



Synthesis of zinc oxide and copper oxide nanoparticles using cow dung extract and evaluation of their effects on seed germination parameters in okra

Pawan Kumar Kansotiya¹, Bhupendra Tanwar¹, Anupama Goyal*

¹ Research Scholar, Department of Science and Technology, Faculty of Education and Methodology, Jayoti Vidyapeeth Women's University, Vedant Gyan Valley, Jharna, Jaipur, Rajasthan, India

Professor, Department of Science and Technology, Faculty of Education and Methodology, Jayoti Vidyapeeth Women's University, Vedant Gyan Valley, Jharna, Jaipur, Rajasthan, India

Abstract

The study aims to investigate the phytostimulatory effects of zinc oxide and copper oxide nanoparticles biosynthesized using cow dung extract by analysing the effect of different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on different germination and plant development parameters in okra. The results show retardation in seed germination in presence of all the treatments. Furthermore, seedling shoot length and root length was found to be increased in plants exposed to both the nanoparticles (ZnONPs and CuONPs), however, the effect was much higher than their metallic counterparts (ZnSO₄ solution and CuSO₄ solution) as well as cowdung extract. Furthermore, the plants exposed to green nanoparticles (ZnONPs and CuONPs) showed higher fresh weight as well as dry weight in comparison to plants exposed to ZnSO₄ solution, CuSO₄ solution and cow dung extract. On the contrary, plants exposed to ZnSO₄ solution, CuSO₄ solution and cow dung extract showed lesser dry weight in comparison to control, while, nanoparticle exposed plants showed high dry weight.

Keywords: Cow dung extract biosynthesized nanoparticles, ZnONPs, CuONPs, okra, seed germination

Introduction

The last few years have been marked by major scientific developments and globalization, however, the problem of population explosion and food scarcity to feed the millions of growing mouths still stands the same. The two main reasons contributing to decline in food productivity include effect of biotic and abiotic stressors on the food crops. The scientific innovations over the years have tried to address this problem by usage of chemical and synthetic fertilizers, that aim to maximize agricultural output. The advent of green revolution led to discovery of a number of agricultures boosting chemically synthesized fertilizers. However, their use is limited owing to widespread concerns about their environmental toxicity and their malicious effects on different components of the ecosystem.

Usage of conventional fertilizers and manures such as cowdung seems to be insufficient for providing the much-needed agricultural boost. This fuelled the need for usage of sustainable means for boosting agricultural productivity. Based on this, the current study has been drafted to investigate the role of cowdung biosynthesized green nanoparticles (ZnONOs and CuONPs) in boosting plant growth and productivity. Nanoparticles, owing to their exceptionally small size and physicochemical attributes are capable of deep penetration within the biological systems and exert profound effects (Jiang *et al*, 2022; Singh *et al*, 2015) [16, 17]. However, owing to serious concerns about environmental toxicity of synthetic fertilizers, the current study explored the phytostimulatory effect of green nanoparticles biosynthesized using cowdung extract (Aslam *et al.*, 2022; Mahawar *et al*, 2018) [18, 19]. The findings of the study showed potential phytostimulatory effect of both ZnONPs and CuONPs on okra seedlings, as evident from increased seedling shoot length, higher root length and

increased seedlings fresh weight as well as dry weight. The phytostimulatory effects exerted by nanoparticles are of higher magnitude in comparison to their bulk metallic counterparts as well as the parent derivative cowdung extract. The findings of the study bear the potential to revolutionize the field of agriculture by boosting plant productivity and provide a permanent solution to the ever-existent problem of food scarcity.

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

CuSO₄ solution was used to prepare CuO nanoparticles. In 90 ml of CuSO₄ 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 shaken 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 ZnSO₄ solution was used.

Characterization of The Synthesized Nanoparticles

Ftir Analysis

Fesem Analysis

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.

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 petriplate, 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.

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 structure of the synthesized nanoparticles are given in Figure 5-6 for ZnONPs and CuONPs respectively. These showed spherical shapes of both nanoparticles.

