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Eco-Friendly Synthesis of ZnO Nanoparticles from *Calotropis procera* and their *in vivo* Nematicidal Potential

Renu Sharma[1*], Hanuman Sharma[1], Apexa Pareek[2], Payal Lodha[3]

[1,2]Research Scholar, Department of Botany, University of Rajasthan, Jaipur-302004, Rajasthan, India
[2]Assistant Professor, Department of Botany, University of Rajasthan, Jaipur-302004, Rajasthan, India
[3]Professor, Department of Botany, University of Rajasthan, Jaipur-302004, Rajasthan, India

***Address for Correspondence:** Renu Sharma, Research Scholar, Department of Botany, University of Rajasthan, Jaipur-302004, India
E-mail: renu.chotia88@gmail.com **& ORCID ID:** https://orcid.org/0009-0008-3965-3902

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ABSTRACT

Background: Conventional chemical nematicides provide significant hazards to environmental and human health. This study evaluates the effectiveness of ZnO nanoparticles, synthesized from the leaf extract of *Calotropis procera*, against Root-Knot Nematodes, particularly *Meloidogyne incognita*, which cause severe impact on the agricultural economy.

Methods: This study presents a sustainable and economical approach for the synthesis of ZnO nanoparticles through the utilization of an aqueous extract derived from *Calotropis* leaves in conjunction with 0.1 M zinc acetate dihydrate. The obtained ZnO nanoparticles were analyzed by using FT-IR, UV-visible spectroscopy (with a significant absorption band observed at 296 nm), XRD, SEM, and DLS techniques.

Results: The *in vivo* application of ZnO NPs at varying concentrations (50, 100, 200, and 500 ppm) resulted in a notable reduction in gall formation and an improvement in plant growth in *Momordica charantia* affected by *M. incognita*. The statistical analysis conducted through Duncan's Multiple Range Test that revealed significant differences ($p<0.05$) among the treatments. Notably, the 500-ppm treatment demonstrated the most substantial suppression of root-knot nematode infection and the most considerable enhancement in plant growth characteristics after 8 weeks, in comparison to the untreated, inoculated control.

Conclusion: This study demonstrated that green-synthesized ZnO NPs are powerful nematicides that can effectively control Root-Knot nematodes while decreasing dependence on harmful chemical nematicides in agriculture.

Key-words: *Calotropis*, Green synthesis, *Meloidogyne*, Nematicides, Root Knot Nematode, Zinc oxide nanoparticles

INTRODUCTION

Nanotechnology is rapidly developing and has diverse applications across numerous fields, including medicine, pharmaceuticals, engineering, agriculture, the food industry, and electronics [1]. The application of nanotechnology in botany emphasizes the utilization of nanoparticles to enhance plant growth and productivity. This approach allows for accurate regulation of agro-chemicals, reducing detrimental impacts, and supports the alteration of plant biomolecules such as proteins and nucleotides. This developing area of study is attracting interest as it contributes to decreasing dependence on harmful chemicals in agriculture.

Metal oxide nanoparticles stand out among the diverse range of nanoparticles, recognized for their distinctive characteristics, including shape, size, concentration, composition, and physicochemical properties [2]. The influence of these factors is significant in assessing how metal oxide nanoparticles affect various plant species. These nanoparticles, including iron oxide, zinc oxide, copper oxide, and titanium dioxide, are known for their stability and catalytic activity [3]. Among nanometal oxides, ZnO nanoparticles are recognized for their antimicrobial [4] and anti-inflammatory [5] properties. Various methods have been developed to synthesize nanoparticles, each with its advantages and limitations.

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These methods can be broadly categorized into physical, chemical, and biological approaches, with further subdivisions based on specific techniques [6].

Green synthesis uses biological components including plant extracts, enzymes, and microbes to create nanoparticles. This method is environmentally friendly and sustainable [7]. Integrating plant-derived ZnO nanoparticles into agricultural pesticides, can help with the development of highly efficient, biodegradable, and non-toxic pesticides that reduce dependence on synthetic chemicals [8].

