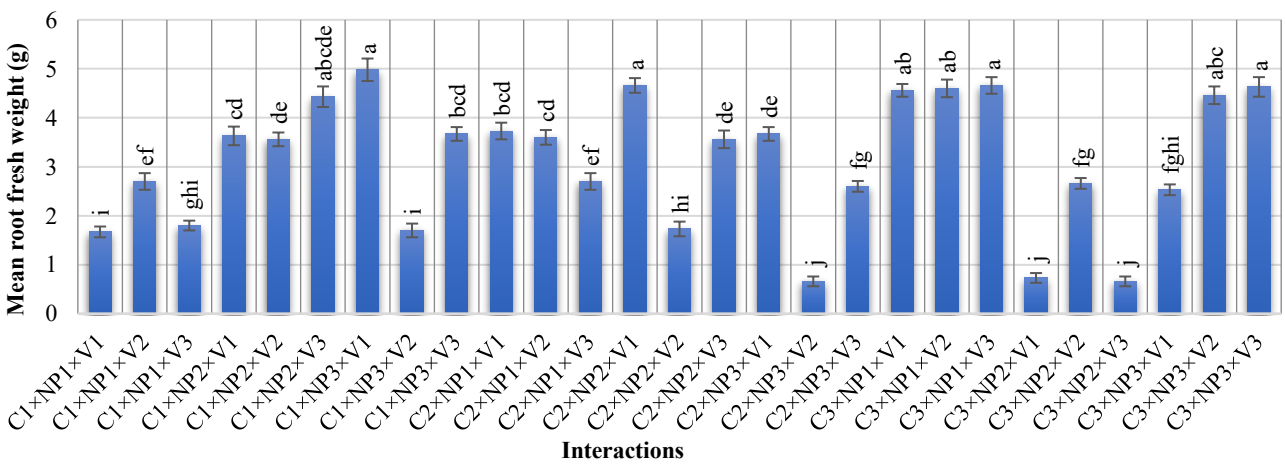

Fig. 10. Interactive effect of the different nanoparticles and concentrations for mean root mean root fresh weight of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. NP1 = Green-synthesized nanoparticles of *Eucalyptus globulus* extract, NP2 = Green-synthesized nanoparticles of *Azadirachta indica* extract, C = concentration, NP3 = Green-synthesized nanoparticles of *Syzygium cumini* extract V = variety, V1 = Ajmeri variety of soybean, V2 = Faisal variety of soybean, V3 = Rawal-1 variety of soybean.

