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OPTIMIZING ZINC OXIDE NANOPARTICLES FOR SEED QUALITY AND VIGOUR IN COWPEA: A DOSE–RESPONSE STUDY

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ABSTRACTS

Background: Cowpea (*Vigna unguiculata* L.) is an important grain legume whose productivity largely depends on seed physiological quality, which governs germination, seedling vigour and crop establishment. Zinc plays a vital role in regulating metabolic and enzymatic processes associated with seed development; however, its availability is often limited in soils. Recent advances in nanotechnology have introduced zinc oxide nanoparticles (ZnO-NPs) as a promising alternative to conventional zinc fertilizers due to their enhanced bioavailability and nutrient use efficiency.
Methods: A pot experiment was conducted under a completely randomized design with thirteen treatments and three replications to evaluate the effect of foliar application of ZnO nanoparticles and bulk zinc on seed quality parameters of cowpea. Zinc was applied at 400, 500 and 600 ppm in both nanoparticles and bulk forms, with single and double foliar spray schedules. Seed quality traits including germination percentage, test weight, seedling length, seedling dry weight, vigour indices, and enzymatic activities (catalase and dehydrogenase) were assessed using standard procedures.
Results: Zinc application significantly improved all seed quality parameters compared with the control, with ZnO nanoparticles outperforming bulk zinc treatments. The treatment involving 500 ppm ZnO nanoparticles applied through two foliar sprays (25 and 50 DAS) recorded the highest test weight (11.30 g), germination (96.78%), vigour index-I (3032.11) and vigour index-II (27864.90), along with enhanced catalase (42.10 µmol H_2O_2 decomposed min^{-1} g^{-1} seed) and dehydrogenase activity (2.18 µg TPF g^{-1} seed h^{-1}). Efficiency analysis confirmed superior nutrient use efficiency of nano-zinc over bulk zinc. Quadratic regression analysis revealed a significant dose–response relationship, identifying ~500 ppm ZnO nanoparticles as the optimum concentration, beyond which the response plateaued.

Keywords: Cowpea; Zinc oxide nanoparticles; Seed germination; Seedling vigour; Enzymatic activity; Nano-fertilizers; Dose–response; Nutrient use efficiency

INTRODUCTION

Cowpea (*Vigna unguiculata* L.) is a nutritionally important grain legume widely cultivated in tropical and subtropical regions due to its adaptability, high protein content and significant contribution to sustainable cropping systems (Boukar *et al*., 2019). In addition to providing an inexpensive source of dietary protein and essential micronutrients, cowpea plays a crucial role in improving soil fertility through biological nitrogen fixation, thereby supporting sustainable agricultural productivity. The success of crop establishment and subsequent productivity in cowpea is largely dependent on seed physiological quality, which governs rapid germination, uniform emergence and vigorous seedling growth under field conditions (Finch-Savage & Bassel, 2016). Seed quality is commonly evaluated through key physiological and biochemical attributes such as germination percentage, test weight, seedling vigour indices and enzymatic activities, which collectively reflect seed metabolic efficiency and physiological integrity during germination and early growth stages. Micronutrient nutrition, particularly zinc (Zn), plays a pivotal role in regulating seed development and germination by influencing several metabolic and biochemical processes (Roychoudhury *et al.,* 2025). Zinc functions as a structural and catalytic component of numerous enzymes involved in protein synthesis, carbohydrate metabolism and hormonal regulation, thereby directly influencing seed formation and seedling development. Adequate zinc availability enhances seed germination, improves seedling vigour and maintains cellular integrity through activation of antioxidant and enzymatic systems (Iqbal *et al*., 2021). However, zinc deficiency remains a widespread constraint in agricultural soils, particularly in intensively cultivated regions, leading to impaired physiological processes, reduced seed quality and lower crop productivity (Singh *et al*., 2020).

Recent advances in nanotechnology have led to the development of zinc oxide nanoparticles (ZnO-NPs) as an efficient alternative to conventional bulk zinc fertilizers. Due to their nanoscale dimensions, higher surface-to-volume ratio and enhanced physicochemical reactivity, ZnO nanoparticles exhibit improved solubility, mobility and bioavailability, resulting in more efficient nutrient uptake and utilization by plants (Sharma *et al*., 2025). Furthermore, nanoscale characterization studies have confirmed that the small particle size and increased surface energy of ZnO nanoparticles

significantly enhance their interaction with plant tissues, thereby improving nutrient delivery and metabolic efficiency (Khan *et al.,* 2022). Recent experimental studies have demonstrated that nano-zinc application significantly improves seed germination, seedling growth, vigour indices and enzymatic activity, highlighting its potential to enhance seed physiological quality and stress tolerance (Geremew *et al*., 2025).