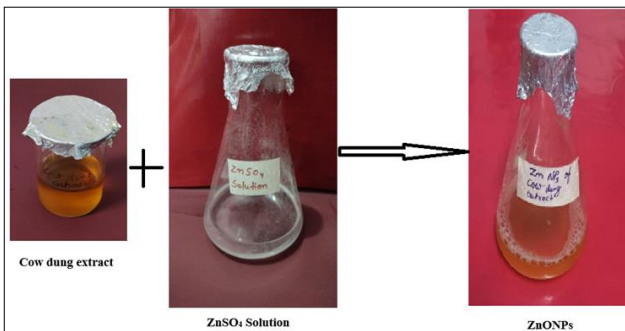


Fig 1: Green synthesis of ZnONPs using cow dung extract

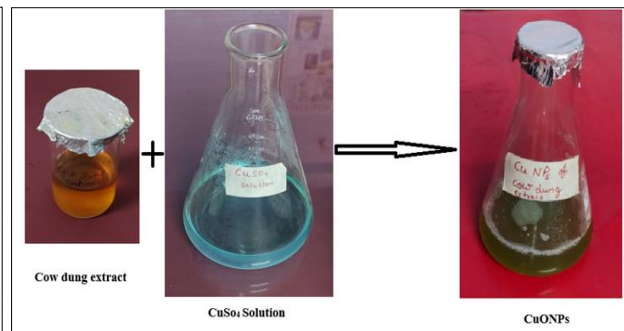


Fig 2: Green synthesis of CuONPs using cow dung

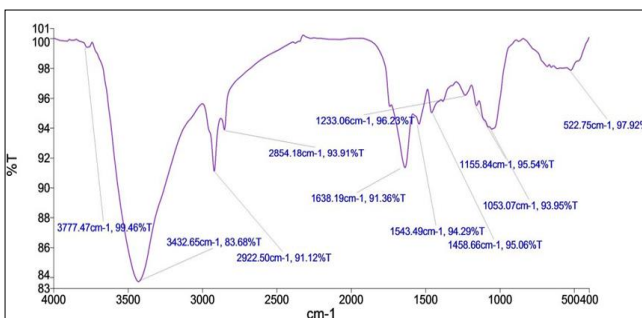


Fig 3: FTIR analysis of the synthesized ZnONPs

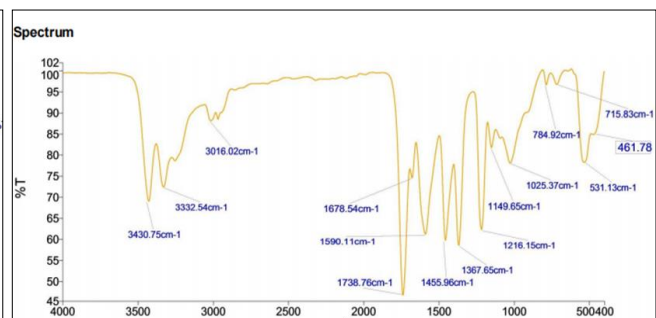


Fig 4: FTIR analysis of the synthesized CuONPs

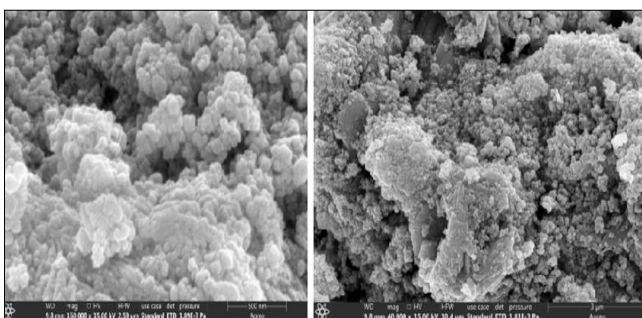


Fig 5: FESEM images of the synthesized ZnONPs

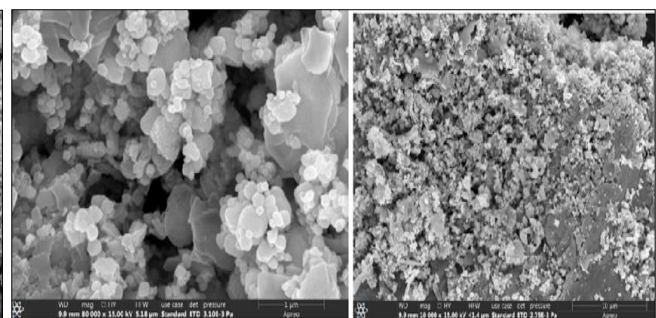


Fig 6: FESEM images of the synthesized CuONPs

Effects of Nanoparticles on Seed Germination in Okra

The results in Table 1(a) and its corresponding graph show effect of different treatments including, different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on seed germination in okra. The results show consistent decrease in seed germination percentage of okra seedlings in all the treatments, with percentage decrease in each case being, 33.33% (Cow Dung Extract 200 ppm), 20% (Cow Dung Extract 100 ppm), 33.33% (Cow Dung Extract 50 ppm), 20% (ZnSO₄ Solution 200 ppm), 26.67% (ZnSO₄ Solution 100 ppm), 33.33% (ZnSO₄ Solution 50 ppm), 0% (ZnONPs 200 ppm), 20% (ZnONPs 100 ppm), 26.67% (ZnONPs 50 ppm), 26.67% (CuONPs 200 ppm), 0% (CuONPs 100 ppm), 0% (CuONPs 50 ppm), 33.33% (CuSO₄ Solution 200 ppm), 33.33% (CuSO₄ Solution 100 ppm) and 6.67% (CuSO₄ Solution 50 ppm).

Effects of Nanoparticles on Seedling Shoot Length in Okra

The results in Table 1(b) and its corresponding graph show effect of different treatments including, different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on seedling length in okra. The results show consistent increase in shoot length of okra seedlings in all the treatments except in case of cow dung treatment (cow dung extract 100 ppm and 50 ppm), where a small decrease was observed. In all other cases, a significant percentage increase was observed in seed length of okra seedlings. The highest percentage increase was observed in case of ZnONPs, showing % increase of 68%, 36.7% and 70.5% (in case of ZnONP 200 ppm, 100 ppm and 50 ppm respectively) followed by CuONPs, showing % increase of 29%, 15% and 10.24% (in case of CuONP 200 ppm, 100 ppm and 50 ppm respectively). Both ZnSO₄ solution and CuSO₄ solution also showed significant percentage increase in seed length, however, the effect was more pronounced in case of respective nanoparticles.

