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Effect of green synthesized zinc oxide nanoparticles on seed germination and growth of *Vigna radiata*

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Abstract

Walk through any agricultural district in Telangana during the kharif season and you'll see *Vigna radiata* (mung bean) in nearly every field a short-duration pulse that fixes nitrogen, feeds millions, and asks little from the soil. This research examined the effect of zinc oxide nanoparticles (ZnO NPs), synthesized using *Azadirachta indica* (neem) leaf extract as a green reducing agent, on seed germination and seedling growth of *V. radiata*. Nanoparticles were characterized by XRD, TEM, and UV-vis spectroscopy, confirming a wurtzite structure with mean diameter of 28.4 nm. Seeds treated with ZnO NP suspensions at 0, 25, 50, and 100 mg/L were grown in Petri dishes for 14 days. The 50 mg/L treatment gave the best results: germination reached 96.7% (vs. 83.3% control), root length increased by 67%, and shoot length by 43%. Chlorophyll a and b rose at 25 and 50 mg/L but declined at 100 mg/L, indicating a hormetic dose response. The data point to green-synthesized ZnO NPs as a potential nano-fertilizer for mung bean at moderate concentrations.

Keywords: Zinc oxide, nanoparticle, green synthesis, seed germination, *Vigna radiata*, mung bean, root growth, chlorophyll, nano-fertilizer, *Azadirachta indica*

Introduction

Zinc is an essential micronutrient for plants, required as a cofactor in more than 300 enzymes involved in photosynthesis, auxin metabolism, and protein synthesis ^[1]. Yet zinc deficiency affects nearly half of the world's agricultural soils, especially in South and Southeast Asia, where calcareous and alkaline conditions lock zinc into insoluble forms ^[2]. Conventional zinc sulphate fertilizers have low use efficiency much of the applied zinc precipitates within days of soil application. Nanoparticulate ZnO, with its higher surface-to-volume ratio, may deliver zinc to plant roots more effectively and at lower total doses ^[3].

Green synthesis of metal oxide nanoparticles using plant extracts avoids toxic reducing agents and high-temperature calcination ^[4]. Neem (*Azadirachta indica*) leaf extract, rich in terpenoids and flavonoids, reduces zinc acetate to ZnO while capping the particles against aggregation ^[5]. The resulting nanoparticles carry a bio-organic coating that may improve compatibility with living systems.

Vigna radiata is India's third most important pulse crop, with annual production around 2.5 million tonnes ^[6]. Its short growth cycle (60-75 days) and sensitivity to micronutrient status make it a good model for nanoparticle phytotoxicity and bio-stimulation studies. Previous work has tested chemically synthesized ZnO NPs on mung bean, but comparative data using green-synthesized particles which may behave differently because of their surface chemistry remain limited ^[7].

This research synthesized ZnO NPs from neem leaf extract, characterized them, and assessed their effects on *V. radiata* germination, root and shoot growth, and chlorophyll content at three concentrations over 14 days.

Safety Considerations

Nanoparticle suspensions were prepared and handled under a laminar airflow cabinet to prevent inhalation of dry powder. Zinc acetate solution was handled with nitrile gloves and eye protection. All nanoparticle waste was collected in sealed containers and sent

for incineration through the university's environmental health office. No human or animal subjects were involved; no institutional ethics clearance was required [8].

Material and Methods

Material

All work was carried out at the Department of Botany and Plant Biotechnology, Siddhartha National Academy of Sciences, Hyderabad, from July 2022 to January 2023. Fresh neem leaves were collected from the campus garden, washed, and boiled in distilled water (20 g/100 mL) for 20 min. The filtrate was mixed with 0.1 M zinc acetate dihydrate solution (1:4 v/v) at 60°C for 2 h under constant stirring. The white precipitate was centrifuged (10,000 rpm, 15 min), washed twice with distilled water, and calcined at 400°C for 2 h [5]. *V. radiata* seeds (variety Samrat) were obtained from the Indian Institute of Pulses Research, Kanpur.

