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MORPHO-STRUCTURAL AND FUNCTIONAL ASSESSMENT OF DUAL-SYNTHESIZED ZNO AND SIO_2 NANOPARTICLES FOR ENHANCED SEED GERMINATION AND ANTIFUNGAL EFFICACY

R.R. Dhutmal[1], K.S. Baig[2], H.V. Kalpande[3], S. G. Shinde[*4], D. K. Zate[5], J. D. Deshmukh[6], M. P. Wankhade[7], A. R. Gaikwad[8]

[1] Associate Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: dhutmalrajesh@gmail.com
ORCID: 0000-0002-4234-5264
[2] Director of Research, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: drkhizerbaig@gmail.com
ORCID: 0000-0001-6499-0515
[3] Head, Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: hvkalpande@gmail.com
ORCID: 0009-0004-6360-6146
[4] Assistant Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: sandeepshinde@vnmkv.ac.in ORCID: 0009-0009-1611-4428
[5] Associate Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India.
Email: dilip20176@gmail.com ORCID: 0009-0002-8148-2867
[6] Assistant Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: jaydeshmukh55@gmail.com ORCID: 0000-0001-8808-4322
[7] Assistant Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: meenawankhade81@gmail.com ORCID: 0009-0006-6980-1887
[8] Assistant Professor (GPB), Department of Genetics & Plant Breeding, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra – 431402, India. Email: arungaikwad_2008@rediffmail.com ORCID: 0000-0001-6695-7292
*Corresponding Author: Dr. Sandeep Govindappa Shinde, sandeepshinde@vnmkv.ac.in

ABSTRACT

The production of high-quality and climate-resilient seeds is critical for ensuring food security under changing environmental conditions. Nanotechnology offers a promising, sustainable alternative to conventional agrochemical-based approaches for improving seed germination, vigor, and health. The present study aimed to synthesize zinc oxide (ZnO) nanoparticles using a sol–gel method and bio-synthesize silica (SiO_2) nanoparticles from sugarcane bagasse through a green approach, to characterize their physicochemical properties, and to evaluate their effects on germination, seedling vigor, and seed health of soybean (Glycine max L.).
ZnO nanoparticles were synthesized using zinc nitrate and potassium hydroxide, followed by calcination, while SiO_2 nanoparticles were obtained from sugarcane bagasse through acid extraction and thermal processing. The nanoparticles were characterized using SEM, TEM, SEM–EDX, and UV–Visible spectroscopy, confirming nano-scale size, characteristic morphology, and elemental purity. Uniformly dispersed nanoparticle suspensions were prepared at different concentrations (30–1200 ppm) and applied as seed treatments.
Results revealed a clear dose-dependent response in soybean seed performance. Lower concentrations, particularly 50 ppm, significantly enhanced germination percentage, shoot and root length, dry seedling weight, and vigor indices (VI-I and VI-II), while simultaneously reducing diseased seed incidence compared to the control. In contrast, higher concentrations (≥800 ppm) resulted in reduced growth and vigor, along with increased disease incidence, indicating phytotoxic effects at elevated doses. Multivariate analyses, including principal component analysis (PCA) and correlation heat maps, demonstrated strong positive associations among germination, growth, and vigor traits, and a strong negative association with diseased seeds.
The study highlights the dual functionality of ZnO and bio-derived SiO_2 nanoparticles in promoting seedling growth and improving seed health when applied at optimized concentrations. The use of sugarcane bagasse as a silica source further underscores the sustainability and circular economy potential of the approach. Overall, nanoparticle-based seed treatment at low concentrations represents a viable strategy for enhancing soybean seed quality and early crop establishment while reducing reliance on conventional agrochemicals.

Key words: Zinc and silicon nanoparticles, seed treatment, soybean, germination percentage, vigor

INTRODUCTION

Ensuring food security under changing climatic conditions requires the production of resilient, high-quality seeds capable of rapid germination, uniform establishment, and enhanced tolerance to biotic and abiotic stresses. Conventional agricultural practices largely depend on repeated applications of chemical fertilizers, pesticides, plant growth regulators (PGRs), and fungicides to sustain crop productivity. Although effective in the short term, excessive reliance on these agrochemicals has raised concerns related to environmental degradation, declining soil health, residue accumulation, and increasing production costs. Consequently, there is a growing need for innovative, sustainable, and precision-based approaches to improve seed and crop performance.

In recent years, nanotechnology has emerged as a promising tool for target-oriented and resource-efficient agriculture. Nanoparticles (NPs), owing to their small size, high surface area, and enhanced reactivity, can interact efficiently with plant systems even at very low concentrations. Several studies have demonstrated the potential of metal and metalloid nanoparticles such as zinc (Zn), silicon (Si), silver (Ag), and titanium (Ti) to enhance seed germination, seedling vigor, nutrient use efficiency, stress tolerance, and disease resistance in different crops, while simultaneously reducing the dependence on conventional agrochemicals, herbicides, fungicides, and insecticides (Nair et al., 2010; Raliya et al., 2015; Tripathi et al., 2017).

Among these, ZnO nanoparticles are of particular importance due to the essential role of zinc as a micronutrient involved in enzyme activation, protein synthesis, membrane integrity, and antioxidant defense. Similarly, silicon-based nanoparticles (SiO_2 NPs) are gaining attention for their ability to strengthen cell walls, improve seed coat integrity, enhance water uptake, and confer tolerance against mechanical damage and pathogens. The use of bio-derived silica, especially from agricultural residues such as sugarcane bagasse, offers an environmentally benign and value-added route for nanoparticle synthesis, aligning with principles of circular economy and sustainable agriculture.