Effects of Nanoparticles on Seedling Root Length in Okra

The results in Table 1(c) and its corresponding graph show effect of different treatments including, different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on seedling root length in okra. The results show consistent increase in root length of okra seedlings in all the treatments. The highest percentage increase in root length

was observed in case of CuSO₄ Solution (50 ppm) (204%) followed by ZnONPs (183%, 76% and 132%) and CuONPs (105%, 74% and 36.5%). Cow dung extract, ZnSO₄ solution and CuSO₄ solution also showed increase in seedling root length, with % change values in each case being (36%, 17.6% and 27.2% for cow dung extract, (82%, 39% and 57% for ZnSO₄ solution) and (41%, 45% and 204% for CuSO₄ solution). As per the results, the percentage increase in root length was higher in case of both the nanoparticle treatments (ZnONPs and CuONPs) in comparison to their bulk ionic counterparts (ZnSO₄ solution and CuSO₄ solution).

Effects of Nanoparticles on Seedling Fresh Weight in Okra

The results in Table 1(d) and its corresponding graph show effect of different treatments including, different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on seedling fresh weight in okra. The results show consistent increase in seedling fresh weight of okra seedlings in all the treatments except cow dung extract (200 ppm and 100 ppm). The highest increase decrease was observed in ZnONPs (73.06%, 12.69% and 82.97% for 50 ppm, 100 ppm and 200 ppm) followed by CuONPs (64.70%, 31.57% and 24.45%). ZnSO₄ Solution and CuSO₄ solution treatments also showed increase in fresh weight, 22.91-33.74% for ZnSO₄ Solution and 9.90-34.05 % for CuSO₄ solution. As per the results, the percentage increase in seedling fresh weight was higher in case of both the nanoparticle treatments (ZnONPs and CuONPs) in comparison to their bulk ionic counterparts (ZnSO₄ solution and CuSO₄ solution).

