Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

Journal of Molecular Science

Journal of Molecular Science

www.jmolecularsci.com ISSN:1000-9035

Green Synthesis of Zinc And Copper Oxide Nanoparticles By Cow Dung Extract And Their Effects On Seed Germination In Tomato.

Pawan Kumar Kansotiya[1], Bhupendra Tanwar[1] and Anupama Goyal[*]
[1]Research Scholar , Department of Science and Technology, Jayoti Vidyapeeth Women's University, Vedant Gyan Valley, Jharna, Jaipur Rajasthan 303122.
[2]Professor, Department of Science and Technology, Jayoti Vidyapeeth Women's University, Vedant Gyan Valley, Jharna, Jaipur Rajasthan 303122.

Article Information
Received: 04-09-2025
Revised: 12-10-2025
Accepted: 08-11-2025
Published: 16-12-2025

Keywords
Cow dung extract biosynthesized nanoparticles, ZnONPs, CuONPs, tomato, seed germination etc.

ABSTRACT
By examining the effects of various concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), $ZnSO_4$ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm), and $CuSO_4$ solution (200 ppm, 100 ppm, 50 ppm) on various germination and plant development parameters in tomatoes, the study seeks to explore the phyto-stimulatory effects of zinc oxide and copper oxide nanoparticles biosynthesised using cow dung extract. The findings indicate that all treatments resulted in a delay in seed germination. Additionally, it was shown that plants exposed to both ZnONPs and CuONPs had longer seedling shoots and roots; however, the effect was significantly greater than that of their metallic equivalents, $ZnSO_4$ solution and $CuSO_4$ solution, as well as cow dung extract. Additionally, compared to plants treated to $ZnSO_4$ solution, $CuSO_4$ solution, and cow dung extract, plants exposed to green nanoparticles (ZnONPs and CuONPs) displayed increased fresh weight and dry weight. Conversely, plants exposed to ZnSO4 solution, CuSO4 solution, and cow dung extract had lower dry weights than the control group, whereas plants exposed to nanoparticles had higher dry weights.

INTRODUCTION:

One of the most exciting new developments in contemporary agricultural research is nanotechnology, which offers creative ways to increase crop yield, seed vigor, and stress tolerance **(Gandhi *et al.,* 2019).** Metal oxide nanoparticles, like copper oxide (CuONPs) and zinc oxide (ZnONPs), have drawn a lot of interest among different nanomaterials because of their special physicochemical, antimicrobial, and growth-promoting qualities. These nanoparticles have historically been created by physical and chemical means, but these processes frequently require hazardous chemicals, a lot of energy, and produce dangerous byproducts. Green synthesis, on the other hand, which uses biological materials, is a cost-effective, environmentally friendly, and sustainable method that avoids environmental hazards while creating bioactive nanostructures **(Roy *et al.,* 2019; Sharma *et al.,* 2021; Acharya *et al.,* 2025).**

Rich in organic matter, microbial communities, and phytochemicals like proteins, flavonoids, and phenolics, cow dung is a plentiful and renewable natural resource. Cow dung extract is a novel and understudied medium for the biosynthesis of nanoparticles because of these bioactive compounds, which function as natural reducing and stabilizing agents. In addition to adding value to agricultural waste, using this resource to create nanomaterials promotes sustainable practices that are consistent with the ideas of the circular bioeconomy**(Raj *et al.,* 2014; Anamayi *et al.,***

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

2016).

One of the most extensively grown vegetables in the world, the tomato (*Solanum lycopersicum* L.) is prized for both its economic significance and nutritional value. However, low germination rates and seedling establishment, especially in nutrient-deficient environments, frequently limit its productivity. Utilizing biosynthesized nanoparticles as seed priming agents presents a viable strategy to improve tomato biomass accumulation, root and shoot elongation, and seed germination.

In this work, ZnONPs and CuONPs will be synthesized and characterized using cow dung extract. Their effects on tomato seed germination and early seedling growth will be compared to those of their respective metal salts ($ZnSO_4$ and $CuSO_4$) and cow dung extract alone. The results shed light on the potential of green-synthesised nanoparticles as sustainable bio-growth promoters and substitutes for traditional agrochemicals.

MATERIALS AND METHODS:

Preparation of cow dung extract

Cow dung extract was prepared by dissolving 2 gm of dry cow dry into 20 ml of distilled water. After shaking at magnetic stirrer for 2 hours at 40°C temperature, it was filtered and some part of filtered extract was kept for evaporation of solvent and other was used for nanoparticle synthesis.

Preparation of nanoparticles:

$CuSo_4$ solution was used to prepare CuO nanoparticles. In 90 ml of $CuSO_4$ solution, 10 ml of cow dung extract was mixed drop by drop within 30 minutes while keeping at magnetic stirrer. After that, the mixture was shaked at magnetic stirrer at room temperature at 1000 rpm for 2 hours (until the change in colour of the solution). appearance of greenish colour indicated synthesis of CuONPs. Those were centrifuged at 10000 rpm for 15 minutes. Pellet was washed 3 times with distilled water and 1 time with ethanol by centrifuging at 5000 rpm for 5 minutes in each cycle. Obtained pellet was kept in muffle furnace at 500°C for 30 minutes for calcination. The obtained nanoparticles were stored for further use. ZnONPs were synthesized using the similar method for CuONPs. In this $ZnSO_4$ solution was used.

Characterization of the synthesized nanoparticles:

The synthesized nanoparticles were characterized using FTIR analysis which was performed by FTIR spectrometer (PerkinElmer 95163). The dried nanoparticles were mixed with KBr and pressed into pellets. The spectra were recorded in the range of 400-4000 cm^{-1} to identify functional groups associated with the nanoparticles. The morphology of the synthesized ZnONPs was examined using FESEM (Zeiss). A small amount of ZnONPs was mounted on a carbon tape and coated with gold before imaging to observe particle size and shape. Characterization was done at Accuphysem analytics.

Preparation of treatment solutions:

Seeds were purchased from local market of Jaipur, Rajasthan. 50 ppm, 100 ppm and 200 ppm concentration of each of all cow dung extract, CuONPs, ZnONPs, $CuSO_4$, and $ZnSO_4$ solutions were prepared. Along with all treatments, control (untreated) group was also included.