Soybean (Glycine max L.) is one of the most important oilseed crops in India, particularly in Maharashtra, contributing significantly to edible oil production, protein supply, and farm income. However, the demand for high-quality soybean seed is steadily increasing, while the demand–supply gap continues to widen due to issues such as below-standard germination of seed lots, poor seed vigor, seed-borne diseases, and mechanical damage to the relatively thin seed coat during harvesting, processing, and handling. These constraints often lead to poor field emergence, uneven crop stand, and yield penalties.

Although nanoparticles have been reported to exert beneficial effects on seed germination, seedling growth, and yield, systematic studies evaluating the effect of realistic concentrations and modes of application in soybean are limited, particularly for bio-synthesized SiO_2 nanoparticles and comparatively less-explored materials such as lignin-based nanoparticles. Moreover, the dual functionality of nanoparticles, combining growth promotion with seed health protection (reduced disease incidence and improved seed coat integrity), remains inadequately investigated in soybean.

In this context, the present study was undertaken with the objectives to synthesize ZnO nanoparticles and bio-synthesize SiO_2 nanoparticles from sugarcane bagasse, to characterize their physicochemical properties using advanced analytical techniques, and to evaluate their effects on germination percentage, seedling vigor, and seed health of soybean. The study aims to generate scientific evidence on the potential of nano-enabled seed treatments as a sustainable strategy for producing climate-resilient, high-quality soybean seeds.

MATERIALS AND METHODS

Experimental material and seed source

Seeds of soybean (Glycine max L.) variety MAUS 612 were obtained from AICRP on Soybean, VNMKV, Parbhani and stored under ambient laboratory conditions prior to experimentation. Seeds having uniform sorted size were out and observed under stereoscopic microscope for any visible mechanical damage and fungal infestation.

Seed cleaning and sterilization

To eliminate surface-borne contaminants, soybean seeds were initially wiped with distilled water followed by rinsing with distilled water. Seeds were then surface-sterilized using 1% (v/v) sodium hypochlorite (NaOCl) solution for 2 minutes, followed by thorough wiping three times with sterile distilled water to remove residual sterilant. Finally, seeds were air-dried on sterile blotting paper under laminar airflow conditions before nanoparticle treatment. This protocol ensured effective sterilization without adversely affecting seed viability.

I. Synthesis of zinc oxide (ZnO) nanoparticles (Sol–Gel Method)

Zinc oxide nanoparticles were synthesized using a controlled sol–gel method. An aqueous solution of zinc nitrate hexahydrate [$Zn(NO_3)_2 \cdot 6H_2O$] (0.2 N) was prepared by dissolving the required quantity of salt in 100 mL of deionized water under continuous magnetic stirring.

Separately, potassium hydroxide (KOH) solution (0.4 N) was prepared in deionized water. The KOH solution was added dropwise (≈1 mL min^{-1}) to the zinc nitrate solution under constant stirring at 40°C, maintaining alkaline conditions to facilitate hydrolysis and gel formation. The reaction was continued until a uniform white precipitate was obtained.

The gel was allowed to age and subsequently centrifuged at 7,000 rpm for 5 minutes. The precipitate was washed 2-3 with deionized water to remove excess ions and impurities followed by washing with 70% ethanol. The washed material was oven-dried and then calcined at 500 °C for 4 hours in a muffle furnace to obtain crystalline ZnO nanoparticles.

II. Bio-Synthesis of Silica (SiO_2) Nanoparticles from Sugarcane Bagasse

Silica nanoparticles were synthesized through a green biosynthesis approach using sugarcane bagasse. Fresh sugarcane bagasse was thoroughly washed with distilled water, air-dried, and finely chopped. The dried bagasse was treated with 1 N sulfuric acid (H_2SO_4) using a solid-to-liquid ratio of 1:10 (w/v) and heated at 65^0c in water bath to extract silicate fractions.

The acid-treated slurry was filtered with Whatman paper to obtain a sodium silicate-rich solution, which was allowed to stand for silica precipitation. The precipitate was separated by centrifugation and washed repeatedly with distilled water until neutral pH was achieved finally washing with 70% ethanol. The recovered silica was oven-dried and subsequently calcined at 600°C to remove organic residues. The resulting material was finely powdered to obtain bio-synthesized SiO_2 nanoparticles.

III. Preparation of nanoparticle suspensions

Fine powders of ZnO and SiO_2 nanoparticles were dispersed separately in deionized water to prepare treatment concentrations of 30, 50, 100, 200, 500, 800, 1000, and 1200 ppm. Each suspension was subjected to ultrasonication at 40 W and 45 °C for 30 minutes to ensure uniform dispersion and prevent agglomeration. Fresh suspensions were prepared prior to seed treatment.

IV. Characterization of nanoparticles

The synthesized ZnO and SiO_2 nanoparticles were characterized at Nanotechnology laboratory at Tamil Nadu using Scanning Electron Microscopy (SEM)and Transmission Electron Microscopy (TEM) for surface morphology and particle size determination. SEM–EDX analysis was performed for elemental confirmation, while UV–Visible spectroscopy was used to assess optical properties and validate nanoscale formation.

V. Seed Treatment and Germination Test

Sterilized soybean seeds were soaked in the respective nanoparticle suspensions for a fixed duration under laboratory conditions. Untreated seeds soaked in deionized water served as the control. Treated seeds were placed in sterile Petri dishes lined with moist filter paper and incubated under controlled conditions.