Effects of Nanoparticles on Seedling Dry Weight in Okra

The results in Table 1(e) and its corresponding graph show effect of different treatments including, different concentrations of cow dung extract (200 ppm, 100 ppm, 50 ppm), ZnSO₄ solution (200 ppm, 100 ppm, 50 ppm), ZnONPs (200 ppm, 100 ppm, 50 ppm), CuONPs (200 ppm, 100 ppm, 50 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm) on seedling fresh weight in okra. The results show variable effect of different treatments on seedling dry weight. Highest dose (200 ppm) of cow dung treatment and ZnSO₄ led to slight increase in seedling dry weight. Similarly, ZnONPs treatment also increased seedling dry weight by 218%, 177% and 59% (200 ppm, 100 ppm and 50 ppm). However, in all other cases, which include, cow dung treatment (100 ppm and 50 ppm), ZnSO₄ solution (100 ppm, 50 ppm), CuONP (100 ppm) and CuSO₄ solution (200 ppm, 100 ppm, 50 ppm), a decrease in seedling dry weight was observed.

Table 1: Effects of the synthesized nanoparticles on seed germination parameters in Okra

Treatment Name	Treatment	% Seed Germination (a)	Seedling Shoot Length (cm) (b)	Seedling Root Length (cm) (c)	Seedling Fresh Weight (g) (d)	Seedling Dry Weight (g) (e)
Distilled Water (Control)	Control	100.00±0.00	1.66±0.07	0.323±0.008	0.323±0.006	0.061±0.005
Cow Dung Extract (200 ppm)	T1	66.67±23.09 (-33%)	1.76±0.08 (+6.02%)	0.441±0.004 (+36.39%)	0.423±0.003 (+30.97%)	0.062±0.003 (+1.64%)
Cow Dung Extract	T2	80.00±20.00 (-)	1.57±0.006 (-5.42%)	0.380±0.003	0.299±0.002 (-)	0.043±0.006 (-)

(100 ppm)		20%)		(+17.68%)	7.44%)	29.51%)
Cow Dung Extract (50 ppm)	T3	66.67±11.54 (-33%)	1.60±0.007 (-3.61%)	0.411±0.005 (+27.21%)	0.319±0.008 (-1.23%)	0.054±0.002 (-11.48%)
ZnSO ₄ Solution (200 ppm)	T4	80.00±0.00 (-20%)	2.13±0.04 (+28.31%)	0.589±0.006 (+82.67%)	0.432±0.006 (+33.74%)	0.074±0.005 (+21.31%)
ZnSO ₄ Solution (100 ppm)	T5	73.33±11.54 (-26.6%)	1.74±0.05 (+4.81%)	0.449±0.003 (+39.53%)	0.376±0.004 (+16.44%)	0.044±0.004 (-27.87%)
ZnSO ₄ Solution (50 ppm)	T6	66.67±11.54 (-33.3%)	1.73±0.08 (+4.21%)	0.509±0.006 (+57.66%)	0.397±0.005 (+22.91%)	0.055±0.003 (-10.16%)
ZnONPs (200 ppm)	T7	100.00±0.00 (0%)	2.80±0.6 (+68.67%)	0.917±0.004 (+183.95%)	0.559±0.006 (+73.06%)	0.194±0.005 (+218.03%)
ZnONPs (100 ppm)	T8	80.00±0.00 (-20%)	2.27±0.5 (+36.5%)	0.569±0.007 (+76.24%)	0.364±0.006 (+12.69%)	0.169±0.003 (+177.05%)
ZnONPs (50 ppm)	T9	73.33±11.54 (-26.67%)	2.83±0.45 (+70.48%)	0.750±0.006 (+132.48%)	0.591±0.006 (+82.97%)	0.097±0.005 (+59.02%)
CuONPs (200 ppm)	T10	73.33±11.54 (-26.67%)	2.15±0.97 (+29.51%)	0.664±0.005 (+105.53%)	0.532±0.005 (+64.70%)	0.085±0.004 (+40.98%)
CuONPs (100 ppm)	T11	100.00±0.00 (0%)	1.91±0.35 (+15.06%)	0.564±0.004 (+74.91%)	0.425±0.008 (+31.57%)	0.057±0.005 (-6.56%)
CuONPs (50 ppm)	T12	100.00±0.00 (0%)	1.83±0.56 (+10.24%)	0.441±0.004 (+36.5%)	0.402±0.004 (+24.45%)	0.077±0.003 (+26.23%)
CuSO ₄ Solution (200 ppm)	T13	66.67±11.54 (-33.3%)	1.87±0.55 (+12.65%)	0.456±0.008 (+41.34%)	0.355±0.008 (+9.90%)	0.060±0.007 (-1.64%)
CuSO ₄ Solution 0 (100 ppm)	T14	66.67±11.54 (-33.3%)	1.88±0.43 (+13.25%)	0.469±0.003 (+45.31%)	0.398±0.005 (+23.26%)	0.053±0.003 (-13.11%)



