

## Research Article

# Efficacy of Biogenically Synthesized ZnO Nanoparticles by *Hyptis suaveolens* Leaf Extract against Cowpea Weevil (*Callosobruchus maculatus*) for Pesticidal Activity

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**Abstract :** This study reports the green synthesis and multifunctional evaluation of zinc oxide nanoparticles (ZnO NPs) using *Hyptis suaveolens* leaf extract. Fresh leaves were shade-dried, pulverized, and extracted to prepare the bio-reducing precursor, with zinc sulfate used as the metallic source. A color shift from deep green to pale yellow confirmed nanoparticle formation. The synthesized ZnO NPs were characterized by XRD, SEM, EDX, and FTIR, revealing crystalline, spherical particles stabilized by phytochemicals. Antibacterial assays demonstrated strong inhibitory activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, with Gram-positive strains showing higher sensitivity. Pesticidal bioassays against *Callosobruchus maculatus* showed dose-dependent mortality, reduced adult emergence, seed damage, and weight loss, along with enhanced seed protection. At higher concentrations (750–1050 ppm), ZnO NPs achieved complete adult suppression; however, 1050 ppm slightly reduced seed germination. These findings highlight *H. suaveolens*-mediated ZnO NPs as eco-friendly, dual-functional agents for sustainable pest and microbial management in agriculture.

**Keywords:** *Hyptis suaveolens*; zinc oxide nanoparticles; green synthesis; antibacterial activity; pesticidal activity; *Callosobruchus maculatus*.

## Introduction

Nanotechnology has emerged as a transformative interdisciplinary field, enabling the design and manipulation of materials at the nanoscale with physicochemical properties distinct from their bulk counterparts. Nanoparticles (NPs), typically ranging from 1 to 100 nm, exhibit enhanced surface reactivity, catalytic efficiency, optical tunability, and biological interactions, supporting their application across medicine, environmental remediation, energy systems, and agriculture [1]. In recent years, increasing attention has been directed toward the development of sustainable nanomaterials that address agricultural productivity while minimizing ecological and health risks. Among available synthesis strategies, green or biogenic synthesis has gained prominence due to its environmental compatibility, cost-effectiveness, and avoidance of toxic reagents. Unlike conventional physical and chemical methods, biological synthesis utilizes plant-derived phytochemicals as natural reducing, capping, and stabilizing agents, enabling the production of stable nanoparticles under mild reaction conditions. Plant-mediated synthesis is particularly attractive

because of its scalability, rapid reaction kinetics, and the presence of bioactive compounds such as flavonoids, phenolics, terpenoids, and alkaloids that enhance nanoparticle stability and biological efficacy [1].

Agricultural nanotechnology has emerged as a promising approach for improving crop protection, post-harvest management, and food security. Nanomaterials have been investigated for applications including nanoformulated pesticides, controlled-release fertilizers, antimicrobial coatings, and biosensors for monitoring soil health and pest incidence [2]. However, the widespread use of synthetic pesticides has resulted in pesticide resistance, environmental contamination, and adverse effects on non-target organisms. Storage pests, particularly *Callosobruchus maculatus* (cowpea weevil), pose a significant threat to leguminous grains, causing extensive post-harvest losses through seed damage, weight reduction, and diminished germination potential. These challenges underscore the urgent need for eco-friendly, multifunctional alternatives for pest and microbial management. Zinc oxide nanoparticles (ZnO NPs) have attracted considerable interest due to their broad-spectrum antibacterial and pesticidal properties, chemical stability, and biocompatibility. Their wide band gap (3.37 eV) and high exciton binding energy contribute to reactive oxygen species (ROS) generation, which is lethal to microbial cells and insect pests. Importantly, ZnO NPs exhibit relatively low toxicity toward non-target organisms, supporting their application in seed protection, stored-grain pest control, and crop disease management [3]. When synthesized through green routes, ZnO NPs offer the additional advantage of sustainability and reduced environmental impact.