Observations were recorded for germination percentage, shoot length, root length, seedling length, dry seedling weight, vigor index-I (VI-I), vigor index-II (VI-II), and diseased seeds percentage, following standard seed testing protocols.

VI. Statistical Analysis

The experiment was conducted in a completely randomized design (CRD) with three replications. Data were subjected to analysis of variance (ANOVA) to test the significance of treatment effects. Mean values were expressed as mean ± standard deviation (SD) and visualized using bar charts with error bars.

To understand multivariate relationships among seed quality traits and treatments, Principal Component Analysis (PCA) was performed using standardized data. Trait associations were further examined through Pearson's correlation coefficients, which were visualized using a correlation heat map. All statistical analyses were performed using R statistical software (R Core Team, Vienna, Austria), while graphical visualizations were carried out using python (Python Software Foundation, USA) with scientific libraries NumPy, SciPy, Matplotlib and Seaborn).

RESULTS AND DISCUSSION

Table 1. ANOVA of seed quality and seedling vigor traits of soybean as influenced by nanoparticle concentrations

Source	df	Shoot length (cm)	Germination (%)	Root length (cm)	Dry seedling weight (mg)	Vigor Index-I	Vigor Index-II	Diseased seeds (%)
Treatments	8	7.972	142.5	4.613	23.603	427510.20	366920.20	911.583
Error	18	0.380	10.825	0.472	1.398	30285.99	26192.98	3.862
Total	26							

The significant treatment effects observed in ANOVA for germination, seedling growth, vigor indices, and diseased seed percentage confirm the strong influence of nanoparticle application on soybean seed performance. Similar enhancements in soybean germination and early seedling growth due to ZnO and SiO_2 nanoparticles at lower concentrations have been reported earlier and attributed to improved water uptake, enzyme activation, micronutrient availability, and antioxidant regulation (Raliya et al., 2015; Bansal & Kaushik, 2021). Reduction in diseased seeds at optimal concentrations further suggests a protective role of nanoparticles through antimicrobial activity and improved seed coat integrity (Singh et al., 2016). However, the decline in performance at higher concentrations supports earlier findings that excessive nanoparticle doses induce oxidative stress and phytotoxic effects, emphasizing the need for dose optimization in soybean seed treatment (Singh et al., 2020; Tripathi et al., 2017).

Characterization of Zinc (Zno) and Silica (SiO_2) nanoparticles

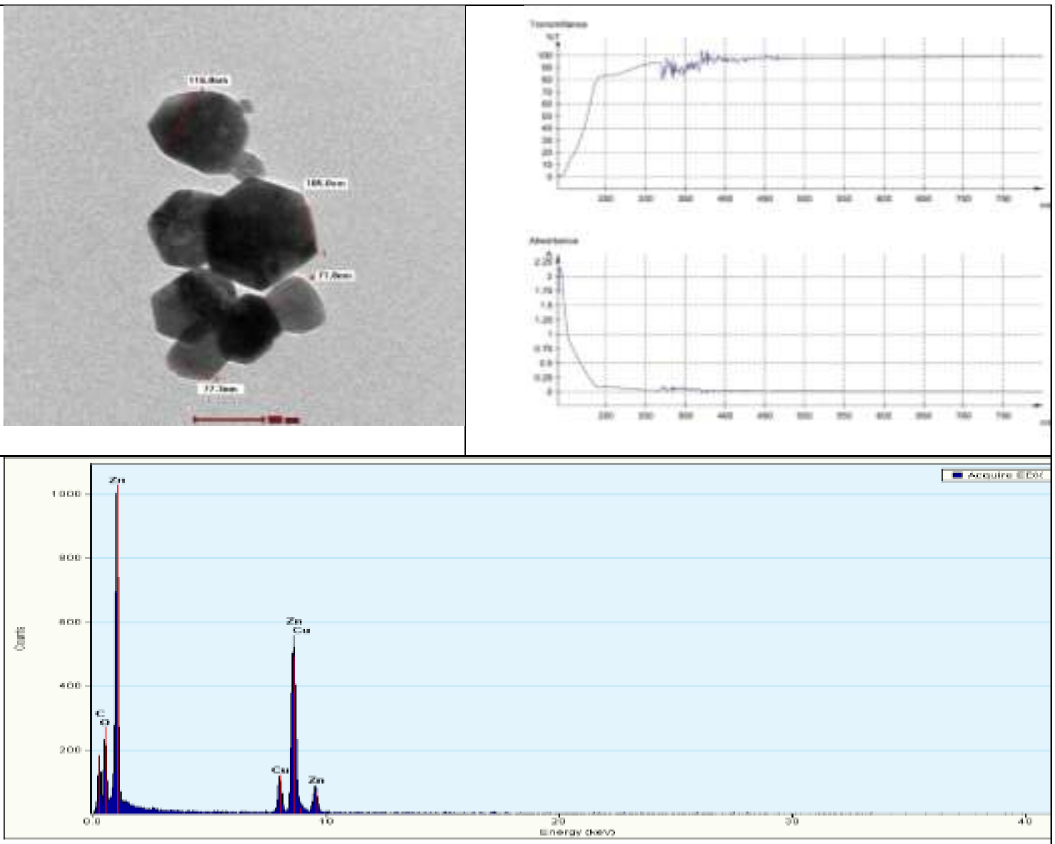

Fig 1. Characterization of synthesized nanoparticles showing (a) TEM micrograph depicting particle morphology and size distribution, (b) UV–Visible absorption and transmittance spectra indicating optical behavior, and (c) EDX spectrum confirming elemental composition with zinc as the predominant element.