Fig 7: Germinated okra seeds

Discussion

The current study aims to explore the biostimulatory effect of green nanoparticles (ZnONPs and CuONPs) biosynthesized using cow dung extract on different parameters of okra seedlings, including their germination kinetics, shoot length, root length, fresh weight, and dry weight. The results for the above-mentioned parameters have also been analysed if bulk metallic counterparts of these nanoparticles (ZnSO₄ solution and CuSO₄ solution) are used as treatment. The study shows consistent decrease in seed germination in case of all the treatments, however, nanoparticle exposure in certain cases seemed to unaffected the seed germination kinetics. Decreased seed germination in presence of cow dung extract may occur due to presence of high level of organic matter in it, which leads to induction of osmotic stress in the seeds, leading to disrupted water regulation and decreased germination (Lv *et al.*, 2013; Pratap *et al.*, 2010; Chen *et al.*, 2020) [2, 4, 3].

Furthermore, the germination process by cowdung maybe retarded due to nutrient imbalance causing nutrient toxicity in seeds. Also, ZnSO₄ solution and CuSO₄ solution may lead

to decreased seed germination owing to zinc and copper induced oxidative stress, zinc induced disruption of protein synthesis and enzyme activities as well as copper induced disruption to cellular DNA, RNA and proteins, all of which impede seed germination process (Zhang *et al.*, 2015; Mir *et al.*, 2021) [1, 5]. However, nanoparticle treatment seemed to neither retard or promote seed germination unlike other treatments, which maybe attributed to their exceptional size, surface characteristics and biocompatibility at the used concentrations, all of which allow the nanoparticles to interact with the seeds without interfering with germination process.

In the next part, the results show significant increase in seedling shoot length in all the treatment modalities, including, cow dung extract, ZnSO₄ solution and CuSO₄ solution, CuONPs as well as ZnONPs. The percentage increase in shoot length was the highest in case of nanoparticles. Similar effect was observed in case of root length, where too, treatment with CuONPs as well as ZnONPs showed the highest increase in okra root length.

The increased shoot and root length in case of nanoparticles in comparison to their metal counterparts maybe attributed to the following reasons:

- Both ZnONPs and CuONPs, due to their extremely small size, serve as crucial candidates for augmented bioavailability of zinc and copper ions to plants in comparison to their metal counterparts. Both these ions play a crucial role in physiological processes in plants, including protein synthesis and enzymatic activation for photosynthesis.
- Nanoparticles ensure slow and sustained release of the micronutrients (Zinc and copper), which ensure maintenance of stable nutrient supply, eliminating over burst of nutrients which may otherwise retard plant growth.
- Nanoparticles interact with phytohormones, auxin and cytokinin, ensuring proper cell division and elongation in plants.
- Nanoparticles possess inherent antioxidant activity, which is particularly useful for mitigation of oxidative stress in plants, ensuring healthy plant growth and development.
- Nanoparticles may somewhat augment the ability of roots to absorb water and nutrients from the soil, ensuring development of a healthy plant shoot (Tripathi *et al.*, 2022; Ranjan *et al.*, 2024; Yusefi-Tanha *et al.*, 2020; Priyanka *et al.*, 2019; Shah *et al.*, 2023) ^[6, 7, 8, 9, 10].