*Hyptis suaveolens* (L.) Poit., a medicinal and aromatic herb belonging to the family Lamiaceae, is rich in phytochemicals such as terpenoids, flavonoids, saponins, and phenolic compounds. Traditionally used for treating respiratory, gastrointestinal, dermatological, and febrile conditions, the plant exhibits well-documented antimicrobial, antioxidant, and insect-repellent properties [4-7]. The abundance of bioactive constituents makes *H. suaveolens* an effective biological resource for nanoparticle synthesis, where its extracts can simultaneously facilitate metal ion reduction and nanoparticle stabilization. Despite growing interest in plant-mediated ZnO NPs, studies integrating both antibacterial and pesticidal applications—particularly against storage pests—remain limited. The dual-functional potential of *H. suaveolens*-mediated ZnO NPs offers a sustainable strategy for addressing microbial contamination and insect infestation simultaneously, aligning with green chemistry principles and sustainable agriculture goals.

Therefore, the present study aims to synthesize zinc oxide nanoparticles using *Hyptis suaveolens* leaf extract, characterize the nanoparticles using XRD, SEM, EDX, and FTIR techniques, and evaluate their antibacterial activity against selected human pathogens and pesticidal efficacy against *Callosobruchus maculatus*. This work seeks to highlight the synergistic role of ethnobotany and green nanotechnology in developing eco-friendly solutions for

sustainable pest and microbial management in agriculture.

## **Materials and Methods**

### **Plant material collection and authentication**

Fresh, healthy, and disease-free leaves of *Hyptis suaveolens* (L.) Poit. were collected from naturally growing populations along roadsides and fallow lands near Senganur, Salem District, Tamil Nadu, India. The plant material was taxonomically authenticated by a qualified botanist, and voucher specimens were deposited for future reference. Only mature leaves without visible insect damage or fungal infection were selected to ensure uniform phytochemical composition.

### **Preparation of *Hyptis suaveolens* leaf extract**

The collected leaves were thoroughly washed under running tap water to remove adhering dust and debris, followed by rinsing with distilled water. The cleaned leaves were shade-dried at ambient temperature ( $28 \pm 2$  °C) for 7–10 days to preserve thermolabile phytochemicals. The dried leaves were then ground into a fine powder using a sterile mechanical grinder and sieved through a 0.5 mm mesh to obtain uniform particle size.

The powdered material was stored in airtight sterile containers at 4 °C until use. For aqueous extraction, a known quantity of leaf powder was mixed with distilled water and heated gently, followed by filtration to obtain a clear extract. The extract was used immediately as a bio-reducing and stabilizing agent for nanoparticle synthesis.

### **Green synthesis of zinc oxide nanoparticles**

Zinc oxide nanoparticles (ZnO NPs) were synthesized using the aqueous leaf extract of *H. suaveolens*. Briefly, 50 mL of freshly prepared leaf extract was heated at 60–80 °C under continuous magnetic stirring. A 1 mM zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ) solution was prepared separately using double-distilled water and added dropwise to the heated extract under constant stirring. The reaction mixture was maintained at 60 °C for 30–40 minutes until a visible color change was observed, indicating nanoparticle formation. The suspension was cooled to room temperature and centrifuged at 10,000 rpm for 15 minutes. The pellet was washed repeatedly with distilled water and ethanol to remove residual impurities including unreacted phytochemicals and precursor residues and then oven-dried at 80 °C for 12 hours. The dried ZnO nanoparticles were stored in airtight containers for further characterization and biological evaluation.

### **Characterization of ZnO nanoparticles**

#### **X-ray diffraction (XRD)**

The crystalline structure and phase purity of the biosynthesized zinc oxide nanoparticles were analyzed using X-ray diffraction (XRD). Dried ZnO nanoparticle powder was subjected to XRD analysis using a powder X-ray diffractometer equipped with Cu K $\alpha$  radiation ( $\lambda = 1.5406$  Å). The diffractometer was operated under standard voltage and current conditions, and diffraction

patterns were recorded over a  $2\theta$  range of  $20^{\circ}$ – $80^{\circ}$  at a suitable scanning rate. The obtained diffraction data were used to assess crystallinity, phase composition, and structural characteristics of the synthesized nanoparticles.

#### **Scanning electron microscopy (SEM)**

The surface morphology and microstructural features, including particle shape, surface texture, and aggregation behavior of the synthesized ZnO nanoparticles, were examined using scanning electron microscopy (SEM, Carl Zeiss) at various magnifications. Prior to analysis, the dried ZnO nanoparticle powder was gently dispersed on carbon-coated aluminum stubs and sputter-coated with a thin layer of gold to enhance electrical conductivity. SEM observations were carried out under high vacuum conditions at an accelerating voltage of 10–20 kV. Micrographs were recorded at different magnifications to assess particle morphology and aggregation characteristics.