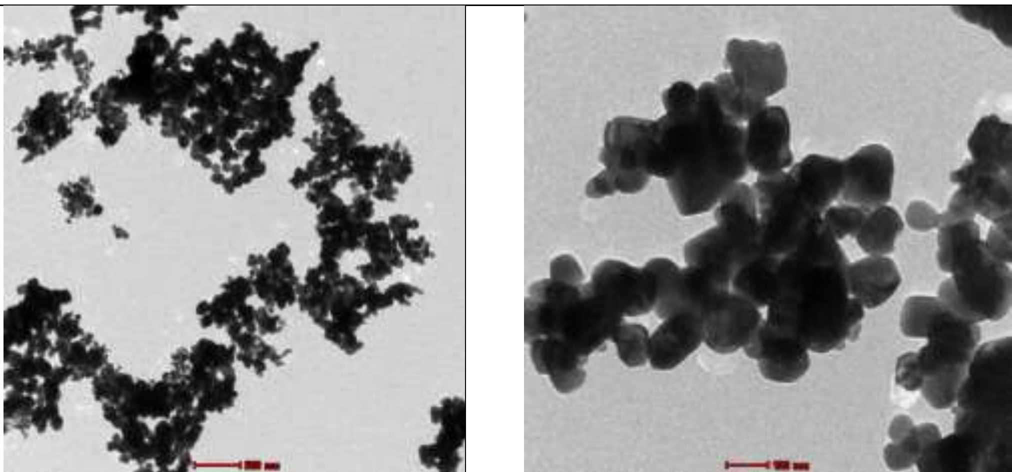

Fig 2. Transmission electron microscopy (TEM) images of synthesized SiO_2 nanoparticles showing (a) low-magnification view illustrating agglomerated nanoparticle clusters and overall dispersion (scale bar: 500 nm) and (b) high-magnification view depicting particle morphology and nanoscale size distribution (scale bar: 100 nm).

The bio-synthesis and characterization of silica (SiO_2) nanoparticles derived from sugarcane bagasse and zinc oxide (ZnO) nanoparticles synthesized for seed treatment applications revealed distinct morphological, structural, and optical features that confirm successful nanoparticle formation.

Silica nanoparticles (SiO_2) synthesized from sugarcane bagasse exhibited well-defined nano-scale characteristics as evidenced by SEM analysis. The micrographs revealed that the particles were predominantly spherical in shape**,** occurring largely as agglomerates with visible porous structures**.** Individual particle sizes ranged from 30 to 80 nm**,** indicating effective size reduction through the bio-extraction route. The porous nature of the silica matrix is attributed to the removal of organic constituents of bagasse during calcination, resulting in a high-surface-area amorphous silica network. Such agglomeration is common in biosynthesized silica due to hydrogen bonding and van der Waals interactions among surface hydroxyl groups. The observed morphology is highly desirable for agricultural applications, as porous silica nanoparticles facilitate better adsorption, water retention, and gradual release of nutrients or bioactive compounds during seed treatment.

The ZnO nanoparticles, characterized using SEM, SEM-EDX, and UV–Visible spectroscopy, showed hexagonal crystalline morphology**,** confirming the formation of wurtzite-structured ZnO. SEM images indicated particle agglomeration with individual crystallites in the size range of 80–110 nm**.** Agglomeration of ZnO nanoparticles is frequently reported and is associated with high surface energy and electrostatic interactions among nano-crystallites. SEM-EDX analysis confirmed the elemental purity of ZnO, with strong signals corresponding to zinc and oxygen, indicating the absence of major impurities. The UV–Visible absorption spectra exhibited a characteristic absorption peak in the 350–380 nm range**,** further validating the nanoscale nature of ZnO particles due to quantum confinement effects.

The distinct physicochemical properties of both nanoparticles justify their suitability for seed treatment applications**.** SiO_2 nanoparticles, owing to their porous structure and biocompatibility, are known to enhance seed hydration, enzyme activation, and mechanical strength of cell walls. ZnO nanoparticles, on the other hand, act as a source of zinc—an essential micronutrient—and are reported to stimulate antioxidant activity, improve germination, and enhance early seedling growth at optimal concentrations. The agglomerated yet nano-sized morphology observed in both materials is consistent with previous reports and does not limit their biological efficacy, particularly under seed priming conditions where gradual dissolution and interaction with seed tissues occur.

Overall, the successful synthesis of bio-derived SiO_2 nanoparticles from agricultural waste (sugarcane bagasse) and chemically synthesized ZnO nanoparticles demonstrates an eco-friendly and functionally effective approach for developing nanomaterials suitable for sustainable seed quality enhancement.

Dose-dependent effects of nanoparticle seed treatments on seed quality parameters

Fig 3. Laboratory germination assay of soybean seeds treated with different nanoparticle concentrations, showing variation in germination and early seedling development across treatments under controlled conditions

Table 2. Dose-dependent effects of nanoparticle seed treatments on germination, seedling growth, vigor indices, and disease incidence in soybean (Glycine max L.)”