Seed treatment:

Seeds were surface sterilized using 0.1% of NaOCl and then soaked with the treatment solutions for 2 hours. Control seeds were soaked in distilled water. In sterile Petri plates, Whatmann filter papers were kept. In each plate, 5 seeds were placed. In each group, 3 plates were taken. Plates were wrapped in aluminium foil and kept in dark for 5 days. Seed germination and various parameters like seedling shoot length, seedling root length, fresh weight and dry weight were measured**(Rawat *et al.,* 2018).**

RESULTS:

Synthesis and characterization of nanoparticles

Presence of cream-ish colour indicated synthesis of ZnONPs while green coloured appearance indicated presence of CuONPs as shown in Figure 1 and 2 respectively. FTIR spectrum of the synthesized ZnONPs and CuONPs are given in figure 3 and 4 respectively. Both spectra show various corresponding peaks. In spectrum of ZnONPs, peaks were obtained at 3777.47 cm^{-1}, 3432.65 cm^{-1}, 2922.50 cm^{-1}, 2854.18 cm^{-1}, 1638.19 cm^{-1}, 1233.06 cm^{-1}, 1543.49 cm^{-1}, 1458.66 cm^{-1}, 1053.07 cm^{-1}, 522.75 cm^{-1}. Similarly, FTIR spectrum of CuONPs exhibited presence of 3430.75 cm^{-1}, 3332.54 cm^{-1}, 3016.02 cm^{-1}, 1678.54 cm^{-1}, 1590.11 cm^{-1}, 1738.76 cm^{-1}, 1455.96 cm^{-1}, 1367.85 cm^{-1}, 1218.15 cm^{-1}, 1149.65 cm^{-1}, 1025.37 cm^{-1}, 784.92 cm^{-1}, 715.83 cm^{-1}, 461.78 cm^{-1}. These peaks confirmed presence of O-H, N-H and R-COOH groups. FESEM structures of the synthesized nanoparticles are given in Figure 5-6 for ZnONPs and CuONPs respectively. These showed spherical shapes of both nanoparticles **(Fakhari *et al.,* 2019).**

Journal of Molecular Science

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

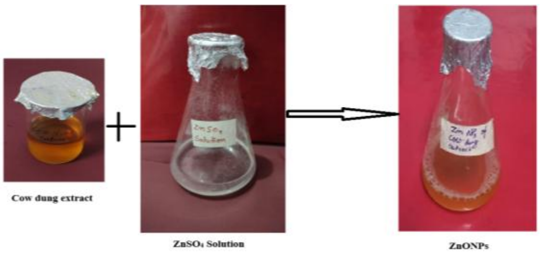


Figure 1: Green synthesis of ZnONPs using cow dung extract.

Figure 2: Green synthesis of CuONPs using cow dung extract.

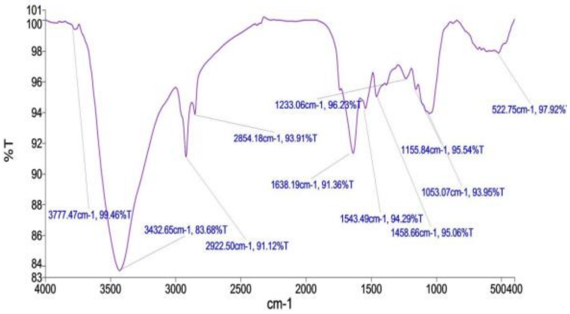


Figure 3: FTIR analysis of the synthesized ZnONPs.

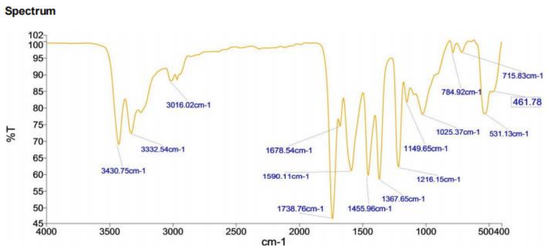


Figure 4: FTIR analysis of the synthesized CuONPs.

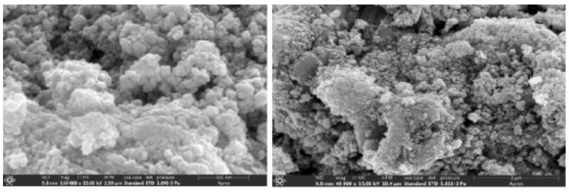


Figure 5: FESEM images of the synthesized ZnONPs.

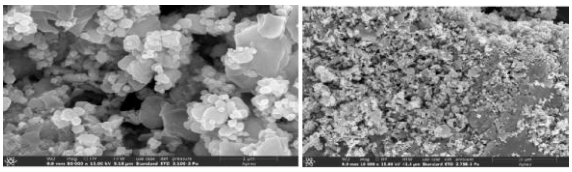


Figure 6: FESEM images of the synthesized CuONPs.

Effects of nanoparticles on seed germination in tomato

% Seed germination

The results of the experiment to assess the effect of synthesized ZnO and CuO nanoparticles using cow dung extract on the seed germination percentage of tomato seeds are presented in detail below:

In the control group (distilled water), the seed germination percentage was 56.67% with a standard deviation of 5.77. When cow dung extract alone was applied, germination improved across all concentrations. Specifically, the 100 ppm concentration (T2) showed the highest germination at 73.33%, indicating a 22.74% increase over the control. The 200 ppm (T1) and 50 ppm (T3) concentrations also resulted in improved germination rates of 70.00% and 60.00%, reflecting increases of 19.06% and 5.57%, respectively. This suggests that cow dung extract itself has bioenhancing properties at optimal concentrations.

In the case of $ZnSO_4$ solutions (non-nano forms), the results were inconsistent. The highest germination was observed at 50 ppm (T6) with a value of 66.67% and a 15.01% increase compared to the control. At 100 ppm (T5), germination was slightly lower at 63.33% (a 10.54% increase). However, a significant decline was seen at 200 ppm (T4), where germination dropped to 46.67%, representing a 21.41% decrease. These findings indicate a concentration-dependent inhibitory effect of higher $ZnSO_4$ doses.

Application of biosynthesized ZnO nanoparticles showed highly encouraging results. At 100 ppm (T8), seed germination peaked at 93.33%, the highest among all treatments, showing a remarkable 39.29% increase over the control. Similarly, 50 ppm (T9) resulted in 83.33% germination (32.01% increase), and 200 ppm (T7) also showed improvement at 66.67% (15.01% increase). These results highlight the strong stimulatory effect of ZnONPs, especially at mid-range concentrations.