In this context, the present study was undertaken to evaluate the effect of foliar application of zinc oxide nanoparticles and conventional bulk zinc on seed quality parameters of cowpea, including germination percentage, test weight, seedling vigour indices and enzymatic activities such as catalase and dehydrogenase. The objective was to assess the influence of different zinc treatments on seed physiological performance and to determine the effectiveness of nano-zinc fertilization in enhancing seed quality and early crop establishment in cowpea.

MATERIALS AND METHODS

The present investigation was conducted as a pot experiment during the summer season of 2023-24 at the Research Farm of the Department of Vegetable Science, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India with Kashi Kanchan variety of cowpea. During the crop season, the average temperature ranged from 18-40 °C with relative humidity 21-48 %. Earthen pots of 30 cm diameter were used for raising the crop.The soil used for filling the pots was sandy loam in texture with pH 7.8, organic carbon 0.25%, available nitrogen 132 kg/ha, phosphorus 13.80 kg/ha and potassium 238 kg/ha. Foliar spray of three doses of zinc oxide (400, 500 and 600 ppm) was done in both nanoparticles and bulk form along with a recommended dose of fertilizer taking control (no zinc oxide application) as a check. Zinc oxide nanoparticles (ZnO-NPs; 97% purity, particle size <50 nm) were procured from Sigma-Aldrich, USA, while bulk zinc was applied as zinc sulphate heptahydrate ($ZnSO_4 \cdot 7H_2O$) of analytical grade. For nano zinc treatments, the required quantity of ZnO-NP powder was dispersed in distilled water and subjected to ultrasonication for 25–30 minutes using a bath sonicator to ensure uniform dispersion and minimize agglomeration. For bulk zinc treatments, the calculated amount of $ZnSO_4 \cdot 7H_2O$ was dissolved in distilled water and the solution was also ultrasonicated for the same duration to maintain uniformity in spray preparation. After sonication, the volume was adjusted to obtain the desired zinc concentrations as per treatment details. A non-ionic surfactant (Tween-20 @ 0.05%) was added to both nano ZnO suspension and bulk $ZnSO_4$ solution after sonication, followed by gentle mixing without further sonication. These fertilizers were applied as foliar spray at two crop growth stages *i.e.,* 25 days after sowing (pre-flowering) and 50 days after sowing (flowering) using a fine mist hand sprayer during early morning hours. All recommended agronomic practices were followed timely for successfully raising the crop. Recommended dose of fertilizers *i.e.,* 20 kg ha^{-1} of nitrogen and 40 kg ha^{-1} of phosphorus was applied as basal dose.

Different treatment combinations to be followed:

No. of treatment	Treatment detail	Time of application
T_1	Recommended dose of fertilizer (RDF)	As Basal dose
T_2	RDF + 400 ppm ZnO NPs	1 spray: - At 25 DAS
T_3	RDF + 400 ppm Bulk Zn	1 spray: - At 25 DAS
T_4	RDF + 400 ppm ZnO NPs	2 sprays: - At 25 DAS + 50 DAS
T_5	RDF + 400 ppm Bulk Zn	2 sprays: - At 25 DAS + 50 DAS
T_6	RDF + 500 ppm ZnO NPs	1 spray: - At 25 DAS
T_7	RDF + 500 ppm Bulk Zn	1 spray: - At 25 DAS
T_8	RDF + 500 ppm ZnO NPs	2 sprays: - At 25 DAS + 50 DAS
T_9	RDF + 500 ppm Bulk Zn	2 sprays: - At 25 DAS + 50 DAS
T_{10}	RDF + 600 ppm ZnO NPs	1 spray: - At 25 DAS
T_{11}	RDF + 600 ppm Bulk Zn	1 spray: - At 25 DAS
T_{12}	RDF + 600 ppm ZnO NPs	2 sprays: - At 25 DAS + 50 DAS
T_{13}	RDF + 600 ppm Bulk Zn	2 sprays: - At 25 DAS + 50 DAS

The experimental data were analyzed under Completely Randomized Design with three replications and thirteen treatments to test the significance of treatment effects, analysis of variance and regression using OPSTAT and KAU Grapes Software. Test weight (100 seeds weight), standard germination, seedling length, seedling dry weight, vigour indices and catalase & dehydrogenase enzyme activity of the cowpea seeds were determined using standard laboratory analytical procedures.