Treatment	Shoot length (cm)	Germination (%)	Root length (cm)	Dry seedling weight (mg)	Vigor Index-I	Vigor Index-II	Diseased seeds (%)
Control	11.11	68.00	14.20	18.30	1723.20	1246.42	25.00
30 PPM	13.10	70.00	16.00	22.10	2039.50	1549.52	10.00
50 PPM	14.20	88.00	16.20	25.01	2678.50	2204.47	0.00
100 PPM	13.50	80.00	16.00	24.30	2362.87	1947.13	12.00
200 PPM	12.77	78.00	15.20	23.10	2184.31	1804.68	21.00
500 PPM	11.20	75.00	14.40	22.80	1922.35	1712.77	35.00
800 PPM	11.80	74.00	14.60	20.30	1956.02	1504.66	48.00
1000 PPM	10.40	70.00	13.80	18.20	1696.09	1276.07	32.00
1200 PPM	9.10	66.00	12.40	17.50	1420.74	1156.86	52.00
SEm±	0.36	1.90	0.40	0.68	100.48	93.44	1.13
SEd	0.50	2.69	0.56	0.97	142.09	132.14	1.60
CD (5%)	1.06	5.64	1.18	2.03	298.53	277.62	3.37
CV (%)	5.18	4.43	4.66	5.55	8.71	10.11	7.53

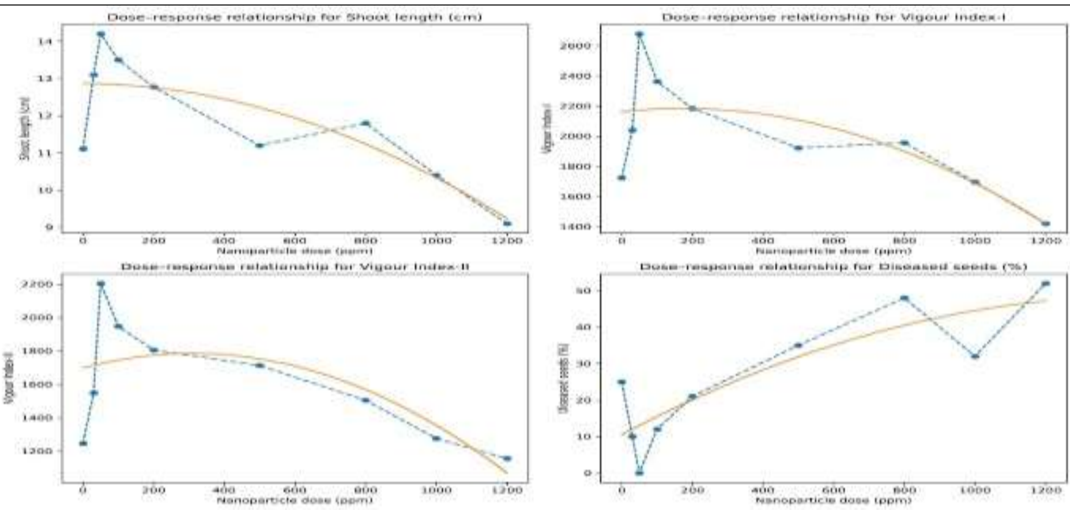

Fig 4. (a) **Dose–response curve illustrating the effect of nanoparticle concentration on shoot length (cm).** (b) **Dose–response relationship between nanoparticle concentration and Vigor Index-I.** (c) **Dose–response relationship showing the influence of nanoparticle concentration on Vigor Index-II.** (d) **Dose–response relationship depicting changes in diseased seeds (%) across nanoparticle concentrations.**