Calotropis serves a variety of functions, being used for fuel, fodder, timber, fiber, and medicinal applications. The therapeutic properties arise from secondary metabolites including tannins, alkaloids, and phenols, and it is commonly recognized as an environmental weed [9]. The leaves of *Calotropis* serve as natural reducing and stabilizing agents, and their aqueous extract facilitates nanoparticle formation [10]. The extract from *Calotropis* leaves exhibits potent nematicidal effects, significantly diminishing the root penetration of *M. incognita*, a prominent root-knot nematode [11].

Root-knot nematode presents a significant challenge in agriculture, resulting in worldwide crop losses estimated at US$157 billion each year [12]. The presence of their infestation leads to diminished crop yields, underscoring the necessity for robust management strategies to protect food security and ensure economic stability. Conventional chemical nematicides present several challenges, such as environmental toxicity, regulatory limitations, elevated production expenses, and potential risks to human health. Moreover, their effectiveness is compromised by the capacity of nematodes to inhabit plant tissues, rendering soil-based treatments less impactful [13]. The purpose of this research is to investigate the *in vivo* effects of green-synthesized ZnO NPs on nematode control, with *C. procera* serving as the reducing agent.

This study aims to provide an eco-friendly, sustainable alternative to traditional nematicides by assessing the efficacy of these nanoparticles against PPNs, thereby contributing to the advancement of comprehensive pest control approaches that prioritize crop protection and environmental health.

MATERIALS AND METHODS

Biological synthesis of ZnO NPs- The Nps are synthesized using the previously mentioned process with certain changes.[14] Fresh and healthy leaves of *Calotropis procera* were collected, carefully rinsed with distilled water, shade-dried, and finally ground into fine powder. 10 g of leaf powder was boiled in 100 ml of distilled water for 30 min. at 80°C. The extract was cooled and filtered using Whatman No. 1 filter paper to obtain a clear solution. To synthesize environment-friendly ZnO NPs, the leaf extract of the *Calotropis* plant was used which works as both a reducing and stabilizing agent.

A solution of 0.1 M zinc acetate dihydrate was prepared and mixed with *Calotropis* extract in a 9:1 ratio while maintaining continuous stirring. The pH was set to 11 by adding 0.02 M NaOH dropwise. The reaction mixture was kept at 80°C for two hours, resulting in the production of a yellow precipitate that indicates effective nanoparticle synthesis. The precipitation was washed by centrifugation at 4000 rpm for 20 minutes, repeated three to four times, and then dried in a hot air oven.

Characterization- UV–vis spectral analysis was conducted using a Thermo-Scientific Genesys 180 spectrophotometer. The morphology of the synthesized ZnO NPs was analyzed by Scanning electron microscopy (JSM-7610Plus). The composition, size and structure of ZnO nanoparticles were examined using X-ray diffraction (SMARTLAB). Additional characterization was conducted via FTIR (Spectrum100, Perkin Elmer). The Anton Paar Litesizer 500 DLS was used to analyze hydrodynamic size and size distribution.

Infectivity bioassay and experimental design (Pot study)

Seed sterilization- The seeds were first rinsed under running tap water before being surface sterilized with 5% sodium hypochlorite (NaOCl) for five minutes. To eliminate any fungal or bacterial contamination, thorough washing was conducted two to three times using distilled water. The seeds were sterilized with a 0.01% mercuric chloride ($HgCl_2$) solution for two minutes, then thoroughly washed with autoclaved water and dried on sterilized filter paper.[15]

Pot experiments- The autoclaved soil was mixed with manure and filled into 2.5 kg pots. Three seeds of the Bitter gourd plant were sowed in each plastic pot. The