RESULTS AND DISCUSSION

Effect of Zinc Oxide Nanoparticles and Bulk Zinc on Seed Quality Parameters of Cowpea

Seed quality parameters are critical determinants of seed viability, physiological strength and field establishment potential. Attributes such as test weight, germination percentage, seedling vigour indices and enzymatic activities collectively determine the planting value of seeds and the ability of seedlings to establish rapidly under field

conditions. In the present investigation, foliar application of zinc in both nanoparticle and bulk forms significantly influenced seed quality traits of cowpea, with nano-zinc treatments consistently outperforming bulk zinc fertilizers and the control as illustrated in Table-1.

Test weight, which reflects seed size and efficiency of seed filling during the reproductive phase, was significantly influenced by zinc fertilization. In the present study, test weight varied from 10.08 g to 11.30 g among treatments. The lowest test weight was recorded under the control treatment (T_1), whereas the highest value was obtained under T_8. This treatment remained statistically comparable with T_{12} which recorded a test weight of 11.29 g. Among the bulk zinc treatments, the maximum test weight (10.66 g) was recorded under T_{13} (600 ppm bulk zinc with two sprays). The improvement in test weight under nano-zinc fertilization may be attributed to enhanced photosynthetic activity and improved assimilate translocation towards developing seeds (Saleem *et al*., 2022). Zinc plays an essential role in enzyme activation, carbohydrate metabolism and protein synthesis, all of which contribute to efficient seed filling and accumulation of dry matter in seeds. Furthermore, the nanoscale characteristics of ZnO nanoparticles, including high surface area and enhanced reactivity, facilitate improved nutrient uptake and metabolic efficiency. Similar improvements in seed weight have been reported in cowpea and other legumes by Alasvand and Shokuhfar (2017) and Al-Hadeethi *et al.* (2022).

Seed germination is a key indicator of seed viability and physiological quality. In the present investigation, germination percentage ranged from 88.30% to 96.78% across the treatments. The lowest germination was recorded under the control treatment, whereas the highest germination percentage was observed under T_8 followed closely by T_{12} (96.76%). Bulk zinc treatments recorded comparatively lower germination values ranging between 89.83% and 92.25%. The enhancement in germination under nano-zinc fertilization may be attributed to improved seed maturation and greater accumulation of nutrients during seed development. Zinc is involved in several metabolic pathways including nucleic acid synthesis, enzyme activation and energy metabolism. Adequate zinc supply during seed formation enhances embryo viability and promotes efficient mobilization of stored food reserves during germination, ultimately leading to higher germination percentage (Teiz *et al.,* 2015). Similar observations were reported by Srinivasan *et al.* (2017), Suriyaprabha *et al.* (2018) and Kurdekar *et al.* (2025) who observed improved seed germination in legume crop on zinc micronutrient application.

Table 1: Effect of foliar application of zinc oxide nanoparticles and bulk zinc on seed quality parameters

Treatments	Test Weight (g)	Germination (%)	Seedling length (cm)	Seedling dry weight (mg)	Vigour Index-I	Vigour Index-II	Catalase Enzyme	Dehydrogenase Enzyme
T1	10.08	88.30	27.39	259.00	2418.53	22869.70	35.48	1.86
T2	10.94	94.51	30.20	279.08	2854.20	26375.85	40.21	2.10
T3	10.27	89.83	28.07	263.50	2521.52	23670.21	36.58	1.92
T4	11.21	96.12	30.99	285.50	2978.75	27442.26	41.55	2.16
T5	10.52	91.36	28.78	268.72	2629.34	24550.26	37.76	1.99
T6	11.07	95.19	30.55	281.68	2908.05	26813.12	40.80	2.13
T7	10.35	90.33	28.30	265.20	2556.33	23955.52	36.96	1.94
T8	11.29	96.76	31.30	287.90	3028.58	27857.20	42.06	2.18
T9	10.59	91.83	29.00	270.34	2663.07	24825.32	38.00	2.00
T10	11.07	95.21	30.56	281.75	2909.61	26825.42	40.85	2.13
T11	10.42	90.79	28.50	266.80	2587.51	24222.77	37.31	1.96
T12	11.30	96.78	31.33	287.92	3032.11	27864.90	42.10	2.18

T13	10.66	92.25	29.17	271.88	2690.93	25080.93	38.31	2.01
C. D. at 5%	0.06	0.41	0.21	1.60	26.94	242.18	0.26	0.01