#### **Energy-dispersive X-ray spectroscopy (EDX)**

Elemental composition and purity of the ZnO nanoparticles were determined using energy-dispersive X-ray spectroscopy (EDX) coupled with SEM. The EDX analysis provided qualitative and semi-quantitative information on the elemental distribution present on the nanoparticle surface. Spectra were collected from selected regions of the sample to confirm the presence of zinc and oxygen and to evaluate elemental purity.

#### **Fourier transform infrared spectroscopy (FTIR)**

Fourier transform infrared (FTIR) spectroscopy was employed to identify the functional groups involved in the biogenic synthesis and stabilization of zinc oxide nanoparticles. FTIR spectra of the *Hyptis suaveolens* leaf extract and the synthesized ZnO nanoparticles were recorded using an FTIR spectrometer in the range of  $4000$ – $400$   $\text{cm}^{-1}$ . Samples were prepared using the KBr pellet method, and the obtained spectra were analyzed to identify characteristic absorption bands corresponding to biomolecules associated with nanoparticle reduction and capping.

#### **Antibacterial activity**

The antibacterial activity of the biosynthesized ZnO nanoparticles was evaluated against *Bacillus subtilis* and *Enterococcus faecalis* (Gram-positive), and *Pseudomonas aeruginosa* (Gram-negative). Pure cultures of the test microorganisms were obtained from a recognized microbiology laboratory and maintained on nutrient agar slants at  $4^{\circ}\text{C}$  until further use. Antibacterial activity was assessed using the agar well diffusion method. Nutrient agar medium was prepared according to standard protocols, sterilized, and poured into sterile Petri dishes. Overnight bacterial cultures were uniformly spread over the agar surface using sterile cotton swabs. Wells of 10 mm diameter were aseptically punched into the agar, and ZnO nanoparticle suspensions at different concentrations (20, 40, and 60  $\mu\text{L}$ ) were introduced into the wells. The plant extract without nanoparticles served as the control. The plates were initially kept at room temperature for 1 hour to allow proper diffusion of the nanoparticles into the agar matrix. The plates were incubated at  $37^{\circ}\text{C}$  for 18–

24 hours, after which the zones of inhibition were measured in millimeters. All assays were conducted in triplicate to ensure reproducibility.

### **Pesticidal activity**

#### **Insect culture maintenance**

A laboratory culture of the cowpea weevil (*Callosobruchus maculatus*) was established from naturally infested cowpea seeds collected from local storage facilities. Adult insects were carefully separated and reared in clean, transparent plastic jars (1 kg capacity) containing uninfested and sterilized cowpea seeds. The mouths of the jars were covered with fine muslin cloth and secured with rubber bands to allow adequate aeration while preventing insect escape and external contamination. The cultures were maintained under controlled laboratory conditions of  $27 \pm 2$  °C temperature and  $65 \pm 5\%$  relative humidity, which are optimal for the growth and reproduction of cowpea weevils. Fresh cowpea seeds were supplied periodically to ensure continuous oviposition and larval development. The cultures were monitored regularly, and newly emerged, healthy, uniform-aged, unmated adults were collected and used for bioassays following established protocols [2].

#### **Contact toxicity bioassay**

Contact toxicity assays were conducted to evaluate the pesticidal activity of biosynthesized zinc oxide nanoparticles (ZnO NPs) against adult *C. maculatus*. ZnO nanoparticles were tested at concentrations of 150, 300, 450, 550, 750, and 1050 ppm per kg of cowpea seeds. For each treatment, 100 g of cowpea seeds were placed in screw-capped plastic jars and treated individually with the respective nanoparticle concentration. The jars were manually shaken for approximately 60 seconds to ensure uniform distribution of nanoparticles on the seed surface. Twenty unmated adult *C. maculatus* were introduced into each jar, and the jar openings were covered with muslin cloth. Untreated seeds without nanoparticles served as the control. The experiments were arranged in a completely randomized design with three replications and maintained at  $30 \pm 1$  °C and  $65 \pm 5\%$  relative humidity [2].