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undesired seedlings were meticulously removed after germination, leaving the healthiest one in every pot. We then made 2-3 small holes around the seedling's root and inoculated it with 2500 J2s and treatments were initiated 7 days after inoculation. Using a pipette, 10 ml of *Calotropis* ZnO NPs at various concentrations (50, 100, 200, and 500 ppm) were applied around the seedling's root. For control, treatment of Zno NPs was not applied on inoculated plants. The treatment was implemented weekly for eight weeks. The plants were harvested after 60 days. Plant height was measured at both 30 and 60 days. The time to flowering was observed daily by monitoring the plants. Post-harvest, assessments were conducted to shoot fresh mass, root length, and the number of galls found on the roots.[16]

RESULTS

FTIR Analysis- FTIR analysis was used to identify the functional groups in *Calotropis* leaf extract and their involvement in the formation of ZnO nanoparticles (NPs) (Fig. 1(A) and (B)). The leaf extract's ftir spectra showed absorption peaks at 3421 and 2928 cm^{-1}, representing stretching vibrations of primary and secondary amines, O-H groups in alcohols, and C-H bonds in alkanes. An absorption peak at 1636 cm^{-1} was linked to C=C stretching (aromatic), signifying the presence of an aromatic ring system. A stretching vibration at 1095 cm^{-1} was to C–N attributed. The FTIR spectra of green-synthesized ZnO NPs exhibited an absorption band at 611cm a certain wavenumber, conforming to the Zn–O link, thereby proving the presence of zinc oxide. Additionally, bands at 1578, 1057, and 1416 cm^{-1} were associated with the aromatic rings and functional groups present in organic molecules.

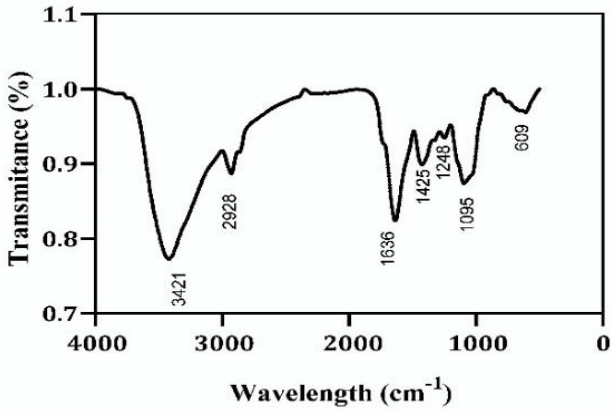

Fig. 1 (A): FTIR spectra of *Calotropis* Leaf Powder

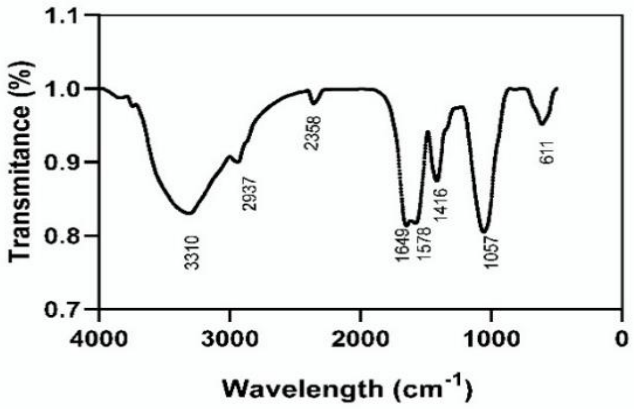

Fig. 1 (B): FTIR spectra of green synthesized ZnO NPs

XRD Analysis- The XRD profile of the formulated ZnO NPs demonstrates their crystalline structure (Fig. 2). Sharp diffraction peaks were observed at 2θ values of 27.93, 32.32, 38.24, 46.39, 54.91, 57.69, 64.71, 67.60, 74.67,76.81 and 85.78 degrees. These peaks represent the diffraction lattice planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202), respectively, confirming the hexagonal wurtzite structure of the synthesized nanoparticles. This pattern aligns with the standard peaks provided by the JCPDS 36-1451. The average size of the ZNPs was determined using the Debye–Scherer equation. XRD analysis indicated that the average size of the nanoparticles, synthesized using zinc acetate, was 18.20 nm.