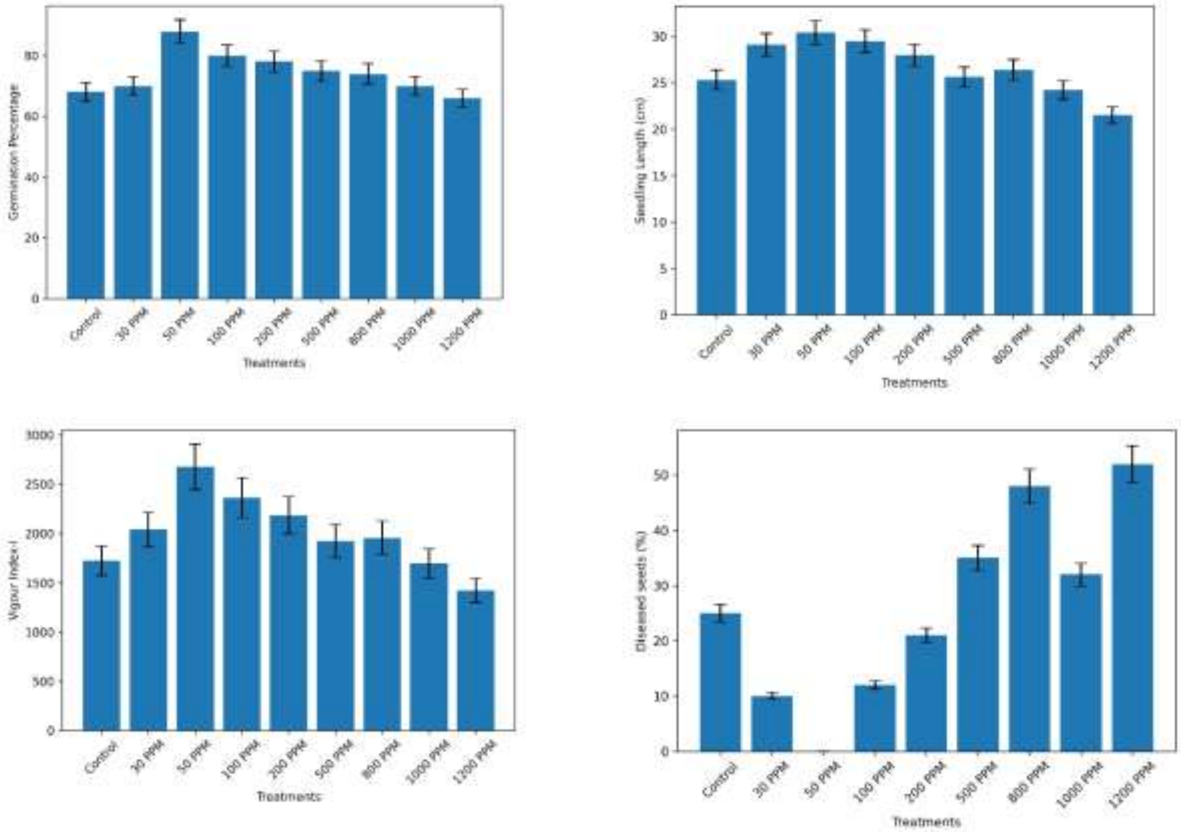

Fig 5. (a) **Germination percentage as influenced by different nanoparticle concentrations.** (b) **Seedling length (cm) under varying nanoparticle doses.** (c)**Vigor Index-I in response to nanoparticle treatments.** (d) **Percentage of diseased seeds across nanoparticle concentrations.**

Treatment-wise mean responses for germination percentage, shoot length, root length, dry seedling weight, vigor indices were visualized using bar plots, with error bars denoting standard error of the mean (± SE), enabling clear comparison of nanoparticle dose effects on soybean seedling performance. Among all treatments, 50 ppm consistently recorded the highest germination percentage, seedling growth, biomass accumulation, and vigor indices, indicating its strong promotive effect on early seedling establishment. The improvement at this concentration suggests enhanced water imbibition, activation of hydrolytic enzymes, improved nutrient availability, and stimulation of metabolic and physiological processes during germination.

Moderate concentrations (30–100 ppm) also showed improved performance over the control, but the magnitude of response was lower than that observed at 50 ppm. In contrast, higher concentrations (≥500 ppm, particularly 800–1200 ppm) resulted in a gradual decline in germination, seedling length, dry weight, and vigor indices, accompanied by a marked increase in diseased seeds. This decline indicates the onset of phytotoxic effects at elevated nanoparticle doses, possibly due to oxidative stress, membrane damage, and disruption of normal cellular metabolism. Excess nanoparticle accumulation may generate reactive oxygen species (ROS), impair enzymatic activity and weaken seedling defense mechanisms, thereby increasing susceptibility to disease.

The increase in vigor indices at lower concentrations reflects the combined positive effects of improved germination and seedling growth, whereas their reduction at higher doses further confirms stress-induced growth inhibition. The observed response pattern aligns well with the concept of hormesis, where low doses of nanoparticles stimulate growth while higher doses become inhibitory or toxic. Similar dose-dependent effects of nanoparticles on soybean germination and seedling performance have been widely reported, supporting the physiological basis of the present findings.

Overall, the results clearly indicate that nanoparticle application at an optimized low concentration (50 ppm) is beneficial for improving soybean seed quality and early seedling vigor, whereas excessive concentrations adversely affect growth and increase disease incidence. These findings highlight the importance of dose optimization for safe and effective use of nanoparticles in seed priming and seed quality enhancement.

RESULTS AND DISCUSSION

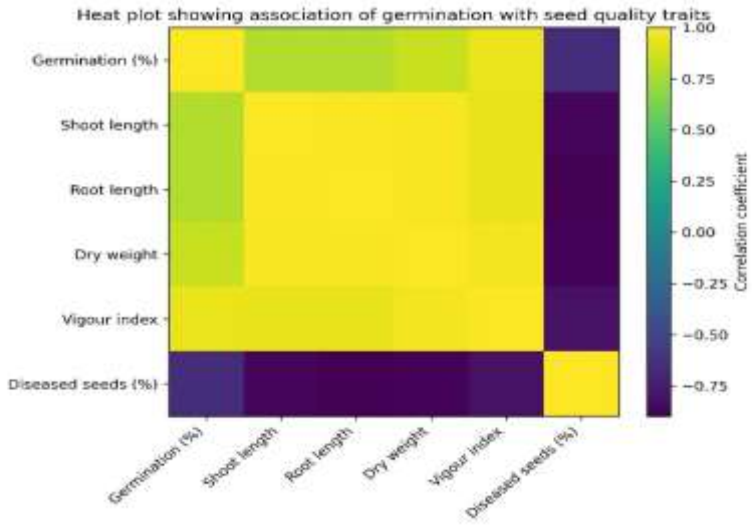

Fig 6. Heat map depicting the correlation between germination percentage and seed quality traits, including shoot length, root length, dry seedling weight, vigor index, and diseased seeds (%), with color intensity representing the strength and direction of correlation coefficients.

The correlation heat plot clearly illustrates the interrelationships between germination percentage and key seed quality traits in soybean. Germination (%) exhibited strong positive associations with shoot length, root length, dry seedling weight, and vigor index, indicating that improved germination is closely linked with enhanced seedling growth, biomass accumulation, and overall physiological vigor. Such strong positive correlations suggest that treatments promoting rapid and uniform germination also stimulate early seedling development and seedling robustness, which are critical determinants of successful crop establishment (ISTA, 2020; Abdul-Baki and Anderson, 1973).