In contrast, CuO nanoparticles showed mixed effects. The 100 ppm concentration (T11) gave an impressive germination percentage of 90.00%, which is a 37.04% increase compared to control, indicating strong stimulatory potential. However, at 50 ppm (T12), germination was 70.00% (19.06% increase), and at 200 ppm (T10), the value dropped to 53.33%, showing a slight negative effect (-6.24%). Thus, CuONPs also showed concentration-dependent effects, with 100 ppm being the most effective.

With regard to $CuSO_4$ treatments, the results

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

followed a similar trend to $ZnSO_4$. The 50 ppm concentration (T15) resulted in 73.33% germination (22.74% increase), and 100 ppm (T14) gave 66.67% (15.01% increase). The 200 ppm concentration (T13) had no significant effect, showing 56.67% germination, equivalent to the control. This again confirms the inhibitory effect of higher salt concentrations and the better efficacy of lower doses.

Overall, the results demonstrate that both ZnO and CuO nanoparticles synthesized using cow dung extracts significantly enhanced seed germination in tomato compared to their respective salt solutions and control. The most effective treatments were ZnONPs at 100 ppm and CuONPs at 100 ppm, which exhibited the highest germination percentages and positive changes. These findings emphasize the potential of biosynthesized nanoparticles as sustainable and efficient alternatives to conventional agrochemicals for improving seed germination and crop establishment.

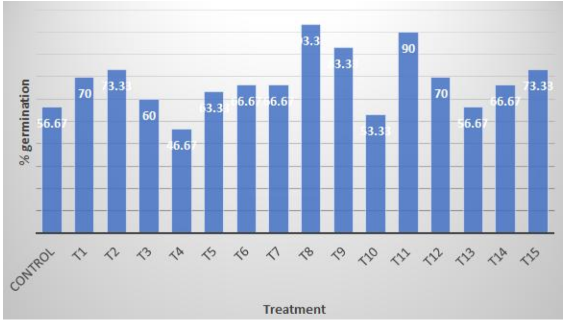


Figure 7: % seed germination in okra.

Effects of nanoparticles on seedling shoot length in okra

The effect of various treatmentsincluding cow dung extracts, metal salts, and biosynthesized nanoparticleson the seedling shoot length of tomato is summarized and interpreted in the following results:

In the control group (distilled water), the mean shoot length of tomato seedlings was 1.03 cm with a standard deviation of 0.09. Among the cow dung extract treatments, the shoot growth varied with concentration. At 100 ppm (T2), shoot length improved to 1.12 cm, showing a modest increase of 7.76%, while 50 ppm (T3) also enhanced the shoot length to 1.08 cm (4.33% increase). Interestingly, the 200 ppm treatment (T1) negatively affected shoot elongation, reducing it to 0.74 cm—representing a 39.19% decrease compared to control. This suggests that while cow dung extract at lower concentrations can enhance shoot growth slightly, higher concentrations may exhibit inhibitory effects.

The zinc sulphate ($ZnSO_4$) treatments demonstrated a concentration-dependent influence. The 100 ppm concentration (T5) produced a substantial increase in shoot length, reaching 1.61 cm (a 36.16% increase), while 50 ppm (T6) also showed a significant enhancement at 1.23 cm (16.49% increase). However, the 200 ppm treatment (T4) resulted in a smaller increase to 1.10 cm (6.36% increase). These findings indicate that moderate concentrations of $ZnSO_4$ can improve shoot growth, but higher doses may be less effective.

The most prominent improvement in shoot length was observed in plants treated with biosynthesized ZnO nanoparticles. The 100 ppm treatment (T8) showed the highest shoot length of 2.12 cm—an impressive 51.42% increase over control. Similarly, 50 ppm ZnONPs (T9) resulted in 2.08 cm (50.48% increase), and even the higher concentration of 200 ppm (T7) achieved 1.66 cm shoot length (37.95% increase). This consistent and marked improvement in shoot growth across all nanoparticle concentrations highlights the strong bio efficacy of ZnONPs, especially at 100 ppm.

CuO nanoparticles also significantly promoted seedling growth. The highest shoot length among CuONP treatments was seen at 100 ppm (T11) with 1.99 cm, marking a 48.33% increase. The 50 ppm (T12) and 200 ppm (T10) treatments also performed well, with shoot lengths of 1.69 cm (39.05% increase) and 1.34 cm (23.13% increase), respectively. These results suggest that CuONPs are highly effective, particularly at mid-range concentrations, for enhancing tomato seedling development.

By contrast, copper sulphate ($CuSO_4$) solutions had variable and less significant effects. The 100 ppm treatment (T14) increased shoot length to 1.27 cm (19.11% increase), while 200 ppm (T13) had a minimal effect, with shoot length at 1.06 cm (3.13% increase). Notably, the 50 ppm treatment (T15) actually reduced the shoot length to 0.96 cm, which corresponds to a 6.92% decrease compared to control.

In conclusion, the data clearly demonstrate that ZnO and CuO nanoparticles synthesized using cow dung extract substantially improved tomato seedling shoot length in a concentration-dependent manner, outperforming both cow dung extract alone and metal salt solutions. Among all treatments, ZnONPs at 100 ppm showed the maximum enhancement, followed closely by CuONPs at the same concentration. These findings support the potential use of green-synthesized nanoparticles as powerful bio-growth stimulants in sustainable agriculture.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

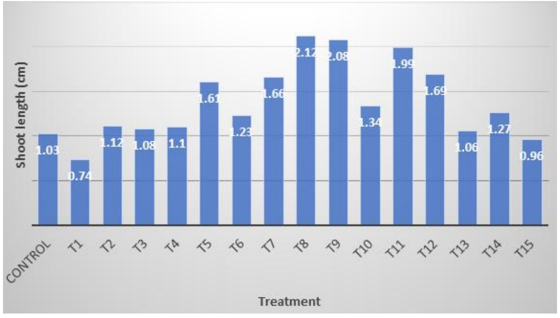


Figure 8: Seedling Shoot Length (cm) in okra.