Seedling length and weight are important indicators of seed vigour, seedling length varied from 27.39 to 31.33 cm while seedling weight ranged from 259.00 to 287.92 mg among treatments. The lowest value was observed under control, while the highest seedling length and weight was recorded under treatment T_{12} (600 ppm ZnO nanoparticles with two sprays) which remaining statistically at par with T_8 (500 ppm ZnO nanoparticles with two sprays). Improved seedling growth under zinc treatments may be associated with enhanced enzymatic activity and auxin synthesis during germination, which promotes cell division and elongation. Further, Zinc plays a vital role in mobilization of seed reserves and greater biomass accumulation during early growth thereby caused high dry matter production (Rizwan *et al.,* 2019). Previous studies by Sorahinobar *et al.* (2023) and Kumar *et al.* (2023) have also reported that zinc fertilization improves seedling growth and early establishment in legume crops.

Seedling vigour is a reliable indicator of seed physiological strength and the ability of seedlings to establish rapidly under field conditions. In the present study, zinc fertilization significantly influenced both vigour index-I and vigour index-II. Vigour index-I ranged from 2418.53 to 3032.11, whereas vigour index-II ranged from 22869.70 to 27864.90 among the treatments. The lowest vigour index values (2418.53 and 22869.70) were recorded under the control treatment, whereas the highest values (3032.11 and 27864.90) were obtained under T_{12} (600 ppm ZnO nanoparticles with two foliar sprays). These values remained statistically comparable with T_8 (500 ppm ZnO nanoparticles with two sprays). The improvement in seedling vigour under nano-zinc treatments may be attributed to enhanced nutrient accumulation in seeds and improved metabolic activity during germination. Zinc is known to regulate several physiological processes including protein synthesis, auxin metabolism and cell division, which collectively promote rapid seedling growth and biomass accumulation. Moreover, higher nutrient reserves in seeds produced under nano-zinc fertilization provide sufficient energy for early seedling development, resulting in more vigorous seedlings (Rizwan *et al.,* 2019). These findings corroborate the results of Choudhary and Khandelwal (2020) and Kumar *et al*. (2023) who reported that zinc fertilization significantly improved seed vigour and seedling growth in leguminous crops.

Enzyme activity serves as an important biochemical indicator of seed metabolic efficiency and viability. In the present investigation, zinc fertilization significantly enhanced both catalase and dehydrogenase enzyme activities. Catalase activity ranged from 35.48 to 42.10 H_2O_2 decomposed min^{-1} g^{-1} seed, while dehydrogenase activity ranged from 1.86 to 2.18 µg TPF g^{-1} seed h^{-1}. The lowest enzyme activities (35.48 and 1.86) were recorded under the control treatment, whereas the highest activities (42.10 and 2.18) were observed under T_{12} (600 ppm ZnO nanoparticles with two sprays) and remained statistically comparable with T_8. The increased enzymatic activity under nano-zinc fertilization may be attributed to the critical role of zinc as a structural and catalytic component of numerous enzymes involved in respiration and energy metabolism. Catalase protects cells from oxidative damage by detoxifying hydrogen peroxide generated during metabolic processes, while dehydrogenase activity reflects improved respiratory metabolism and seed viability. Consequently, higher enzyme activity under ZnO nanoparticle treatments facilitated rapid mobilization of stored food reserves during germination, resulting in enhanced seedling growth and vigour (Marschner, 2012). Comparable results were reported by Tului *et al.* (2021) and Basahi (2022) while estimating the role of nano-zinc in enzyme activation and antioxidant defence mechanisms in legume crops.

Efficiency analysis of zinc treatments on seed quality parameters

Efficiency analysis was performed to evaluate the relative effectiveness of zinc oxide nanoparticles and conventional bulk zinc fertilization in improving seed quality parameters of cowpea. The efficiency values were calculated as the percentage increase in each trait over the untreated control treatment as depicted in Table-2.

Among the evaluated treatments, foliar application of 600 ppm ZnO nanoparticles with two sprays (T_{12}) demonstrated the highest efficiency for most of the seed quality attributes. This treatment resulted in an improvement of approximately 12.10% in test weight and 9.60% in germination percentage over the control treatment, indicating a significant enhancement in seed development and viability. Similarly, seed vigour indices showed marked improvement under nano-zinc fertilization, with vigour index-I increasing by about 25.36% and vigour index-II by 21.84% compared with the control. The enhanced seed vigour under nano-zinc application suggests improved seed metabolic activity and more efficient mobilization of stored reserves during germination (Gupta *et al*., 2024). Enzymatic activities associated with seed metabolism were also considerably enhanced under zinc oxide nanoparticle treatments. Catalase activity increased by approximately 18.65%, while dehydrogenase activity improved by 17.20% over the control. It reflects greater metabolic activity and improved oxidative stress regulation during seed

germination. These enzymatic responses indicate that nano-zinc fertilization stimulates key biochemical processes involved in seed viability and early seedling establishment (Kumar *et al*., 2025). The maximum improvement recorded under bulk zinc fertilization was about 5.75% for test weight, 4.47% for germination, 11.26% for vigour index-I and 9.66% for vigour index-II, indicating relatively lower nutrient use efficiency than nano-zinc treatments. Similarly, enzymatic activities under bulk zinc treatments increased only moderately, further highlighting the superior physiological response induced by zinc oxide nanoparticles.