Adult mortality was recorded at 1, 3, 5, 7, and 10 days after treatment. One month after treatment, parameters including the number of eggs laid per 100 seeds, adult emergence per 100 g of seeds, percentage seed damage, percentage seed weight loss, percentage seed germination, and percentage reduction over control were recorded. Corrected mortality (%) was calculated using Abbott's formula:

$$\text{Corrected mortality (\%)} = \frac{(X-Y)}{(100-Y)} \times 100$$

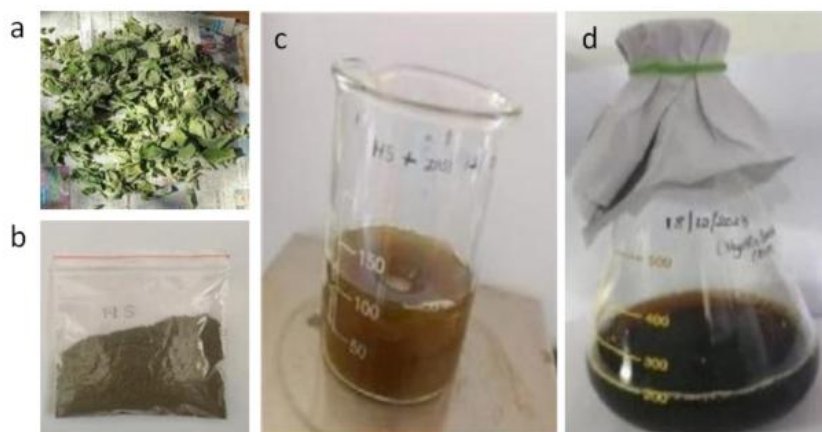
where X = mortality in treatment and Y = mortality in control

### **Results and Discussion**

#### **Role of *Hyptis suaveolens* leaf extract in ZnO nanoparticle formation**

The formation of zinc oxide nanoparticles (ZnO NPs) in the present study was strongly influenced by the phytochemical constituents of *Hyptis suaveolens* leaf extract, which acted as natural reducing, capping, and

stabilizing agents. The visible color change of the reaction mixture from deep green to pale yellow during synthesis served as a preliminary indication of  $Zn^{2+}$  ion reduction and ZnO nanoparticle nucleation mediated by plant-derived biomolecules (Figure 1). Similar visual transitions have been widely reported as characteristic indicators of ZnO nanoparticle formation in plant-mediated green synthesis systems.



**Figure 1.** Sequential steps involved in the green synthesis of zinc oxide nanoparticles using *Hyptis suaveolens* leaf extract: (a) shade-dried leaves, (b) powdered leaf material, (c) aqueous leaf extract, and (d) reaction mixture after ZnO nanoparticle formation, indicated by a visible color change.

*H. suaveolens* is rich in flavonoids, phenolics, terpenoids, alkaloids, and proteins containing functional groups capable of interacting with zinc ions. These phytochemicals facilitate  $Zn^{2+}$  ion reduction and simultaneously stabilize the growing nanoparticles, preventing uncontrolled aggregation. The involvement of these biomolecules in nanoparticle formation and surface stabilization is further supported by FTIR analysis. Thus, the plant extract plays a dual role in ZnO nanoparticle synthesis by driving both nucleation and stabilization under mild, eco-friendly conditions.

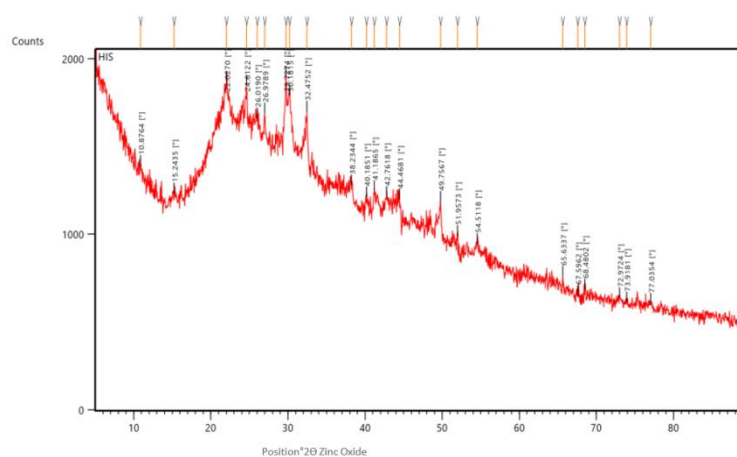
#### **Structural characterization by X-ray diffraction (XRD)**