Effects of nanoparticles on seedling root length in okra

The study on the effect of various treatments on seedling root length in tomato revealed notable differences across cow dung extract, metal salts, and biosynthesized nanoparticles. The control group treated with distilled water showed a root length of 0.36 cm. Cow dung extract at 100 ppm significantly enhanced root growth to 0.58 cm, indicating a 37.57% increase, while 50 ppm also showed a positive effect with 0.47 cm. However, a higher concentration of 200 ppm reduced root length to 0.28 cm, reflecting a 28.57% decline, suggesting potential phytotoxicity at elevated levels. Zinc sulphate treatments demonstrated a concentration-dependent enhancement, with 100 ppm producing a root length of 0.98 cm (63.14% increase), followed by 50 ppm at 0.85 cm and 200 ppm at 0.55 cm. Among all treatments, biosynthesized ZnO nanoparticles were the most effective, with the 100ppm concentration resulting in the maximum root length of 1.21 cma 70.25% increasewhile 200 ppm and 50 ppm also yielded significant improvements of 1.01 cm and 0.92 cm, respectively. CuO nanoparticles also showed strong stimulation, particularly at 100 ppm with a root length of 0.86 cm (57.98% increase), followed by 200 ppm and 50 ppm. In comparison, copper sulphate solutions had a moderate effect, with the best result at 50 ppm (0.74 cm, 51.57% increase). Overall, the results indicate that biosynthesized ZnONPs and CuONPs, especially at 100 ppm, significantly enhance root elongation in tomato seedlings, outperforming both their respective metal salts and cow dung extract alone, underscoring the potential of green-synthesized nanoparticles in sustainable crop enhancement strategies.

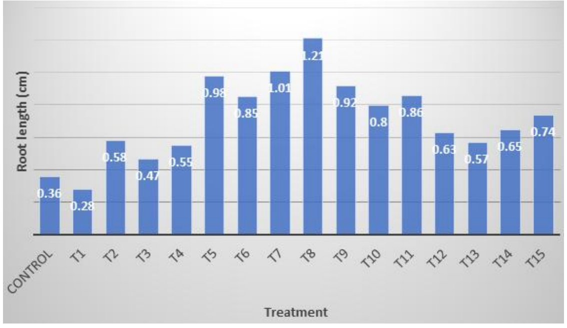


Figure 9: Seedling Root Length (cm) in okra.

Effects of nanoparticles on seedling fresh weight in okra

The evaluation of seedling fresh weight in tomato under various treatments revealed significant enhancements, particularly with biosynthesized nanoparticles and certain salt concentrations. The control treatment with distilled water resulted in a baseline fresh weight of 0.34 g. Among cow dung extract treatments, the 100 ppm concentration (0.53 g) produced the most noticeable improvement, showing a 54.71% increase over control, while 50 ppm (0.50 g) also demonstrated a substantial rise (46.08%). However, at 200 ppm, cow dung extract slightly reduced the fresh weight to 0.33 g, indicating a minor inhibitory effect (-3.73%). Zinc sulphate treatments produced the most dramatic increases, particularly at 100 ppm, where the fresh weight reached 1.06 g—an exceptional 210.78% increase—followed by 50 ppm (0.94 g, 175.49%) and 200 ppm (0.58 g, 70.20%). Biosynthesized ZnO nanoparticles also led to considerable improvements, with the 100 ppm treatment yielding 0.91 g (167.65% increase), followed by 50 ppm at 0.77 g (125.49%) and 200 ppm at 0.75 g (121.57%). CuO nanoparticles similarly showed strong effects; 100 ppm resulted in 0.84 g (147.06%), 200 ppm in 0.81 g (139.22%), and 50 ppm in 0.68 g (99.02%). In contrast, copper sulphate solutions were less effective, with the 200 ppm treatment reducing fresh weight slightly to 0.32 g (-6.86%), while 100 ppm and 50 ppm showed moderate increases of 31.37% and 98.04%, respectively. Overall, the data clearly indicate that $ZnSO_4$ at 100 ppm had the highest impact on fresh biomass accumulation, closely followed by ZnONPs and CuONPs at similar concentrations. These findings underscore the potential of both inorganic salts and green-synthesized nanoparticles, particularly zinc and copper-based, in significantly enhancing seedling biomass production in tomato.

Journal of Molecular Science
Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

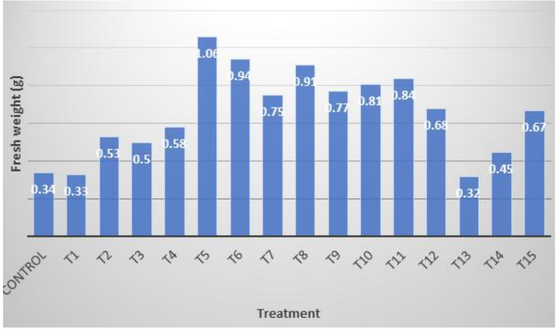


Figure 10: Seedling Fresh Weight (g) in okra.

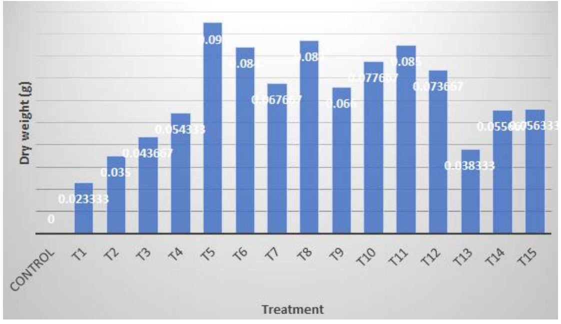
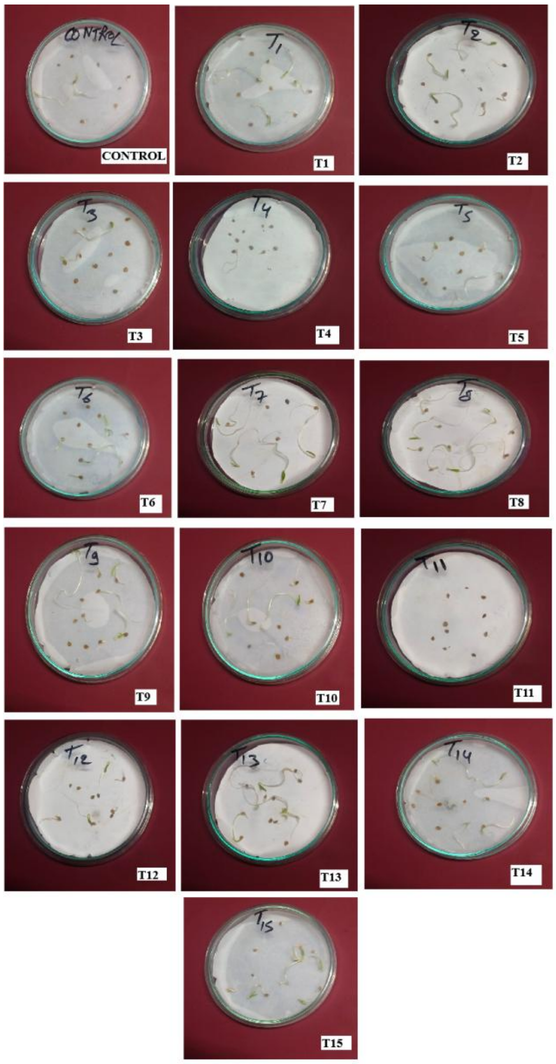