The higher efficiency of ZnO nanoparticles may be attributed to their unique physicochemical properties, including greater surface area, enhanced reactivity and improved bioavailability, which facilitate more efficient absorption and translocation of zinc within plant tissues (Rani *et al.,* 2025).

Table-2: Relative efficiency (%) of zinc treatment on seed quality parameters of cowpea

Treatments	Test Wt. (%)	Germination (%)	Vigour index-I (%)	Vigour index-II (%)	Catalase Enzyme (%)
T_1	-	-	-	-	-
T_2	8.53	7.03	18.01	15.33	13.33
T_3	1.88	1.73	4.25	3.50	3.10
T_4	11.21	8.85	23.16	19.99	17.10
T_5	4.36	3.46	8.71	7.34	6.42
T_6	9.82	7.80	20.24	17.24	14.99
T_7	2.67	2.29	5.69	4.74s	4.17
T_8	12.00	9.58	25.22	21.80	18.54
T_9	5.05	3.99	10.11	8.55	7.10
T_{10}	9.82	7.82	20.30	17.29	15.18
T_{11}	3.37	2.81	6.98	5.91	5.15
T_{12}	12.10	9.60	25.36	21.84	18.65
T_{13}	5.75	4.47	11.26	9.66	7.97

Dose–response modelling using quadratic regression/Regression analysis

Quadratic regression analysis was employed to examine the dose–response relationship between zinc concentration and seed quality parameters of cowpea. The regression models revealed a pronounced curvilinear association between zinc application and key seed physiological attributes, including germination percentage, test weight, seedling vigour index and enzymatic activities as illustrated in Figure-1. The fitted quadratic curves demonstrated that seed quality parameters increased progressively with increasing zinc concentration up to an optimum threshold, beyond which the response tended to stabilize or show a slight decline. This pattern clearly reflects the nonlinear influence of zinc availability on seed physiological performance and highlights the presence of an optimal nutrient level required to maximize seed quality (Jung and Lee*,* 2025).

The regression analysis further indicated that treatments receiving zinc oxide nanoparticles exhibited a markedly stronger response compared with conventional bulk zinc treatments. This was evidenced by steeper regression slopes and higher predicted maxima under nano-zinc application, suggesting improved nutrient use efficiency and enhanced physiological responsiveness (Prasad *et al*., 2017). The superior response observed under ZnO nanoparticle treatments may be attributed to their nanoscale characteristics, including greater surface area, enhanced reactivity and improved mobility within plant tissues, which collectively facilitate more efficient zinc uptake and metabolic utilization during seed development.

Among the concentrations evaluated, the quadratic regression models consistently predicted the optimum zinc dose that recorded the highest seed quality attributes in the experiment to be approximately 500 ppm ZnO nanoparticles. The close agreement between the predicted regression maxima and the experimentally observed peak further supports the reliability of the regression models in identifying the optimal dose (O'Brien and Silcox, 2024). Beyond the optimum concentration, the regression curves exhibited a tendency toward stabilization or marginal decline, indicating that increasing zinc supply above the optimal level does not result in proportional improvements in seed quality traits (Sayed *et al*., 2024). This phenomenon may be associated with reduced nutrient use efficiency or metabolic saturation at higher zinc concentrations, which limits further physiological benefits (Montgomery *et al*., 2021).

CONCLUSION

The findings of the efficiency analysis further confirmed that ZnO nanoparticles provide substantially greater improvements in seed physiological quality compared with bulk zinc fertilizers, highlighting their agronomic superiority. Moreover, the quadratic regression analysis established a clear dose–response relationship and identified 500 ppm ZnO nanoparticles as the optimal concentration for maximizing seed quality, beyond which no significant

additional benefits were observed. From an agronomic and practical perspective, the use of zinc oxide nanoparticles offers a promising and efficient approach for enhancing seed quality, improving seedling establishment and potentially increasing crop productivity in cowpea. Additionally, improved seed physiological traits and enzymatic activity under nano-zinc fertilization indicate its potential role in nutritional biofortification and sustainable crop production. Therefore, foliar application of ZnO nanoparticles at an optimum concentration of 500 ppm with two sprays can be recommended as an effective strategy for improving seed quality and early crop establishment in cowpea under similar agro-climatic conditions. Further field-based validation and long-term studies are suggested to assess its scalability and environmental implications.