The crystalline nature and phase purity of the biosynthesized ZnO nanoparticles were confirmed by XRD analysis (Figure 2). The diffraction peaks correspond well with the standard hexagonal wurtzite structure of ZnO, with no detectable impurity phases. The XRD pattern exhibited distinct diffraction peaks at  $2\theta$  values of approximately  $31.7^\circ$ ,  $34.4^\circ$ ,  $36.2^\circ$ ,  $47.5^\circ$ ,  $56.6^\circ$ ,  $62.8^\circ$ , and  $67.9^\circ$ , corresponding to the (100), (002), (101), (102), (110), (103), and (112) crystallographic planes, respectively. These diffraction peaks matched well with the standard hexagonal wurtzite structure of ZnO (JCPDS card No. 36-1451), confirming the successful synthesis of crystalline ZnO nanoparticles. The absence of additional impurity peaks indicated high phase purity and confirmed that phytochemicals from *H. suaveolens* did not introduce secondary crystalline phases during synthesis. The relatively broad diffraction peaks suggested the nanocrystalline nature of the particles, validating the

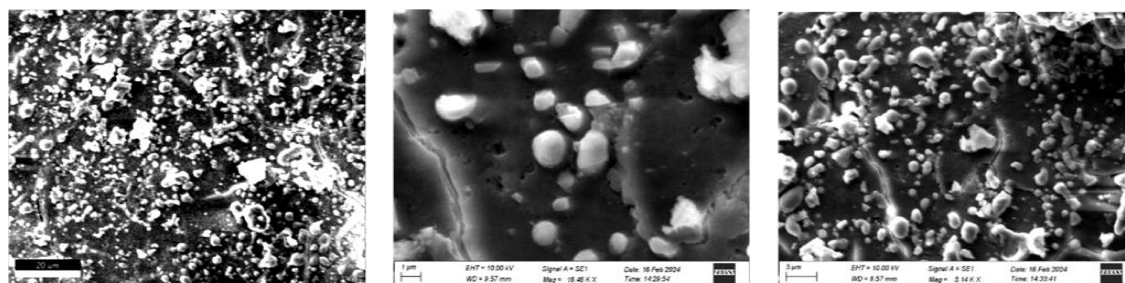
effectiveness of the green synthesis approach.

### Surface morphology analysis by SEM

SEM analysis revealed that the biosynthesized ZnO nanoparticles exhibited predominantly quasi-spherical to irregular morphology with pronounced agglomeration (Figure 3). Such agglomeration is characteristic of green-synthesized metal oxide nanoparticles and can be attributed to high surface energy and bio-organic capping by phytochemicals derived from *Hyptis suaveolens* leaf extract. Although the agglomerates appeared in the micrometer range, higher magnification micrographs clearly demonstrated that they were composed of nanoscale ZnO crystallites, consistent with the crystallite size inferred from XRD analysis. The nanoparticles displayed rough and porous surface features, which are advantageous for biological applications, as increased surface roughness enhances interactions with bacterial cell walls and insect cuticles, thereby contributing to improved antibacterial and pesticidal activity.