Figure 11: Seedling Dry Weight (g) in okra

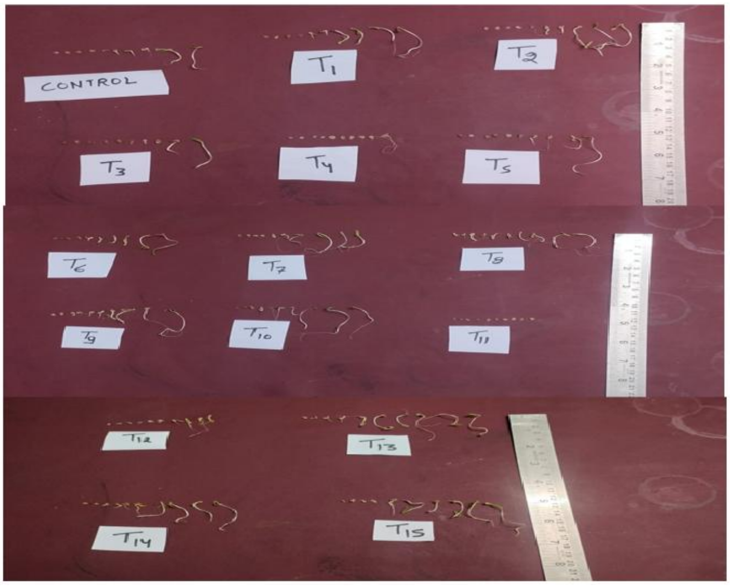
Effects of nanoparticles on seedling dry weight in okra

The data on seedling dry weight in tomato seedlings under different treatments reveal significant improvements with both metal salts and biosynthesized nanoparticles, particularly at optimized concentrations. The control group treated with distilled water had a dry weight of 0.029167 g. Cow dung extract showed varied effects depending on concentration. At 50 ppm (T3), the dry weight was 0.043667 g, reflecting a 35.88% increase over control, while 100 ppm (T2) resulted in a 20% increase (0.035 g). However, 200 ppm (T1) slightly reduced dry weight to 0.023333 g, indicating a 20% decrease, likely due to inhibitory effects at higher doses. $ZnSO_4$ treatments resulted in marked improvements, particularly at 100 ppm (T5), which yielded the highest dry weight of 0.095 g, corresponding to a 70.53% increase. The 50 ppm (T6) and 200 ppm (T4) treatments also showed significant increases of 66.67% and 48.47%, respectively. Among ZnO nanoparticle treatments, the 100 ppm concentration (T8) produced a dry weight of 0.087 g (67.82% increase), followed closely by 200 ppm (T7) at 0.067667 g (58.62%) and 50 ppm (T9) at 0.066 g (57.58%). CuONPs also exhibited a similar trend, with 100 ppm (T11) giving 0.085 g (67.06% increase), 200 ppm (T10) at 0.077667 g (63.95%), and 50 ppm (T12) at 0.073667 g (61.99%). $CuSO_4$ treatments showed moderate but consistent improvements, with dry weight increasing to 0.056333 g (50.30%) at 50 ppm (T15), 0.055667 g (49.70%) at 100 ppm (T14), and 0.038333 g (26.96%) at 200 ppm (T13). Overall, $ZnSO_4$ at 100 ppm showed the maximum improvement in seedling dry biomass, followed closely by biosynthesized ZnONPs and CuONPs, confirming the superior efficacy of nanoparticles and zinc supplementation in enhancing plant growth performance.



Photoplate 1: Petri plate experiment in tomato.

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137
Journal of Molecular Science



Photoplate 2: Seed germination in tomato.

Table 1: Comparative effects of the synthesized nanoparticles on seed germination parameters in tomato.