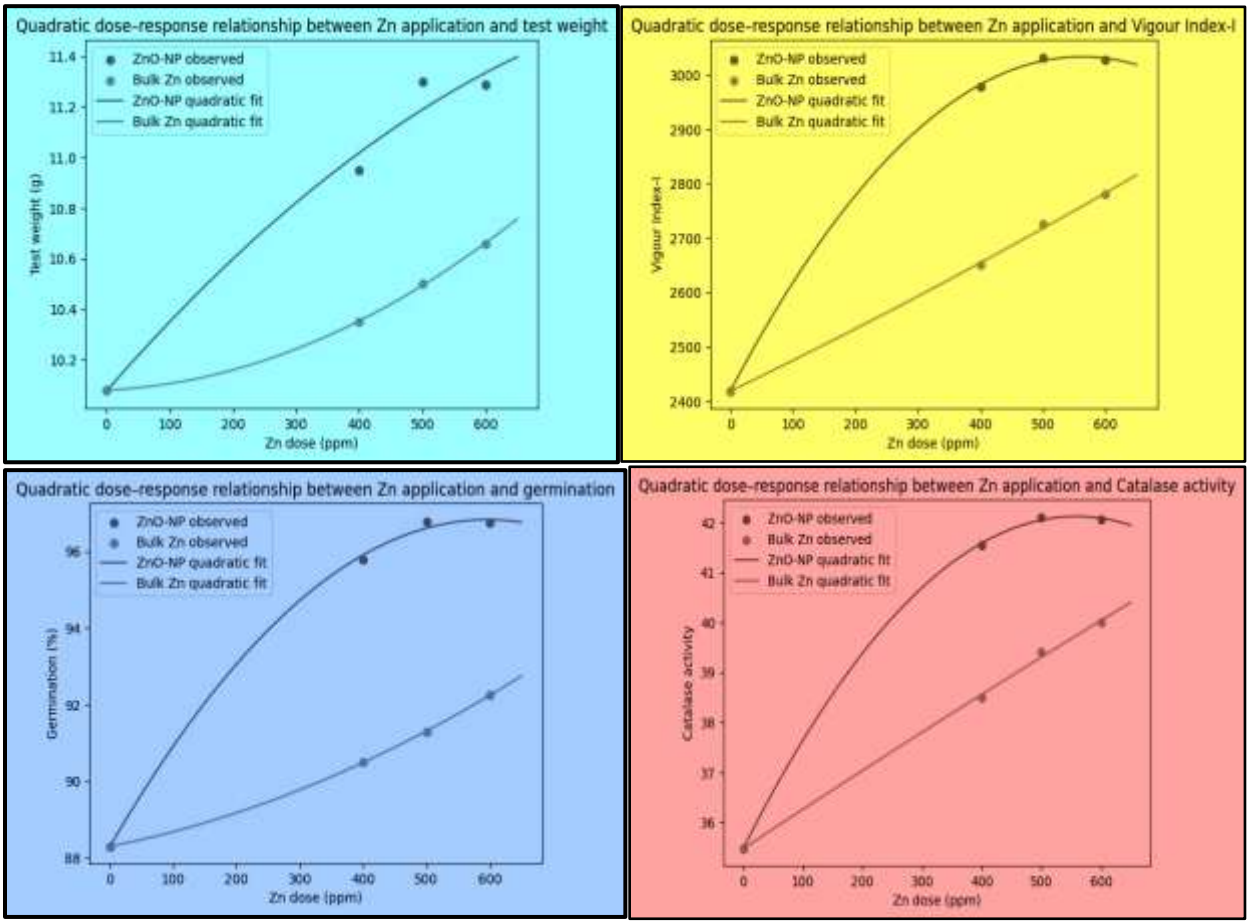


Figure-1: Quadratic dose response relationship between zinc and seed quality parameters

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Disclaimers
The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

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Informed consent
All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