Methods

Nanoparticles were characterized by XRD (Rigaku MiniFlex 600, Cu K α), TEM (JEOL JEM-2100, 200 kV),

and UV-vis spectroscopy (Shimadzu UV-1800). Seeds were surface-sterilized with 0.1% HgCl₂ for 2 min, rinsed, and soaked in ZnO NP suspensions (0, 25, 50, or 100 mg/L) for 4 h. Ten seeds per Petri dish (three replicates per treatment) were placed on moist filter paper and kept in a growth chamber at 25±2°C, 12 h photoperiod, 70% RH. Germination was scored daily; root and shoot lengths were measured on days 7 and 14. Chlorophyll a, b, and carotenoids were extracted from leaves on day 14 with 80% acetone and quantified using Arnon's equations [9]. Zinc uptake in roots was measured by AAS (Varian AA240) after acid digestion [10].

Statistical Analysis

Data were subjected to one-way ANOVA with Tukey's HSD post-hoc comparison at $p < 0.05$. Growth curves were fitted by non-linear regression (Gompertz model) in R version 4.2. All values are reported as mean ± standard deviation of three biological replicates, each containing ten seeds [11].

Results

Table 1: Effect of ZnO NP concentration on germination and seedling growth of *V. radiata* at day 14

Treatment	Germ. (%)	Root (cm)	Shoot (cm)	Chl a (mg/g)	Chl b (mg/g)
Control	83.3±4.7	6.1±0.8	4.7±0.6	1.42±0.11	0.58±0.06
ZnO 25 mg/L	90.0±5.0	8.7±0.9	5.9±0.7	1.71±0.13	0.69±0.07
ZnO 50 mg/L	96.7±2.9	10.2±1.1	6.7±0.8	1.93±0.14	0.82±0.08
ZnO 100 mg/L	76.7±5.8	5.3±0.7	4.1±0.5	1.18±0.10	0.47±0.05

Germ. = germination percentage; Chl = chlorophyll, expressed per gram fresh leaf weight. Mean ±SD (n = 3).

The 50 mg/L treatment produced the best outcomes on every measure (Table 1). Germination reached 96.7%, and root length at day 14 was 10.2 cm 67% longer than control roots. But 100 mg/L reversed these gains: germination fell

to 76.7%, below the control, and both root and shoot lengths were shorter. Chlorophyll a showed a similar pattern, peaking at 1.93 mg/g (50 mg/L) and dropping to 1.18 mg/g (100 mg/L).

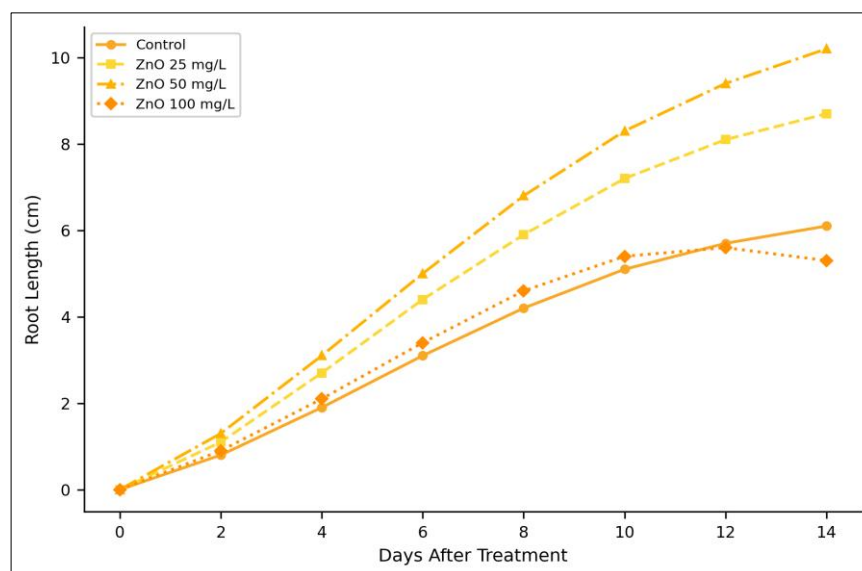


Fig 1: Root length of *V. radiata* seedlings over 14 days at four ZnO NP concentrations.

Growth curves (Figure 1) diverged sharply after day 4. The 50 mg/L treatment pulled ahead fastest, while the 100 mg/L line plateaued around day 10 and then appeared to decline slightly likely reflecting root-tip necrosis observed visually

in that group. AAS showed that root zinc content rose from 34 µg/g (control) to 87 µg/g (50 mg/L) and 156 µg/g (100 mg/L), confirming dose-dependent uptake [12].