Treatment Name	Treatment	% Seed germination	Shoot length (cm)	Root length s (cm)	Seedling fresh weigh (g)	Seedling dry weight (g)
Distilled Water (Control)	**Control**	56.67±5.77	1.03±0.09	0.36±0.06	0.34±0.02	. 0.029±0.002
Cow Dung Extract (200 ppm)	**T1**	70.00±10.00 (19.06%)	0.74±0.08 (-39.19)	0.28±0.07 (-28.57)	0.33±0.03 (-3.73%)	0.023±0.002 (-20%)
Cow Dung Extract (100 ppm)	**T2**	73.33±5.77 (22.74%)	1.12±0.13 (7.76)	0.58±0.04 (37.57)	0.53±0.01 (54.71%)	0.035±0.004 (20%)
Cow Dung Extract (50 ppm)	**T3**	60.00±10.00 (5.57%)	1.08±0.15 (4.33)	0.47±0.07 (23.94)	0.50±0.03 (46.08%)	0.043±0.002 (35.87%)
$ZnSO_4$ Solution (200 ppm)	**T4**	46.67±5.77 (-21.41%)	1.10±0.12 (6.36)	0.55±0.05 (34.55)	0.58±0.06 (70.20%)	0.054±0.003 (48.46%)
$ZnSO_4$ Solution (100 ppm)	**T5**	63.33±5.77 (10.54%)	1.61±0.16 (36.16)	0.98±0.07 (63.14)	1.06±0.08 (210.78%)	0.095±0.002 (70.52%)
$ZnSO_4$ Solution (50 ppm)	**T6**	66.67±15.28 (15.01%)	1.23±0.12 (16.49%)	0.85±0.06 (57.81%)	0.94±0.03 (175.49%)	0.084±0.002 (70.52%)
ZnONPs (200 ppm)	**T7**	66.67±5.77 (15.01%)	1.66±0.24 (37.95%)	1.01±0.09 (64.36%)	0.75±0.04 (121.57%)	0.067±0.003 (58.62%)
ZnONPs (100 ppm)	**T8**	93.33±5.77 (32.01%)	2.12±0.13 (51.42%)	1.21±0.09 (70.25%)	0.91±0.03 (167.65%)	0.087±0.004 (67.81%)
ZnONPs (50 ppm)	**T9**	83.33±5.77 (32.01%)	2.08±0.20 (50.48%)	0.92±0.05 (60.87%)	0.77±0.08 (125.49%)	0.066±0.003 (57.57%)
CuONPs (200 ppm)	**T10**	53.33±5.77 (-6.24%)	1.34±0.10 (23.13%)	0.80±0.03 (55.00%)	0.81±0.04 (139.22%)	0.077±0.004 (63.94%)
CuONPs (100 ppm)	**T11**	90.00±10.00 (37.04%)	1.99±0.15 (48.33%)	0.86±0.07 (57.98%)	0.84±0.04 (147.06%)	0.085±0.003 (67.05%)
CuONPs (50 ppm)	**T12**	70.00±10.00 (19.06%)	1.69±0.07 (39.05%)	0.63±0.08 (42.86%)	0.68±0.06 (99.02%)	0.073±0.002 (61.99%)
$CuSO_4$ Solution (200 ppm)	**T13**	56.67±5.77 (0.01%)	1.06±0.14 (3.13%)	0.57±0.06 (36.84%)	0.32±0.04 (-6.86%)	0.038±0.002 (26.95%)
$CuSO_4$ Solution (100 ppm)	**T14**	66.67±5.77 (15.01%)	1.27±0.05 (19.11%)	0.65±0.04 (44.62%)	0.45±0.03 (31.37%)	0.055±0.005 (49.70%)
$CuSO_4$ Solution (50 ppm)	**T15**	73.33±5.77 (22.74%)	0.96±0.11 (-6.92%)	0.74±0.03 (51.57%)	0.67±0.04 (98.04%)	0.056±0.003 (50.29%)

Note: All experiments were done in triplicates. Values have been represented as Mean±SD. % change in each parameter was calculated in respected to control.

DISCUSSION:

Nanoscience or nanotechnology is a widely used technology in modern translational research. The development of metallic nanoparticles by green chemistry or an eco-friendly approach, using biological materials such as bacteria, fungi, yeast, and plants has gained much research momentum in recent years. By this approach, the problems of environmental toxicity and the use of harsh reaction conditions in the synthesis of nanoparticles can be easily circumvented. Among the various biological materials, plants are well suited for the green synthesis of metallic nanoparticles as they are rich sources of phytochemicals, and are non-pathogenic. In addition, the presence of various phytochemicals, such as tannin, flavonoids, proteins, polysaccharides, etc., render the resulting nanoparticles with numerous properties, including antimicrobial, anticancer, and antioxidant activities. A wide range of metallic and non-metallic nanoparticles, such as silver, gold, copper oxide, zinc oxide, palladium, platinum, titanium dioxide, and iron oxide have been synthesized using the extracts of various plants. The characteristics of the nanoparticles were assessed using UV-Vis absorption spectroscopy, electron microscopy (scanning and transmission), energy dispersive X-ray spectroscopy (EDX), and Fourier transform infrared spectroscopy (FTIR) **(Deen *et al.,* 2022).** Among the metallic nanoparticles, zinc and copper nanoparticles have received considerable attention owing to their attractive physicochemical and antimicrobial properties. These properties allow these nanoparticles to be used in the development of antibacterial surfaces, topical ointments, and wound-healing materials.

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and consumed vegetables globally. It is renowned for its high yield and substantial contribution to human nutrition. The fruit is rich in vitamins (B, C, K, A, and E), carotenoids, and essential minerals such as potassium and phosphorus, making it a valuable source of nutrients. Additionally, widespread tomato cultivation and consumption significantly contribute to economic growth in many regions worldwide. However, tomato production faces numerous challenges, both biological and environmental. Among these, nutrient deficiencies stand out as a major factor that can adversely affect plant growth, fruit quality, and yield **(Wang *et al.,* 2023).** Nanotechnology has brought revolutionary changes across various fields, including agriculture. Nanoparticles (NPs), which have at least one dimension in the nanoscale (1 to 100 nanometers), exhibit unique properties due to their increased surface area and significant effects **(Ahmed *et al.,* 2024).**In the present study, synthesis and characterization of ZnO and CuO nanoparticles was done using cow dung and studied their effects on seed germination, growth and biochemical parameters of tomato.

The results of current study showed that presence of creamish colour indicated synthesis of ZnONPs while green coloured appearance indicated presence of CuONPs. FTIR spectrum of the synthesized ZnONPs and CuONPs show various corresponding peaks. These peaks confirmed presence of O-H, N-H and R-COOH groups. FESEM structure of the synthesized nanoparticles showed spherical shapes of both nanoparticles. These results were in accordance of **Abdelbaky *et al.,* (2022**) and **Takele *et al.,* (2023),** who formed and characterize ZnO and CuO NPs using aqueous leaf extract of *Pelargonium odoratissimum* (L.) and *Zingiber officinale* rhizome extract, respectively.

Overall, the results demonstrate that both ZnO and CuO nanoparticles synthesized using cow dung extracts significantly enhanced seed germination in tomato compared to their respective salt solutions and control. The most effective treatments were ZnONPs at 100 ppm and CuONPs at 100 ppm, which exhibited the highest germination percentages and positive changes. These findings emphasize the potential of biosynthesized nanoparticles as sustainable and efficient alternatives to conventional agrochemicals for improving seed germination and crop establishment.

The enhanced germination noted in seeds subjected to nanoparticles can be ascribed to their minute dimensions, facilitating rapid absorption into the seed coat. This, in turn, augments the absorption and assimilation of nanoparticles by the seeds, as explained by **Savithramma *et al.,* (2012),** additionally, the technique of seed priming represents a distinct and innovative approach with the capacity to enhance the enzymatic activity of α-amylase, facilitate the absorption of iron, and induce the generation of reactive oxygen species (ROS). Consequently, such physiological responses culminate in elevated concentrations of soluble sugars, thereby providing crucial support for seed germination processes. The heightened seed vigor is likely a consequence of the comprehensive enhancement in both seed germination and seedling growth **(Walia, 2024).** Additionally, the quenching of free radicals by nanoparticles contributes to the observed increase in seed vigor, as elucidated by **Shyla and Natarajan (2014).** The outcomes align consistently with the investigations conducted by **Rai and Jajoo (2021)** in wheat, **Thunugunta *et al.,* (2018)** in brinjal, **Raskar *et al.,* (2014)** in onion and Younes et al. (2020) in tomato, eggplant and pepper.