REFERENCES

1. Al– Hadeethi, M. S. B., Al-Dulaimi, O. I., & Al-,Hayani, M. A. H. (2023, December). Evaluation of Several Genotypes of *Vigna radiata* Bean for Spraying with Manganese and Zinc and Their Effects on Growth Characteristics. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1262, No. 5, p. 052026). IOP Publishing.
2. Alasvand P and Shokuhfar A. (2017).Evaluation Effect of Different Levels of Zinc and Manganese Nano-Chelate on Quantitative and Qualitative Traits of Cowpea (*Vigna unguiculata* L.). *Journal of Crop Nutrition Science*. **3(4):**33. https://oiccpress.com/jcns/article/view/5858
3. Basahi, M. A. (2022). Effects of zinc oxide nanoparticles on germination and seedling establishment of pea (*Pisum sativum*) and beans (*Phaseolus vulgaris*). *Applied Ecology & Environmental Research*. ***20*(5):** 3893-3909**.** http://dx.doi.org/10.15666/aeer/2005_38933909
4. Boukar, O., Belko, N., Chamarthi, S., Togola, A., Batieno, J., Owusu, E. & Fatokun, C. (2019). Cowpea (*Vigna unguiculata)*: Genetics, genomics and breeding. *Plant breeding*. ***138*(4):** 415-424. https://doi.org/10.1111/pbr.12589.
5. Choudhary, P. K., & Khandelwal, V. (2020). Comparative efficacy of Zn supplement and zinc oxide nanoparticles over the seed germination of lentil and chick pea. *Journal of Pure and Applied Microbiology*. ***14*(1):** 673-678. https://doi.org/10.22207/JPAM.14.1.69
6. Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: extending performance beyond adaptation. *Journal of Experimental Botany*. **67(3):** 567-591. https://doi.org/10.1093/jxb/erv490
7. Geremew, A., Stovall, L., Woldesenbet, S., Ma, X., & Carson, L. (2025). Nanopriming with zinc oxide: a novel approach to enhance germination and antioxidant systems in amaranth. *Frontiers in Plant Science*. ***16*(1):** 1-15. 10.3389/fpls.2025.1599192
8. Gupta A, Bharati R, Kubes J, Popelkova D, Praus L, Yang X, Severova L, Skalicky M and Brestic M (2024) Zinc oxide nanoparticles application alleviates salinity stress by modulating plant growth, biochemical attributes and nutrient homeostasis in *Phaseolus vulgaris*. *Frontiers of Plant Science*. **15**:1-16. https://doi.org/ 10.3389/fpls.2024.1432258.
9. Iqbal, M. S., Akbar, M., Akhtar, S., Fatima, S., & Ghafoor, A. (2021). Multivariate analysis and its application for characterizing cowpea landraces. *Legume Research: An International Journal*.**44(1).** https://doi.org/10.18805/LR-489.
10. Jung, D. H., & Lee, J. (2025). Establishing a Quadratic Regression Model of the Growth Characteristics and Isoorientin Contents of Wheatgrass Seedlings in Response to Different Temperature Conditions. *Horticultural Science and Technology*, ***43*(1):** 61-69. https://doi.org/10.7235/HORT.20250008
11. Khan, Y., Sadia, H., Ali Shah, S. Z., Khan, M. N., Shah, A. A., Ullah, N., ... & Khan, M. I. (2022). Classification, synthetic, and characterization approaches to nanoparticles, and their applications in various fields of nanotechnology: a review. *Catalysts*. ***12*(11):**1386-1402. https://doi.org/10.3390/catal12111386
12. Kumar, S. M., Yadav, S., Yadav, S., Choudhary, R., LAL, S., Bharadwaj, C., & Kumar, P. R. (2023). Preliminary studies on the effect of nanoparticle seed treatments on seed quality attributes and yield in chickpea. *Turkish Journal of Agriculture and Forestry*.***47*(1):**100-115. https://doi.org/10.55730/1300-011X.3067.
13. Kumar, S.M., Yadav, S., Choudhary, R. (2025).Nanopriming with zinc oxide nanoparticle boosts seed vigour, photosynthesis, osmolytes accumulation and antioxidant activity in tomato. *Scietific Reports*. **15(1):** 37375-37389. https://doi.org/10.1038/s41598-025-09269-4.
14. Kurdekar, A. K., Desai, B. K., Koppalakar, B. G., Sannagoudar, M. S., Rathod, P. S., Hiregoudar, S. & Nazim, M. (2025). Comparative evaluation of green synthesized and commercial iron and zinc nanoparticles on germination, growth and productivity of pigeonpea. *Scientific Reports*. ***15*(1):** 25956-25970.
15. Marschner, H. (Ed.). (2011). *Marschner's mineral nutrition of higher plants*. Academic press.
16. Montgomery, D.C., Peck, E.A., Vining, G.G. (2021). Introduction to Linear Regression Analysis, 6th ed. John Wiley & Sons, Hoboken, NJ, USA.
17. O'Brien, T. E., & Silcox, J. W. (2024). Nonlinear regression modelling: a primer with
18. applications and caveats. *Bulletin of mathematical biology*. ***86*(4):** 40-54. https://doi.org/10.1007/s11538-024-01274-4
19. Prasad, R., Bhattacharyya, A., & Nguyen, Q. D. (2017). Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives. *Frontiers in microbiology*. ***8*(1):** 1014-1027. https://doi.org/10.3389/fmicb.2017.01014
20. Rani, S., Kumari, N., & Sharma, V. (2025). Zinc oxide nanoparticles improve photosynthesis by modulating antioxidant system and psb a gene expression under arsenic stress in different cultivars of Vigna radiata. *BioNanoScience*. ***15*(2):** 242-253. https://doi.org/10.1007/s12668-025-01858-x
21. Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A. & Waris, A. A. (2019). Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and
22. cadmium concentration in wheat. *Chemosphere*. **214(1):** 269-277.