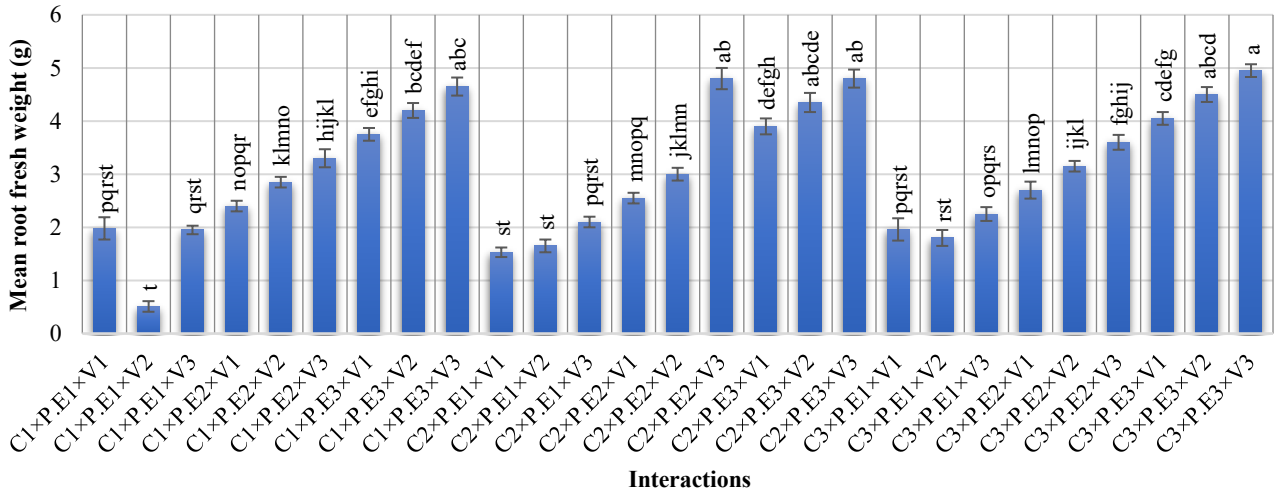

Fig. 11. Interactive effect of the different plant extracts and concentrations for mean root mean root fresh weight of soybean varieties. Vertical bars show error bars for treatments mean. Mean values sharing similar lettering are not significantly different from each other at α = 5%. P.E = Plant extract, $P.E_1$ = *Eucalyptus globulus* extract, $P.E_2$ = *Azadirachta indica* extract, C = concentration, $P.E_3$ = *Syzygium cumini* extract, V = variety, V_1 = Ajmeri variety of soybean, V_2 = Faisal variety of soybean, V_3 = Rawal-1 variety of soybean.

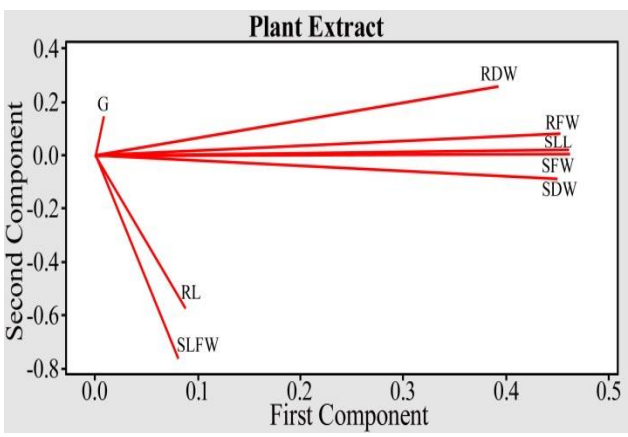

Fig. 12. Principal component analysis of plant extracts for seedling growth parameters.

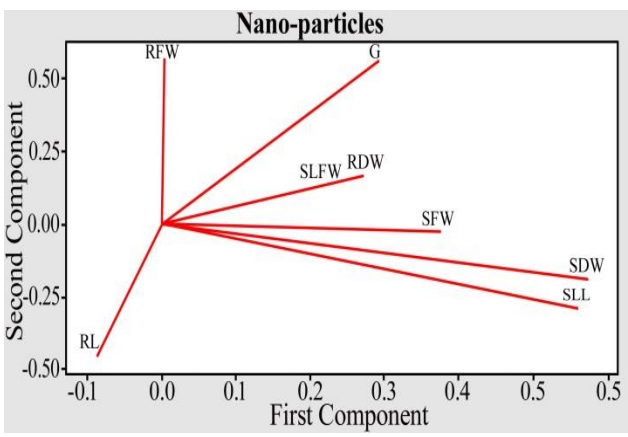

Fig. 13. Principal Component analysis of plant extracts for seedling growth parameters.