Undoubtedly, cow dung extract is rich source of organic matter, which ensures plant growth and development, but such organic treatments are at the mercy of microbes to break down nutrients, which is comparatively slow process. Hence the phytostimulatory effect is not as potent as the nanoparticle exposure.

Similar effect, wherein, nanoparticle treatment led to enhancement in seedling shoot length and seedling root length has been reported in a number of previous studies (Guo *et al.* 2022; Li *et al.*, 2019; Rawat *et al.*, 2018; Das *et al.*, 2018; Javed *et al.*, 2022) ^[11, 12, 13, 14, 15].

Thereafter, in the later part of the study, the researchers showed increase in seedling fresh weight on treatment with ZnONPs and CuONPs. ZnONPs and CuONPs led to significant increase in seedling fresh weight, the percentage increase being 12%-82.3% (ZnONPs) and 24%-64% (CuONPs). ZnONPs have previously been reported to showcase increased bioavailability and higher surface area, thereby improve water and nutrient absorption by seedlings, leading to increased seedling fresh weight. Similar phytostimulatory results of lower magnitude have been observed in case of CuONPs, which too facilitate nutrient absorption by plants. On the contrary, ZnSO₄ and CuSO₄ solutions also showcase increased seedling fresh weight, but of a lower magnitude in comparison to their respective nanoparticles. The comparatively lower efficacy of bulk materials in comparison to their nanoparticle counterparts maybe attributed to their slower uptake by plant roots. In contrast to this, cowdung treatment led to drastic reduction in seedling fresh weight at higher concentrations (200 ppm and 100 ppm), which may have occurred due to induction of osmotic stress in plants at higher cowdung concentrations.

The phytostimulatory effect of ZnONPs in increasing seedling fresh weight translated to seedling dry weight as well, where too, ZnONPs treated seedlings showcased

significantly increased biomass accumulation, indicative of increased dry weight. Similarly, in complete correlation to what was observed earlier, cowdung treatment led to decreased dry weight of seedlings. Also, ZnSO₄ and CuSO₄ treatment showed decrease in seedling dry weight. This does not align with the results obtained in case of seedling fresh weight, and is indicative of the fact that this treatment promoted initial fresh weight gain, which failed to translate to dry weight accumulation. This conversion may have occurred because of several reasons, including, increased water uptake or alterations in metabolic pathways, that perturbed conversion of fresh weight to dry matter accumulation.

Concluding the findings of the study in a nutshell, the current study unveils the augmented phytostimulatory efficacy of cow dung biosynthesized ZnONPs and CuONPs, in comparison to the cowdung extract itself and bulk metallic counterparts (ZnSO₄ and CuSO₄ treatments). The phytostimulatory effect of the nanoparticles is evident from increased seedling shoot length and root length as well as increased seedling fresh weight and dry weight in presence of nanoparticles. On the other hand, cowdung extract itself and bulk metallic counterparts (ZnSO₄ and CuSO₄ treatments) failed to showcase phytostimulatory effects of a comparable magnitude as the ZnONPs and CuONPs. Nonetheless, the study opens avenues for future research exploring the usage of green nanoparticles for optimization of agricultural outputs and obtaining crucial insights into the mechanistic pathways regulating the effect in nanoparticles on plant physiology and development.

Conclusion

The potential of green-synthesized ZnONPs and CuONPs from cow dung extracts as efficient biostimulants in agriculture is highlighted by this study. These nanoparticles have the potential to greatly support sustainable agriculture methods, as seen by the improved seed germination and growth metrics seen in okra. The underlying mechanisms of action, such as the function of particular phytochemicals in cow dung that might enhance the effectiveness of nanoparticles, should be investigated in future studies. All things considered; the results encourage the shift to more sustainable farming methods by encouraging the use of environmentally friendly products in agricultural applications.