https://doi.org/10.1016/j.chemosphere.2018.09.120
23. Roychoudhury, A., Mukherjee, S., & Ghosh, A. (2025). Molecular and physiological studies of the role of micronutrients in sustainable growth and development of crop plants. In *Agricultural crop improvement* (pp. 135-147). CRC Press.
24. Saleem, M., Usman, K., Rizwan, M., Al Jabri, H., & Alsafran, M. (2022). Functions and strategies for enhancing zinc availability in plants for sustainable agriculture. *Frontiers in Plant Science*. **13(1):**1033092-1033105. https://doi.org/10.3389/fpls.2022.1033092.
25. Sayed, M. D., Hosen, M., Rahman, M. H., Morium, M., Islam, M. R., Kubra, M. K & Islam, M. S. (2024). Effect of boron and zinc on growth, yield attributes, yield and nutrient bio-fortification of grass pea (lathyrus sativus l.) In old
26. himalayan piedmont plain. *Applied Ecology & Environmental Research*. **22(3):** 2278-2305. http://dx.doi.org/10.15666/aeer/2203_22772305
27. Sharma, R., Kumar, N., Sharma, P., Yadav, A., & Aggarwal, N. K. (2025). Biosynthesis, characterisation and therapeutic potential of green coconut waste derived zinc oxide nanoparticles. *Results in Surfaces and Interfaces*. **18(1):** 100372-100383. https://doi.org/10.1016/j.rsurfi.2024.100372
28. Singh, O. V., Shekhawat, N., Singh, K., & Gowthami, R. (2020). Assessment of genetic variability and inter-character association in the germplasm of cowpea (*Vigna unguiculata* L. Walp) in hot arid climate. *Legume Research*. **43(3):** 62-366. https://doi.org/10.18805/LR-3983.
29. Sorahinobar, M., Deldari, T., Nazem Bokaeei, Z., & Mehdinia, A. (2023). Effect of zinc nanoparticles on the growth
30. and biofortification capability of mungbean (*Vigna radiata*) seedlings. *Biologia*. **78(4):** 951-960. https://doi.org/10.1007/s11756-022-01269-3
31. Srinivasan, R., Maity, A., Singh, K. K., Ghosh, P. K., Kumar, S., Srivastava, M. K. & Kumari, B. (2017). Influence of copper oxide and zinc oxide nano-particles on growth of fodder cowpea and soil microbiological properties. *Range Management and Agroforestry*. **38(2):** 208-214. https://rmaj.in/rma/article/view/225
32. Suriyaprabha, R., Sreeja, K. A., Prabu, M., Prabu, P. and Rajendran, V. (2018). Bioaccumulation of transition metal oxide nanoparticles and their influence on early growth stages of *Vigna unguiculata* seeds. *Bionano science.* **8(3):** 752-760. https://doi.org/10.1007/s12668-018-0535-2
33. Teiz, L., Zeiger, E., Møller, I. M., & Murphy, A. (2015). Plant physiology and development (6th ed.). Sinauer Associates.
34. Tului, V., Janmohammadi, M., Abbasi, A., Vahdati-Khajeh, S., & Nouraein, M. (2021). Influence of iron, zinc and bimetallic Zn-Fe nanoparticles on growth and biochemical characteristics in chickpea (*Cicer arietinum*) cultivars. *Poljoprivreda i Sumarstvo*. **67(2):** 179-193. https://doi.org/10.17707/AgricultForest.67.2.13.