DLS Analysis- Fig. 3 shows the size distribution of green-synthesized ZnO-NPs based on Dynamic Light Scattering investigation. The mean particle size of the ZnO-NPs was established at 80.5 nm, accompanied by a standard deviation of 14.3 nm. DLS measurements typically indicate particle sizes that exceed those determined by alternative methods, primarily due to the inherent characteristics of the technique. Specifically, DLS determines the hydrodynamic diameter, which reflects the diffusion behavior of particles in suspension. The diameter is typically greater than the actual particle size, especially when nanoparticles are suspended in ultrapure water, due to the attraction forces causing agglomeration indicating that the ZnO nanoparticles in ultrapure water possess high stability.

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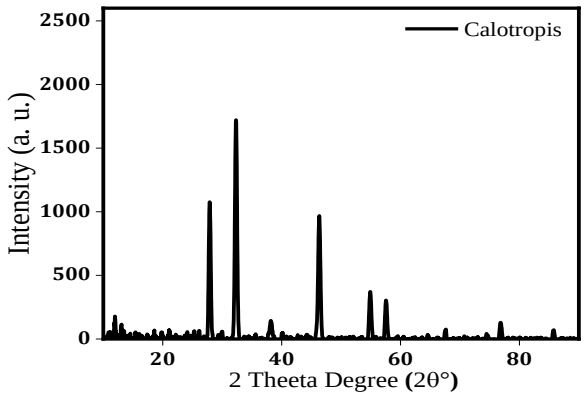

Fig. 2: XRD of green synthesized ZnO NPs

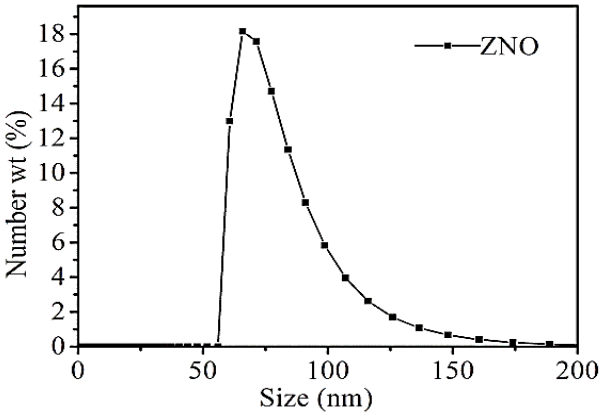

Fig. 3: DLS of green synthesized ZnO NPs

Scanning Electron Microscopy- Fig. 4 displays the SEM image of ZnO NPs formed with the green synthesis method. This examination was conducted to investigate the composition of the reaction products. The SEM picture displays discrete zinc particles in conjunction with various aggregates. The nanoparticles are primarily spherical, having diameters between 26.8 to 55.4 nm.