In contrast, diseased seeds (%) showed a strong negative correlation with germination and all growth- and vigor-related traits. This inverse relationship indicates that increased disease incidence adversely affects seed metabolic efficiency, resulting in reduced germination and inferior seedling performance. Pathogen infection is known to impair reserve mobilization, disrupt enzymatic activity, and induce oxidative stress during germination, thereby limiting seedling growth and vigor (McDonald, 1999; Bradford et al., 2007). Reduced disease pressure likely allows better utilization of seed reserves, resulting in improved growth parameters and vigor index.

The observed association pattern supports the role of nanoparticle-based seed treatments in enhancing soybean germination and seedling quality. At optimum concentrations, nanoparticles have been reported to enhance water uptake, activate hydrolytic enzymes, improve antioxidant defense systems, and facilitate nutrient availability during germination, collectively leading to improved seedling growth and vigor (Rico et al., 2011; Dimkpa and Bindraban, 2017). Zinc oxide and silicon dioxide nanoparticles, in particular, have been shown to improve germination, root–shoot development, and vigor indices in soybean and other legumes when applied at low concentrations (Mahajan et al., 2011; Suriyaprabha et al., 2012).

However, the strong negative linkage between diseased seeds and germination further emphasizes that effective seed treatments must balance growth promotion with disease suppression. Nanoparticles possessing antimicrobial properties can reduce seed-borne pathogen load, thereby indirectly enhancing germination and seedling vigor (Kah et al., 2019; Choudhary et al., 2020). Similar positive correlations among germination, seedling growth, and vigor following nanoparticle application have been widely reported in soybean and other crops, confirming the physiological basis of these responses (Prasad et al., 2012; Raliya et al., 2018).

Overall, the correlation heat plot provides robust evidence that enhanced germination acts as a central driver of seedling vigor, while disease incidence remains a major limiting factor. The results justify the use of optimized nanoparticle doses as seed treatments to improve soybean seed quality, early establishment, and plant health under sustainable crop production systems.

Radar plot

The radar plot provides an integrated comparison of key seedling quality parameters under control, optimum (50 ppm), and higher (1200 ppm) treatment levels. The 50 ppm treatment consistently exhibited the maximum values for shoot length, root length, dry seedling weight, germination percentage, and vigor index (VI-II), indicating a strong positive influence on early seedling establishment and overall vigor. The simultaneous improvement of multiple traits suggests that this concentration effectively enhances physiological efficiency, likely through improved enzymatic activity, nutrient assimilation, and cellular metabolism during germination and early growth.

In contrast, the control treatment showed moderate performance across all traits, reflecting baseline seedling growth under untreated conditions. However, the 1200 ppm treatment showed a pronounced decline in growth and vigor traits along with a higher proportion of diseased seeds**,** indicating a clear inhibitory or phytotoxic effect at elevated concentration. Such reductions at higher doses may be attributed to metabolic imbalance, oxidative stress, and disruption of membrane integrity, which negatively affect germination and seedling development.

The observed response pattern is consistent with the concept of dose-dependent hormesis**,** where low concentrations stimulate growth while higher concentrations exert toxic effects. Similar trends have been reported in studies involving seed treatments with micronutrients and nanoparticles, where optimal doses enhance seedling performance but excess levels impair physiological processes and increase stress susceptibility (Calabrese & Baldwin, 2003; Nair et al., 2010). The increased disease incidence at higher concentration further suggests weakened seedling defense mechanisms, possibly due to stress-induced suppression of normal metabolic pathways (Rico et al., 2011).

Overall, the radar plot clearly demonstrates that 50 ppm represents an optimum concentration for improving seedling growth, vigor, and health**,** whereas higher concentration (1200 ppm) is detrimental. This integrated multivariate assessment strengthens the conclusion that careful dose optimization is critical for achieving beneficial effects without inducing toxicity.

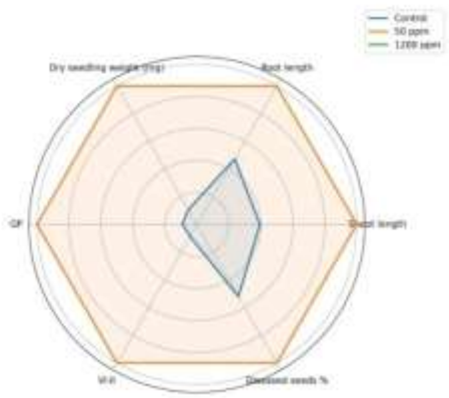

Fig 7. Radar chart showing the comparative effect of treatments (Control, 500 ppm, and 1200 ppm) on seedling growth and quality parameters—shoot length, root length, dry seedling weight, germination percentage (GP), vigor index-II (VI-II), and percentage of diseased seeds.