References

1. Zhang R, Zhang H, Tu C, Hu X, Li L, Luo Y, *et al* Christie P. Phytotoxicity of ZnO nanoparticles and the released Zn II ion to corn *Zea mays* L. cucumber *Cucumis sativus* L. during germination. *Environmental Science and Pollution Research*, 2015;22:11109–17.
2. Chen L, Liu L, Lu B, Ma T, Jiang D, Li J, *et al.* Exogenous melatonin promotes seed germination and osmotic regulation under salt stress in cotton *Gossypium hirsutum* L. *Plos One*, 2020;15(1):0228241.
3. Lv B, Xing M, Yang J, Qi W, Lu Y. Chemical and spectroscopic characterization of water extractable organic matter during vermicomposting of cattle dung. *Bioresource Technology*, 2013;132:320–6.
4. Pratap V, Kumar Sharma Y. Impact of osmotic stress on seed germination and seedling growth in black gram (*Phaseolus mungo*). *Journal of Environmental Biology*, 2010;31(5):721.

5. Mir AR, Pichtel J, Hayat S. Copper uptake, toxicity and tolerance in plants and management of Cu-contaminated soil. *Biometals*,2021:34(4):737–759.
6. Priyanka N, Geetha N, Ghorbanpour M, Venkatachalam P. Role of engineered zinc and copper oxide nanoparticles in promoting plant growth yield present status and future prospects. *Advances in Phytotechnology*,2019:183–201.
7. Yusefi-Tanha E, Fallah S, Rostamnejadi A, Pokhrel LR, Particle size and concentration dependent toxicity of copper oxide nanoparticles (CuONPs) on seed yield and antioxidant defense system in soil grown soybean (*Glycine max* cv. Kowsar). *Science of the Total Environment*,2020:715:136994.
8. Shah IH, Manzoor MA, Sabir IA, Ashraf M, Liaquat F, Gulzar S, Chang L, *et al.* Phytotoxic effects of chemically synthesized copper oxide nanoparticles induce physiological biochemical and ultrastructural changes in *Cucumis melo*. *Environmental Science and Pollution Research*,2023:30(18):51595–606.
9. Tripathi D, Singh M, Pandey-Rai S. Crosstalk of nanoparticles and phytohormones regulate plant growth and metabolism under abiotic and biotic stress. *Plant Stress*,2022:6:100107.
10. Ranjan A, Rajput VD, Prazdnova EV, Gurnani M, Sharma S, Bhardwaj P, *et al.* Augmenting abiotic stress tolerance and root architecture the function of phytohormone-producing PGPR and their interaction with nanoparticles. *South African Journal of Botany*,2024:167:612–29.
11. Guo H, Liu Y, Chen J, Zhu Y, Zhang Z. The effects of several metal nanoparticles on seed germination and seedling growth a meta-analysis. *Coatings*,2022:12(2):183.
12. Li R, He J, Xie H, Wang W, Bose SK, Sun Y, *et al.* Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *International Journal of Biological Macromolecules*,2019:126:91–100.
13. Rawat PS, Kumar R, Ram P, Pandey P. Effect of nanoparticles on wheat seed germination and seedling growth. *International Journal of Agricultural and Biosystems Engineering*,2018:12(1):13–16.
14. Das P, Barua S, Sarkar S, Karak N, Bhattacharyya P, Raza N, *et al.* Plant extract-mediated green silver nanoparticles: efficacy as soil conditioner and plant growth promoter. *Journal of Hazardous Materials*,2018:346:62–72.
15. Javed Z, Tripathi GD, Mishra M, Gattupalli M, Dashora K. Cow dung extract mediated green synthesis of zinc oxide nanoparticles for agricultural applications. *Scientific Reports*,2022:12(1):20371.
16. Jiang Y, Zhou P, Zhang P, Adeel M, Shakoore N, Li Y, *et al.* Green synthesis of metal-based nanoparticles for sustainable agriculture. *Environmental Pollution*,2022:309:119755.
17. Singh A, Singh NB, Hussain I, Singh H, Singh SC, Plant-nanoparticle interaction an approach to improve agricultural practices plant productivity. *International Journal of Pharmaceutical Sciences Invention*,2015:4(8):25–40.
18. Aslam AA, Aslam MS, Aslam AA, An overview on green synthesis of nanoparticles and their advanced applications in sustainable agriculture. *International Journal of Applied Chemical Biological Sciences*,2022:3(2):70–99.
19. Mahawar H, Prasanna R. Prospecting the interactions of nanoparticles with beneficial microorganisms for developing green technologies for agriculture. *Environmental Nanotechnology Monitoring Management*,2018:10:477–85.