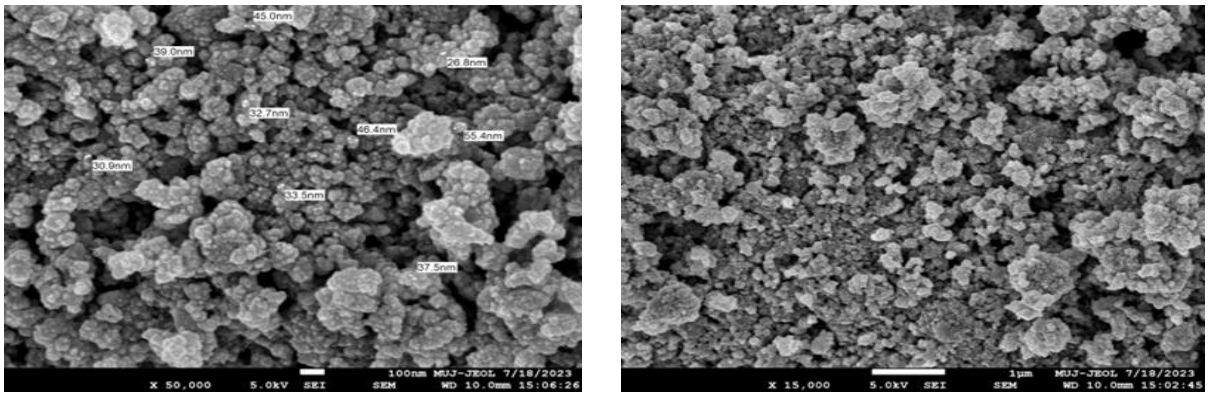

Fig. 4: SEM image of *Calotropis procera* mediated ZnO NPs (Size of 26.8-55.4 ZnO NPs in 100 nm scale)

UV-Vis spectrophotometer- Fig. 5 indicate the UV–Vis spectral measurement of the green-synthesized ZnO nanoparticles, performed within the 200–700 nm range revealed a distinctive absorption peak at around 296 nm, thereby conforming the effective synthesis of the nanoparticles.