Principal component analysis

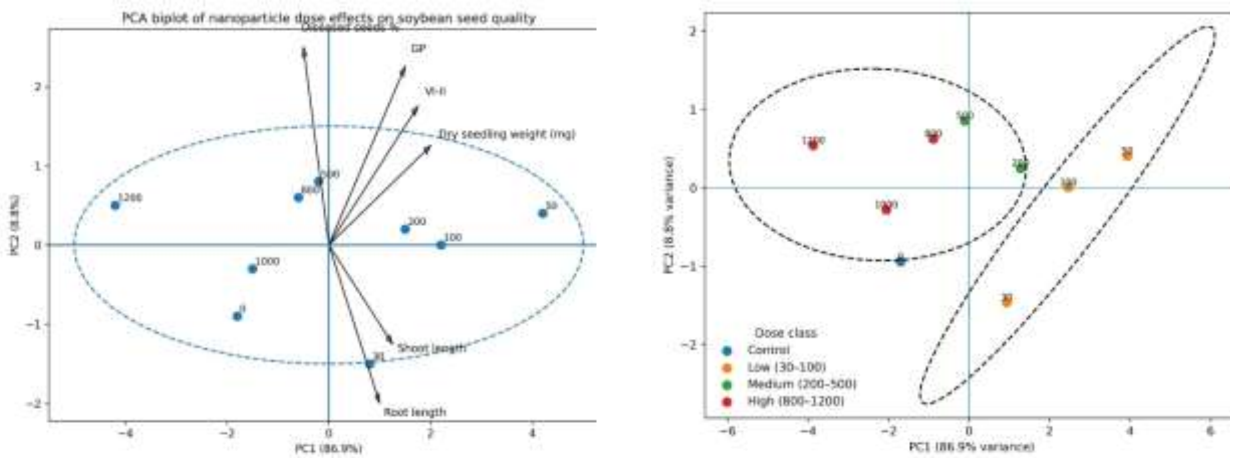

Fig 8. PCA biplot illustrating the influence of different nanoparticle doses on soybean seed quality, showing the relationships between treatments and key parameters (germination percentage, vigor index-II, dry seedling weight, shoot length, root length, and diseased seeds %) along the first two principal components (PC1 and PC2).

Principal component analysis (PCA) effectively summarized the multivariate response of seedling growth, vigor, and disease parameters across different treatment concentrations. The first two principal components explained a substantial proportion of the total variability, with PC1 accounting for 86.9% **and** PC2 for 8.8% of the variation, indicating that the observed responses were largely governed by treatment dose effects. The PCA score plot with 95% confidence ellipses clearly separated treatments into distinct dose-dependent clusters, demonstrating a strong and systematic response pattern.

Low concentrations **(**30–100 ppm) clustered distinctly on the positive side of PC1, reflecting their strong association with enhanced shoot and root length, higher dry seedling weight, improved germination percentage, and increased vigor index. This stimulatory effect at lower doses may be attributed to enhanced metabolic activity, improved nutrient uptake, and activation of growth-promoting physiological processes, a phenomenon commonly described as hormesis**.** Similar low-dose stimulation has been reported in seed treatment studies involving micronutrients and nanoparticles, where optimal concentrations enhance enzymatic activity and early seedling establishment (Calabrese & Baldwin, 2003; Nair et al., 2010).

Medium concentrations **(**200–500 ppm**)** occupied an intermediate position near the origin, indicating a transitional response between stimulation and inhibition. This suggests that while growth promotion was still evident, the beneficial effects began to plateau, possibly due to the onset of mild physiological stress. Such intermediate clustering has been observed in dose–response studies where increasing concentrations gradually shift from beneficial to inhibitory effects (Tripathi et al., 2017).

In contrast, high concentrations **(**800–1200 ppm**)** formed a separate cluster on the negative side of PC1, strongly associated with increased diseased seed percentage and reduced growth and vigor traits. The grouping of these treatments within a distinct confidence ellipse indicates phytotoxic effects at elevated doses, likely caused by oxidative stress, membrane damage, or disruption of normal cellular metabolism. Excessive concentrations are known to induce reactive oxygen species (ROS) accumulation, leading to impaired germination and seedling growth (Rico et al., 2011; Singh et al., 2020).

Overall, the PCA provides robust multivariate evidence that lower concentrations are optimal for enhancing seedling performance**,** whereas higher concentrations exert detrimental effects. The clear separation of dose classes validates the dose-dependent nature of the treatment response and supports the use of lower concentrations for seed quality enhancement.

CONCLUSION

The present investigation demonstrated the successful synthesis of zinc oxide (ZnO) nanoparticles through a sol–gel method and bio-synthesis of silica (SiO_2) nanoparticles from sugarcane bagasse using a green approach, followed by their effective application as seed treatments in soybean. Comprehensive physicochemical characterization confirmed the nanoscale size, distinct morphology, and elemental purity of both nanoparticles, validating their suitability for biological applications.

Seed treatment with ZnO and SiO_2 nanoparticles exerted a clear concentration-dependent effect on germination, seedling growth, vigor, and seed health of soybean. Among the tested concentrations, 50 ppm emerged as the optimum dose, significantly enhancing germination percentage, shoot and root length, dry seedling weight, and vigor indices, while concurrently reducing the incidence of diseased seeds. In contrast, higher concentrations (≥800 ppm) adversely affected seedling performance and increased disease incidence, indicating phytotoxic effects at excessive nanoparticle levels.

Multivariate analyses, including PCA and correlation heat mapping, provided robust evidence that improved germination is strongly associated with enhanced seedling vigor and growth traits, whereas disease incidence is a major limiting factor negatively influencing seed quality. The findings support the concept of hormesis, where low nanoparticle doses stimulate physiological processes, while higher doses induce stress-related inhibition.

Importantly, the bio-synthesis of SiO_2 nanoparticles from sugarcane bagasse highlights a sustainable and value-added utilization of agricultural waste, aligning with principles of circular economy and environmentally responsible agriculture. Overall, the study establishes that nano-enabled seed treatment at optimized low concentrations is a promising strategy for improving soybean seed quality, early seedling establishment, and resilience, while potentially reducing dependence on conventional agrochemicals. Further field-based validation and long-term assessments are warranted to facilitate large-scale adoption of this technology in sustainable crop production systems.

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