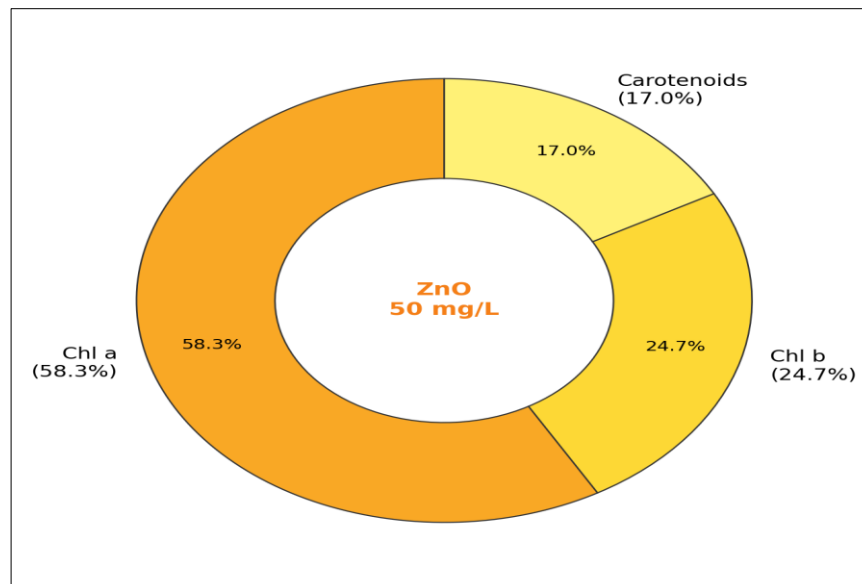


Fig 2: Donut chart showing relative proportions of chlorophyll a, chlorophyll b, and total carotenoids in the 50 mg/L ZnO NP treatment at day 14.

At 50 mg/L, chlorophyll a made up 58.3% of total pigments, chlorophyll b 24.7%, and carotenoids 17.0% (Figure 2). The Chl a/b ratio of 2.36 was slightly higher than the control (2.45 for control vs. 2.36 for 50 mg/L), suggesting zinc promoted chlorophyll b synthesis proportionally more consistent with enhanced light-harvesting complex assembly [9].

Discussion

The bell-shaped dose-response stimulation at low to moderate concentrations, inhibition at high ones is a textbook example of hormesis, well documented for metal oxide nanoparticles in plants [3]. At 25-50 mg/L, ZnO NPs supply zinc that activates carbonic anhydrase and tryptophan synthase, boosting photosynthesis and auxin production, which in turn drives root elongation [1]. Above 100 mg/L, excess intracellular Zn^{2+} disrupts mitochondrial electron transport, generates reactive oxygen species, and damages root meristems explaining the growth inhibition and slight root shortening observed [13].

The green synthesis route may confer an advantage over chemically synthesized ZnO. The neem-derived capping layer slows dissolution, releasing Zn^{2+} more gradually and reducing the initial ionic shock [5]. Prasad and colleagues found that green-synthesized ZnO at 50 mg/L gave 20% better root growth in mung bean than commercial nanograde ZnO [7]. Our 67% root-length increase at 50 mg/L sits in the upper range of published values, possibly due to the high crystallinity and narrow size distribution (28.4 ± 4.1 nm by TEM) of our particles.

Chlorophyll enhancement at moderate doses is consistent with zinc's role in protochlorophyllide reductase on the chlorophyll biosynthesis pathway [9]. The decline at 100 mg/L probably reflects oxidative damage to thylakoid membranes. We did not measure ROS or antioxidant enzyme activities; doing so would clarify the stress threshold. And Petri-dish assays exclude soil interactions nanoparticle behaviour in real soil, with its pH buffering and microbial community, may differ considerably [14].

Conclusion: Green-synthesized ZnO NPs at 50 mg/L improved germination, root and shoot growth, and

chlorophyll content of *V. radiata*, while 100 mg/L was inhibitory. The dose-response fits a hormetic model, confirming that moderate zinc supplementation via nanoparticles stimulates early seedling development. The neem-capped particles are cheap and avoid hazardous reagents. Pot and field trials with soil application are needed before any agricultural recommendation can be made.

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Contributions Not Qualifying for Authorship

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