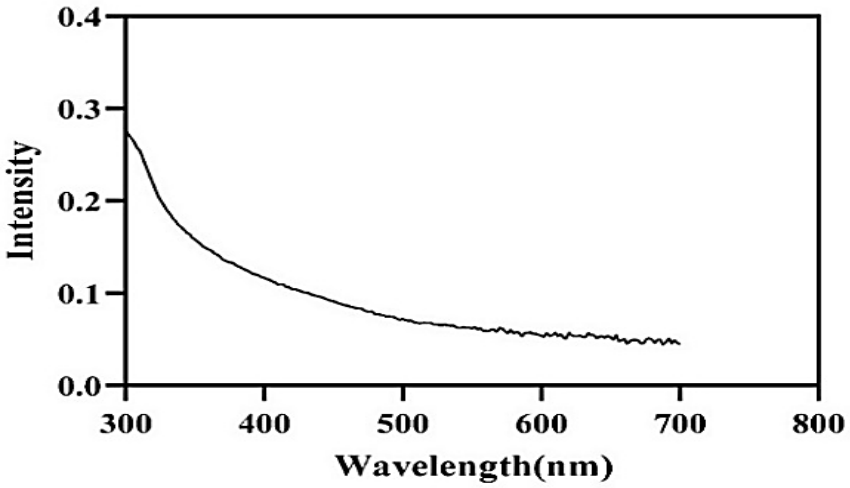

Fig. 5: UV–visible spectra of NPs synthesized from *Calotropis procera*

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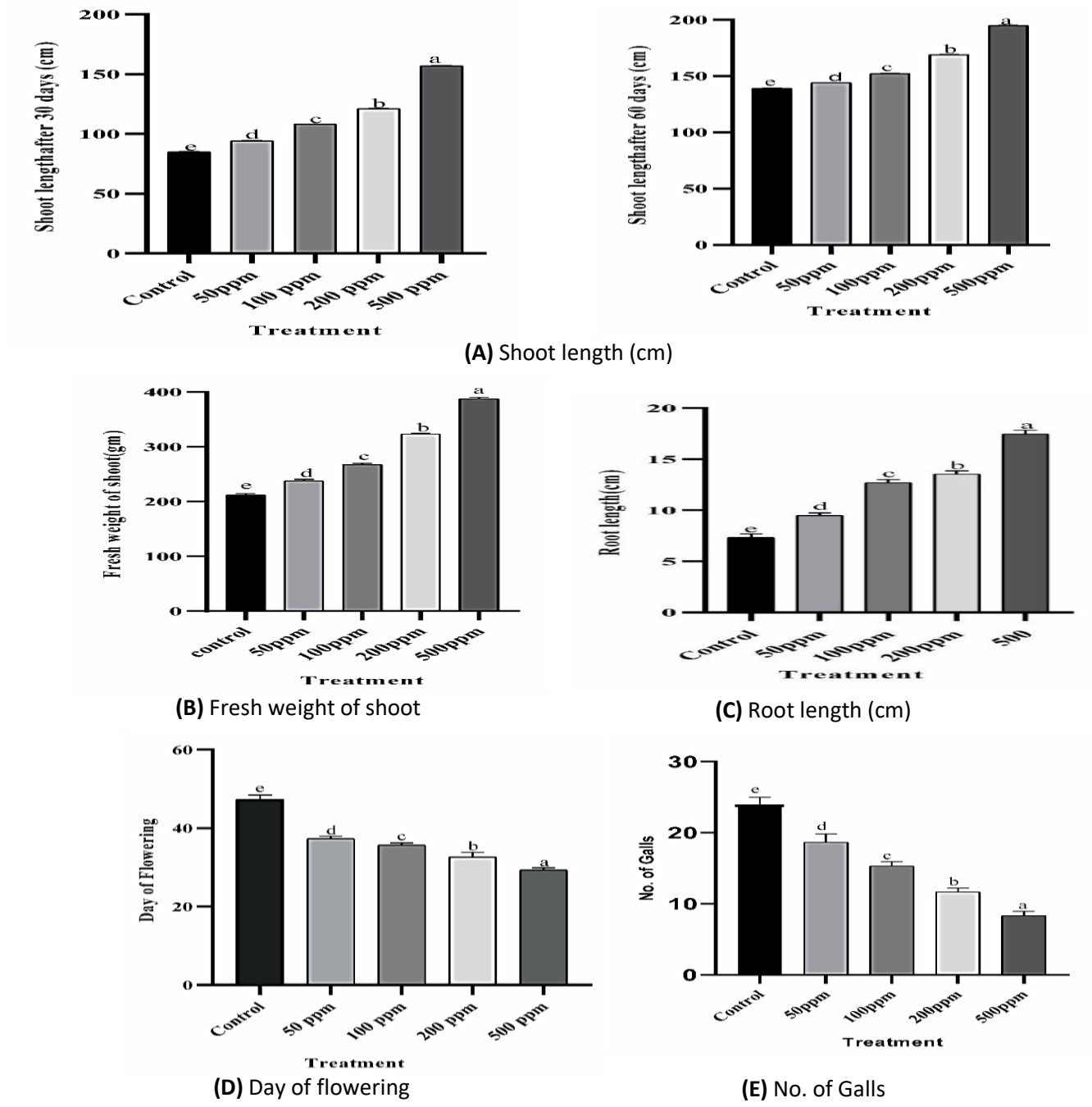
***In-vivo* bioassay-** The application of ZnO NPs to bitter gourd plants resulted in considerable increases in growth indices while considerably reducing the number of root galls. Statistical study indicated a significant difference ($p<0.05$) in plant growth parameters among all treatments. The plants treated with 500ppm exhibited the highest shoot length (197.13cm), fresh shoot weight (387.76 g), root length (17.22 cm), early day of flowering (29 days) and minimum number of galls (8.23 gall) among all growth parameters when compared to the untreated inoculated control and the other concentrations (50, 100 and 200 ppm). Fig. 6 shows the shoot length, fresh shoot weight, root length, flowering time, No. of galls of bitter gourd plants at various concentrations as 50, 100, 200 and 500 ppm to untreated inoculated control. Additionally, symptoms of root-knot disease, such as root gall, were evident in plants inoculated with *M. incognita* juveniles.



(A) Shoot length (cm)

(B) Fresh weight of shoot

(C) Root length (cm)

(D) Day of flowering

(E) No. of Galls

Fig. 6: Effect of various treatment of green synthesized ZnO NPS on various growth parameter of infected bitter gourd plant with nematode. Distinct letters above the bars signify statistically significant differences ($p < 0.05$) according to the Duncan test.