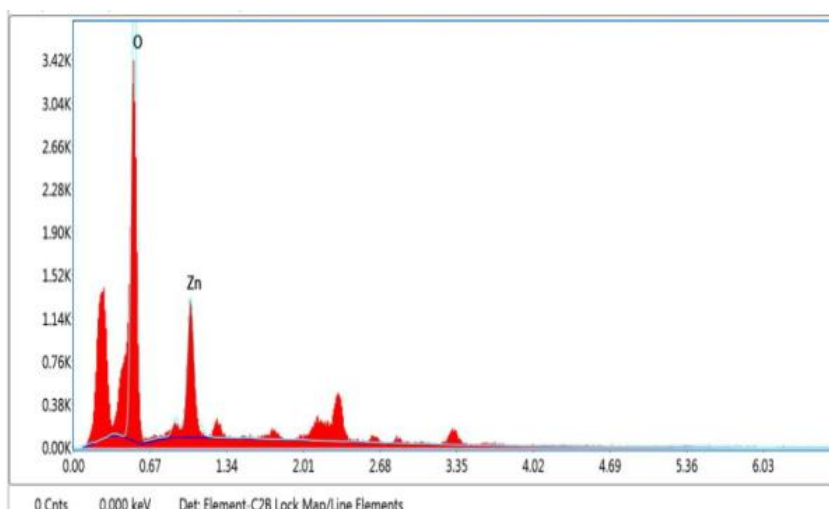
**Figure 2.** X-ray diffraction (XRD) pattern of zinc oxide nanoparticles biosynthesized using *Hyptis suaveolens* leaf extract. The diffraction peaks observed at  $2\theta$  values corresponding to the (100), (002), (101), (102), (110), (103), and (112) planes confirm the formation of crystalline ZnO with a hexagonal wurtzite structure (JCPDS card No. 36-1451).



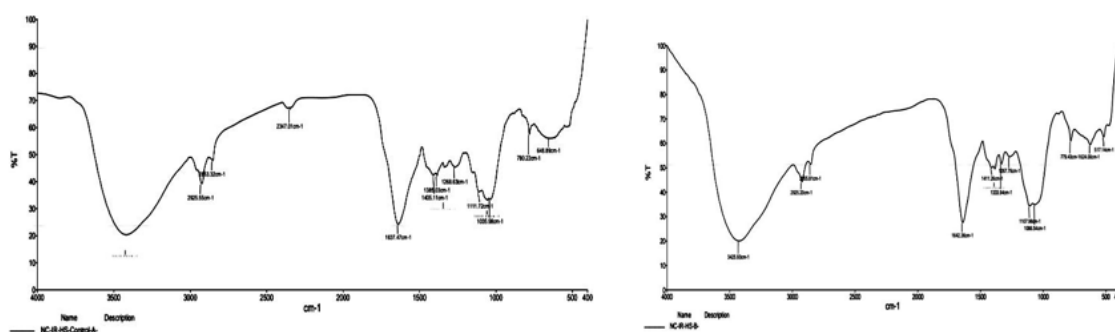
**Figure 3.** Scanning electron microscopy (SEM) images of zinc oxide nanoparticles biosynthesized using *Hyptis suaveolens* leaf extract at different magnifications: (a) low magnification (20  $\mu\text{m}$ ) showing aggregated nanoparticle clusters, (b) high magnification (1  $\mu\text{m}$ ) revealing quasi-spherical ZnO nanograins, and (c) intermediate magnification (5  $\mu\text{m}$ ) illustrating dense nanoparticle distribution and surface roughness. The observed agglomeration is attributed to phytochemical capping and the high surface energy of the biosynthesized nanoparticles.

### Elemental composition analysis by SEM–EDX

The elemental composition and purity of the biosynthesized ZnO nanoparticles were confirmed using SEM-coupled energy-dispersive X-ray spectroscopy, and the corresponding spectrum is shown in Figure 4. The EDX profile displayed strong characteristic peaks corresponding to zinc (Zn) and oxygen (O), confirming the formation of ZnO nanoparticles. The absence of additional elemental peaks indicated high purity of the synthesized nanoparticles. Minor low-intensity signals may be attributed to residual phytochemical capping agents or sample preparation artifacts; however, these did not affect the stoichiometric integrity of ZnO. The near-stoichiometric Zn–O ratio confirmed efficient biogenic conversion of  $Zn^{2+}$  ions into ZnO nanostructures.



**Figure 4.** Energy-dispersive X-ray spectroscopy (EDX) spectrum of zinc oxide nanoparticles biosynthesized using *Hyptis suaveolens* leaf extract. The spectrum shows prominent peaks corresponding to zinc (Zn) and oxygen (O), confirming the formation and elemental purity of ZnO nanoparticles. The absence of additional impurity peaks indicates successful biogenic synthesis without contamination.