Discussion

Soybean is an important cereal as well as oil seed crop worldwide, but the production potential of the soybean is predisposed to many factors which affect the overall germination and growth of the crop. Seed germination can be increased by the priming technique. Priming increases plant antioxidant activity and stress resistance, as well as plant growth and productivity. Priming also improves the viability of seeds, sprouting processes which accelerate and synchronize the germination. It is an inexpensive and potential pre-planting treatment that is done primarily in developing countries today. The present research was executed to probe the relative efficacy of the nanoparticles, plant extracts, moisture levels and packaging materials for different soybean varieties for various growth, quantitative as well qualitative parameters under laboratory conditions. In the current research work, maximum germination (98.00%) was recorded in the case of each of the $C_1{\times}NP_2{\times}V_1$ and $C_2{\times}NP_3{\times}V_1$. Similarly, $C_1{\times}NP_3{\times}V_1$ and $C_3{\times}NP_1{\times}V_3$ triggered equal germination percentage, i.e. 95.00%. $C_2{\times}NP_1{\times}V_3$ and $C_3{\times}NP_2{\times}V_3$ showed equal effects regarding germination (%) of the three soybean varieties. $C_3NP_3{\times}V_3$ caused 90.00% germination while the lowest germination (71.00%) was recorded in case of $C_1{\times}NP_1{\times}V_1$. Maximum germination (86.67%) was noted in the case of $C_1{\times}PE_1{\times}V_1$. Lowest germination (69.00%) was recorded in the case of $C_2{\times}PE_1{\times}V_3$ but statistically not different from $C_3{\times}PE_3{\times}V_2$. The effects of the rest of the interactions are statistically non-significant. Results of root length showed that maximum root length (9.63 cm) was noted in case of $C_3{\times}NP_3{\times}V_1$, $C_2{\times}NP_1{\times}V_1$ was the next effective one (9.53 cm) while lowest mean root length (4.73 cm) was measured in case of $C_2{\times}NP_3{\times}V_3$. Outcomes exhibited that highest root length (14.66 cm) was noted in case of $C_1{\times}PE_2{\times}V_3$ followed by $C_3{\times}PE_3{\times}V_1$ (14.16 cm) while the lowest length (5.63 cm) was recorded in case of $C_1{\times}PE_1{\times}V_1$. Results displayed that maximum root fresh weight (4.70 g) was noted in case of $C_1{\times}NP_3{\times}V_1$ while lowest mean root fresh weight (0.66 g) in each of both $C_2{\times}NP_3{\times}V_2$ and $C_3{\times}NP_2{\times}V_3$. Root dry weight was maximum (0.49 g) in case of $C_1{\times}NP_1{\times}V_3$ whereas lowest mean root dry weight (0.10 g) in case of $C_1{\times}NP_1{\times}V_1$.