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DISCUSSION

The green-synthesized ZnO nanoparticles derived from *C. procera* significantly decreased root gall development and enhanced plant growth *in vivo*. Their treatment showed considerable Nematicidal efficacy against Meloidogyne incognita, emphasizing their contribution to sustained crop protection. The effective synthesis of ZnO nanoparticles was confirmed by many characterization methods. The FTIR study indicates that the soluble components in *Calotropis* leaf extract likely acted as capping agents, inhibiting nanoparticle aggregation in solution and substantially aiding in their extracellular production and morphological shaping [16]. The results indicated the existence of clear and significant peaks (3421, 2928, 2855, 1636, 1578, 1416, 1095, 1578, 1416, 1057, and 611 cm−1), consistent with the findings documented by [10,17].

XRD analysis confirms the hexagonal wurtzite structure of the synthesized nanoparticles, consistent with previous findings [18]. The obtained nanoparticles are larger than the 8-12 nm range described in previous research [14], but less than the 21.8 nm ZnO NPs generated with *C. gigantea* latex [19]. These variations in crystallite size may be influenced by factors such as precursor concentration, reaction time, and the presence of phytochemicals in the plant extract, which can impact the nucleation and growth of nanoparticles.

Al-Darwesh *et al.* [20] generated ZnO NPs using *F. carica* latex and highlighted a spherical morphology with a size of 29.3 nm, as determined by SEM. The UV-Vis spectral analysis of green-synthesized ZnO NPs revealed an absorption peak at 296 nm, which is lower than the 346–397 nm range documented for ZnO nanoparticles derived from *C. gigantea* [10,14,19]. Variations in synthesis conditions, including precursor concentration, reaction time, temperature, and plant extract composition, likely influenced these optical differences. The *in vivo* Nematicidal potential of ZnO NPs was further validated through their application to *M. incognita*-infected bitter gourd plants. The results align with [21], who indicated that biosynthesized ZnO-NPs effectively inhibited *M. incognita*, decreasing galls by 98%, egg masses by 99%, eggs per mass by 99.9%, and females by 95.5%. ZnO-NPs also improved plant growth, increasing leaf area by 118%, fresh weight by 112%, root fresh weight by 107%, stem length by 106%, and root length by 102% when compared to healthy controls.

These results demonstrate ZnO-NPs' dual function of suppressing nematodes and promoting plant growth, hence bolstering its potential as an environmentally friendly pest control method. Similarly, [22] reported that ZnO NPs at concentrations of 100 and 200 mg L^{-1} inhibited nematode hatching and caused juvenile mortality, leading to improved plant growth parameters. Furthermore, [23] found that ZnO NPs significantly reduced gall formation by 81.87% in roots, highlighting their nematicidal properties. This disruption likely interferes with the life cycle of *M. incognita*, preventing the establishment of feeding sites within banana plant roots. The observed improvements in plant growth parameters, including increased shoot and root length, enhanced fresh biomass, and earlier flowering, may be attributed to the role of ZnO NPs in promoting nutrient uptake and activating various plant enzymes [24].

CONCLUSIONS

This research investigation effectively demonstrates the environmentally friendly synthesis of ZnO nanoparticles utilizing *C. procera* leaf extract, validating their structural integrity and biocompatibility through various characterization methods. The *in vivo* application of ZnO NPs resulted in a notable decrease in root-knot nematode infestation and enhanced growth in bitter gourd plants. The results at the highest concentration (500 ppm) were the most effective. These results suggest that ZnO NPs could be used as a potential eco-friendly substitute for chemical nematicides, demonstrating a sustainable approach to crop protection. Future investigations need to explore it in extensive field trials, the interactions between soil and nanoparticles, and their enduring effects on soil microbiota and plant health to enhance agricultural applications.

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CONTRIBUTION OF AUTHORS

Research concept- Renu Sharma
Research design- Renu Sharma
Supervision- Prof. Payal Lodha
Data collection- Renu Sharma
Data analysis and Interpretation- Renu Sharma

Writing article- Renu Sharma, Hanuman Sharma
Critical review- Prof. Payal Lodha, Dr. Apexa Pareek
Article editing- Renu Sharma
Final approval- Prof. Payal Lodha, Apexa Pareek

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