In conclusion, the data clearly demonstrate that ZnO and CuO nanoparticles synthesized using cow dung extract substantially improved tomato seedling shoot length in a concentration-dependent manner, outperforming both cow dung extract alone and metal salt solutions. Among all treatments, ZnONPs at 100 ppm showed the maximum enhancement, followed closely by CuONPs at the same concentration. These findings support the potential use of green-synthesized

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

nanoparticles as powerful bio-growth stimulants in sustainable agriculture.The observed increased physiological performance from the treatment with nanoparticles can be ascribed to the capture of free radicals by the nanoparticles. The smaller dimensions of these nanoparticles enable their penetration through microstructural crevices on seed outer surface, allowing them to engage with and neutralize free radicals, thereby leading to an enhancement in seed vigor. Similar results for increase in shoot length have been observed by **Awan *et al.*, (2021)** in broccoli and **Sabir *et al.*,(2020)** in maize.

The study on the effect of various treatments on seedling root length in tomato revealed notable differences across cow dung extract, metal salts, and biosynthesized nanoparticles. Overall, the results indicate that biosynthesized ZnONPs and CuONPs, especially at 100 ppm, significantly enhance root elongation in tomato seedlings, outperforming both their respective metal salts and cow dung extract alone, underscoring the potential of green-synthesized nanoparticles in sustainable crop enhancement strategies.The apparent improvement in physiological performance resulting from the utilization of nanoparticles can reasonably be ascribed to their ability to neutralize free radicals. The diminished dimensions of these nanoparticles facilitate their penetration through microstructural openings on the external surface of seeds, allowing them to engage with and counteract free radicals. Consequently, this mechanism contributes to an increase in seed vigor. Supporting evidence for the promotion of root growth can be found in the work of **Awan *et al.*, (2021)** in broccoli and **Sabir *et al.*,(2020)** in maize.

The evaluation of seedling fresh weight in tomato under various treatments revealed significant enhancements, particularly with biosynthesized nanoparticles and certain salt concentrations. Overall, the data clearly indicate that $ZnSO_4$ at 100 ppm had the highest impact on fresh biomass accumulation, closely followed by ZnONPs and CuONPs at similar concentrations. These findings underscore the potential of both inorganic salts and green-synthesized nanoparticles, particularly zinc and copper-based, in significantly enhancing seedling biomass production in tomato.The rapid germination may be attributed to the formation of new pores on the seed coat during penetration, facilitating the entry of water into the seed. Alternatively, the nanoparticles may penetrate the seed through surface cracks, activating enzymes in the early stages and thereby accelerating the germination process. Similar results have also been reported by **Senthilkumar (2011)** in black gram, **Sridhar (2012)** in tomato and **Awan *et al.*,(2021)** in broccoli seeds.

The data on seedling dry weight in tomato seedlings under different treatments reveal significant improvements with both metal salts and biosynthesized nanoparticles, particularly at optimized concentrations. Overall, $ZnSO_4$ at 100 ppm showed the maximum improvement in seedling dry biomass, followed closely by biosynthesized ZnONPs and CuONPs, confirming the superior efficacy of nanoparticles and zinc supplementation in enhancing plant growth performance.The improvements observed in seed quality parameters can be attributed to heightened synthesis and activity of hydrolytic enzymes during the initial stages of germination. This enhanced enzymatic activity facilitates the effective utilization of the available nutrient reserves within the seeds, leading to the accelerated emergence and growth of seedlings. Consequently, there is a proportional increase in dry matter production, reflecting the overall beneficial effects of nanoparticles. These positive effects of nanoparticles are likely attributable to their heightened chemical reactivity, enabling them to serve as cofactors for enzymes involved in germination and seedling growth **(Walia, 2024).** Similar findings have also been observed by **Pandey *et al.*, (2010)** on *Cicer arietinum*, **Senthilkumar (2011)** in black gram and **Sridhar (2012)** in tomato.

CONCLUSION:

This study shows that a green and environmentally friendly method for synthesizing ZnO and CuO nanoparticles using cow dung extract can be accomplished. The nanoparticles' spherical shape and the existence of functional groups that support their stability and bioactivity were verified by characterization. When compared to both cow dung extract and traditional salt solutions, biosynthesized ZnONPs and CuONPs dramatically increased tomato seed germination, shoot and root elongation, and seedling biomass, according to a comparative analysis of treatments. The most noticeable stimulatory effects were shown by 100 ppm ZnONPs and CuONPs among the treatments, indicating that these concentrations are ideal for encouraging early plant growth.

The potential of biosynthesized nanoparticles as effective biofertilizers and plant growth stimulants is highlighted by their superior performance over metal salts. The noted increases in seed vigor and seedling establishment were probably caused by their small size, high surface reactivity, and capacity to improve nutrient uptake. These results highlight the importance of green nanotechnology in sustainable agriculture in addition to

Journal of Molecular Science
Volume 35 Issue 3, Year of Publication 2025, Page 1024-1033
DoI-10.004687/1000-9035.2025.137

establishing cow dung as a useful resource for the synthesis of nanoparticles.

Future research should concentrate on long-term physiological effects, field-scale assessments to confirm their safety and effectiveness, and the molecular mechanisms underlying interactions between nanoparticles and plants. A sustainable way to increase agricultural productivity and lessen reliance on chemical fertilizers may be provided by incorporating such green nanomaterials into crop management techniques.