The development and growth of plants are profoundly impacted by a variety of environmental stresses, both biotic and abiotic. Priming seeds is an effective method that can increase the number of seeds that germinate and the overall growth of plants, which will ultimately lead to an increase in productivity despite changes in environmental conditions and stresses. The successful establishment of plants and the development of a robust root system is dependent on the germination rate of their seeds. Traditional methods of seed priming result in rapturing of the seed coat. This rapturing of soybean seed membrane increases free fatty acid and free radical which results in reduced seed vitality. Klarod *et al.,* (2021) studied on coating the seed can help it sprout and grow if it lacks nutrients. Priming treatments help normal and stressed crops germination and seedling growth. Nano-priming is a cutting-edge method of seed priming that gives plants resistance to a range of stressors, which enhances seed germination, growth, and yield. When considering all seed priming techniques combined, nano-priming is a far more effective procedure. The key characteristics of nanoparticles (NPs) in seed priming are their ability to improve surface response capabilities and electron exchange with different plant cells and tissue components. In addition to activating antioxidant and reactive oxygen species (ROS) processes in seeds and forming hydroxyl radicals that loosen cell walls and accelerate starch hydrolysis, nano-priming causes the creation of nanopores in shoots and aids in their uptake. Nano-priming is the most successful technique for priming seeds, principally due to its microscopic size and unique physicochemical features. Because of these physiological differences, the uptake of nanoparticles during the nano-priming process is different for each plant species, and as a result, so is the rate and manner in which they grow (Abbasi *et al.*, 2021). In my research work, 75-90% the germination of soybean varieties was recorded with nanoparticles. The results were in accordance with Wijewardana *et al*., (2018) who also recorded 86% germination of soybean. Seed pretreatment with nanoparticles in the current research work increased the germination of soybean and other growth parameters. These results were in line with Chau *et al*., (2019) who also recorded better germination and other growth parameters of seedlings by seed priming of soybean with nanoscale microelements. However, my results were different with Asif *et al*., (2021) who recorded better germination and growth parameters with KNO_3. The difference may be due to treatments variation and soybean varieties in both studies. Sonawane *et al*., (2021) assessed the efficacy of green-synthesized silver nanoparticles and noted augmented seedling length, root length, root fresh weight and dry weight as was recorded in my research work with the nanoparticles, which endorsed my findings. Results of my research work were concurred by findings Rai-Kalal *et al*., (2021) who noted improved seedling length, seedling fresh and dry weight, root length, root fresh, and dry weight in wheat seeds a plant of Gramineae as was soybean by application of green-synthesized nanoparticles compared with plant extracts priming. Results of my study were corroborated with findings of Omar *et al*., (2023) who executed comparative study of nanoparticles and salts as priming agents and noted improved germination, root length, root fresh weight, root dry weight, seedling length, seedling fresh and dry weight of the soybean seedlings. Kasote *et al.* (2019) used nanoparticles as priming agents on watermelon seeds and recorded improved seedling growth characteristics as were recorded in my research work. Klarod *et al*., (2021) studied on coating the seed can help it sprout and grow if it lacks nutrients. Priming treatments help normal and stressed crops germination and seedling growth. Osmopriming affected seedling dry weight, germination rate, length, and vigor index. Hydropriming decreased germination rate (Rouhi *et al*., 2011). Nano-priming is a cutting-edge method of seed priming that gives plants resistance to a range of stressors, which enhances seed germination, growth, and yield. The key characteristics of nanoparticles (NPs) in seed priming are their ability to improve surface response capabilities and electron exchange with different plant cell and tissue components. In addition to activating antioxidant and reactive oxygen species (ROS) processes in seeds and forming hydroxyl radicals that loosen cell walls and accelerate starch hydrolysis, nano-priming causes the creation of nanopores in shoots and aids in their uptake. Nano-priming is the most successful technique for priming seeds, principally due to its microscopic size and unique physicochemical features. Because of these physiological differences, the uptake of nanoparticles during the nano-priming process is different for each plant species, and as a result, so is the rate and manner in which they grow (Abbasi *et al.*, 2021). In my research work, 75-90% germination of soybean varieties was recorded with nanoparticles. Seed pretreatment with nanoparticles in the current research work increased the germination of soybean and other growth parameters. However, my results were different with Asif *et al*., (2021) who recorded better germination and growth parameters with KNO_3. The difference may be due to treatments variation and soybean varieties in both studies. Sonawane *et al*., (2021) assessed the efficacy of green-synthesized silver nanoparticles and noted augmented seedling length, root length, root fresh weight and dry weight as was recorded in my research work with the nanoparticles, which endorsed my findings.

Selenium nanoparticles have recently been developed for use in agriculture, particularly as a plant fertilizer, to improve bacterial germination, increase crop yield and productivity, and help plants better resist being infected with and transforming into harmful organisms. (Bano *et al.*, 2021).

Results of improved germination percentage, shoot, and root length of soybean seedlings were in line with the findings of Sunny *et al*., (2022) who assessed the impact of green synthesized nanoparticles of titanium oxide on germination and other growth attributes of soybean.

Many other researchers have explored the impacts of nanoparticles on germination and the seedling like Ullah *et al.*, (2021) studied the impact of TiO_2-NP on growth and germination related aspects of *Zea mays* L., Basahi (2021) studied the effect of green-synthesized nanoparticles on *Pisum sativum L.* and Azimi (2021) assessed the impacts of nanoparticles on the growth of *Ziziphora clinopodioides* seedlings and recorded improvement in seedling germination, root length, shoot length and other growth attributes as was recorded in the current research work which supported our findings.

Conclusion

From the current research work, it can be concluded that seed priming is a very useful technique to cope up the problems of reduced seed germination percentages. Traditional techniques have lost their effectiveness due to genetic diversity of plant seeds. Nano-priming can be effective in improving the seed germination and viability of soybean seeds and other crops. Up to 98% germination was achieved using green-synthesized nanoparticles. However, the responses of seed germination and other growth parameters of soybean seedlings were varied with variation in plant species and soybean genotypes.

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