REFERENCES:

1. Abdelbaky AS, Abd El-Mageed TA, Babalghith AO, Selim S, Mohamed AMHA. Green Synthesis and Characterization of ZnO Nanoparticles Using *Pelargonium odoratissimum* (L.) Aqueous Leaf Extract and Their Antioxidant, Antibacterial and Anti-inflammatory Activities. *Antioxidants*. 2022; 11(8):1444. https://doi.org/10.3390/antiox11081444
2. Acharya, S. ., Behera, A. ., CS, A. ., Venugopal, S. ., Thiyagarajan, R. ., & Chinnasamy, T. . (2025). Green-synthesized Titanium Dioxide nanoparticles with Pyrogallol functionalization: A novel approach using cow dung extract for anticancer applications. *International Journal of Nano Dimension*. https://doi.org/10.57647/j.ijnd.2025.1603.17
3. Ahmed M, Marrez DA, Rizk R, Zedan M, Abdul-Hamid D, Decsi K, Kovács GP, Tóth Z. The Influence of Zinc Oxide Nanoparticles and Salt Stress on the Morphological and Some Biochemical Characteristics of *Solanum lycopersicum* L. Plants. Plants (Basel). 2024 May 20;13(10):1418. doi: 10.3390/plants13101418. PMID: 38794488; PMCID: PMC11125107.
4. Anamayi, S. E., Oladele, O. N., Suleiman, R. A., Oloyede, E. O., & Yahaya, U. (2016). Effect of cow dung and NPK fertilizer at different levels on the growth performance and nutrient composition of Moringa oleifera. *Anim Exp Biol*, *4*, 35-39.
5. Awan S, Shahzadi K, Javad S, Tariq A, Ahmad A and Ilyas S. 2021. A preliminary study of influence of zinc oxide nanoparticles on growth parameters of Brassica oleracea var. italica. Journal of the Saudi Society of Agricultural Sciences. 20(1):18-24.
6. Deen GR, Hannan FA, Henari F, Akhtar S. Effects of Different Parts of the Okra Plant (*Abelmoschus esculentus*) on the Phytosynthesis of Silver Nanoparticles: Evaluation of Synthesis Conditions, Nonlinear Optical and Antibacterial Properties. *Nanomaterials*. 2022; 12(23):4174. https://doi.org/10.3390/nano12234174
7. Fakhari, S., Jamzad, M., & Kabiri Fard, H. (2019). Green synthesis of zinc oxide nanoparticles: a comparison. *Green chemistry letters and reviews*, *12*(1), 19-24.
8. Gandhi, A.D., Sathiyaraj, S., Vizhi, D.K., Supraja, N., Babujanarthanam, R., Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp., Biocatalysis and Agricultural Biotechnology (2019), doi: https://doi.org/10.1016/j.bcab.2019.101132.
9. Pandey AC, Sanjay SS and Yadav SR. 2010. Application of ZnO nanoparticles in influencing the growth rate of Cicer arietinum. Journal of Experimental nanoscience. 5(6):488-97.
10. Rai KP and Jajoo A. 2021. Priming with zinc oxide nanoparticles improve germination and photosynthetic performance in wheat. Plant Physiology and Biochemistry. 160:341-351.
11. Raj, A., Jhariya, M. K., & Toppo, P. (2014). Cow dung for eco-friendly and sustainable productive farming. *Environ Sci*, *3*(10), 201-202.
12. Raskar SV and Laware SL. 2014. Effect of zinc oxide nanoparticles on cytology and seed germination in onion. International Journal of Current Microbiology and Applied Sciences. 3(2):467-473.
13. Rawat, P. S., Kumar, R., Ram, P., & Pandey, P. (2018). Effect of nanoparticles on wheat seed germination and seedling growth. *International Journal of Agricultural and Biosystems Engineering*, *12*(1), 13-16.
14. Roy, S.; Rhim, J.W. Carrageenan-Based Antimicrobial Bionanocomposite Films Incorporated with ZnO Nanoparticles Stabilized by Melanin. Food Hydrocoll. 2019, 90, 500–507.
15. Sabir S, Zahoor MA, Siddique MH, Shafique M, Imran M, Hayat S, Malik IR and Muzammil S. 2020. Assessing the Impact of Biologically Synthesized ZnO Nanoparticles as Source of Nutrient on the Growth of Zea mays L. Research square. 12(3): 132-138.
16. Savithramma N, AnkannaS and Bhumi G. 2012. Effect of nanoparticles on seed germination and seedling growth of Boswellia ovalifoliolata an endemic and endangered medicinal tree taxon. Nano Vision. 2(1):2.
17. Senthilkumar S. 2011. Customizing nanoparticles for the maintanence of seed vigour and viability in Blackgram (Vigna mungo) cv. VBN 4. (MSc Thesis), Tamil Nadu Agricultural University, Coimbatore.
18. Shyla KK and Natarajan N. 2014. Customizing zinc oxide, silver and titanium dioxide nanoparticles for enhancing groundnut seed quality. Indian Journal of Science and Technology. 7(9):1376-81.
19. Sridhar C. 2012. Effect of nanoparticles for the maintenance of tomato seed vigour and viability. [MSc (Agri.) Thesis] submitted to Tamil Nadu Agricultural University, Coimbatore.
20. Takele, Elias, Feyisa Bogale, Raji, Shumi, Gemechu, Kenasa, Girmaye, Green Synthesis, Characterization, and Antibacterial Activity of CuO/ZnO Nanocomposite Using Zingiber officinale Rhizome Extract, *Journal of Chemistry*, 2023, 3481389, 15 pages, 2023. **https://doi.org/10.1155/2023/3481389**
21. Thunugunta T, Reddy AC, Seetharamaiah SK and Hunashikatti LR. 2018. Impact of zinc oxide nanoparticles on eggplant (Solanum melongena): studies on growth and the accumulation of nanoparticles. IET Nanobiotechnology. DOI: 0.1049/iet-nbt.2017.0237.
22. Walia ak. Effect of nanoparticles seed priming and foliar spray on growth, seed yield and quality attributes in okra [abelmoschus esculentus (l.) Moench]. Thesis. Dr. Yashwant singh parmar university of horticulture and forestry. Seed science and technology department of seed science and technology college of horticulture. 2024.
23. Wang Q., Xu S., Zhong L., Zhao X., Wang L. Effects of Zinc Oxide Nanoparticles on Growth, Development, and Flavonoid Synthesis in Ginkgo Biloba. Int. J. Mol. Sci. 2023;24:15775. doi: 10.3390/ijms242115775. - DOI - PMC - PubMed
24. Younes NA, Hassan HS, Elkady MF, Hamed AM and Dawood MFA. 2020. Impact of synthesized metal oxide nanomaterials on seedlings production of three Solanaceae crops. Heliyon. 6:1. DOI:10.1016/j.heliyon.2020.e03188