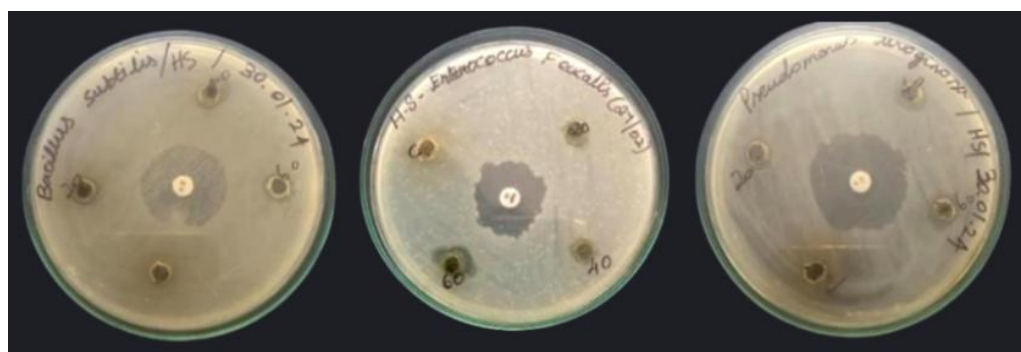
**Figure 5.** Fourier transform infrared (FTIR) spectra of (a) *Hyptis suaveolens* leaf extract (control) and (b) *Hyptis suaveolens*-mediated zinc oxide nanoparticles. The shift and reduction in intensity of characteristic functional group vibrations in the ZnO nanoparticle spectrum indicate the involvement of plant-derived phytochemicals in nanoparticle reduction and surface stabilization. The appearance of Zn–O stretching vibrations in the low wavenumber region confirms the formation of ZnO nanostructures.

### Functional group analysis by FTIR

FTIR spectroscopy was employed to identify the functional groups involved in ZnO nanoparticle formation and stabilization (Figures 5). The *H. suaveolens* leaf extract (Figure 5a) exhibited characteristic absorption bands around 2921  $\text{cm}^{-1}$  (C–H stretching), 1629  $\text{cm}^{-1}$  (N–H bending or C=O stretching), and 1023  $\text{cm}^{-1}$  (C–N stretching), indicating the presence of flavonoids, phenolics, proteins, and alkaloids. In the FTIR spectrum of the synthesized ZnO nanoparticles (Figure 5b), several functional groups showed peak shifting and intensity reduction, confirming their involvement in nanoparticle formation. Absorption bands at approximately 2920–2850  $\text{cm}^{-1}$  indicated residual C–H stretching vibrations, while bands around 1630–1650  $\text{cm}^{-1}$  corresponded to amide I or N–H bending vibrations, suggesting adsorption of proteins and polyphenolic compounds on the ZnO NP surface. Peaks in the range of 628–700  $\text{cm}^{-1}$  were assigned to Zn–O stretching vibrations, confirming ZnO nanoparticle formation. These results demonstrate that *H. suaveolens* phytochemicals play a crucial role in both reduction and surface stabilization of ZnO nanoparticles.

### Antibacterial activity of ZnO nanoparticles

The antibacterial activity of the biosynthesized ZnO nanoparticles was evaluated against *Bacillus subtilis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* (Figure 6). The ZnO nanoparticles exhibited clear zones of inhibition against all tested bacterial strains, with antibacterial activity increasing with nanoparticle concentration. Gram-positive bacteria (*B. subtilis* and *E. faecalis*) showed higher susceptibility than the Gram-negative bacterium (*P. aeruginosa*), likely due to the absence of an outer membrane barrier in Gram-positive bacteria [8]. The antibacterial activity of ZnO nanoparticles may be attributed to reactive oxygen species generation, membrane disruption,  $\text{Zn}^{2+}$  ion release, and synergistic effects of plant-derived phytochemicals adsorbed on the nanoparticle surface



**Figure 6.** Antibacterial activity of biosynthesized zinc oxide nanoparticles evaluated by the agar well diffusion method. Representative inhibition zones observed against (a) *Bacillus subtilis*, (b) *Enterococcus faecalis*, and (c) *Pseudomonas aeruginosa* at different concentrations of ZnO nanoparticles (20, 40, and 60  $\mu\text{L}$ ). The central disc represents the standard control. Increased zone diameter with increasing concentration indicates dose-dependent antibacterial activity.

### Adult mortality of *Callosobruchus maculatus*

The insecticidal activity of ZnO nanoparticles against adult *Callosobruchus maculatus* was evaluated over a 10-day exposure period, and the results are presented in Table 1. A concentration- and time-dependent increase in mortality was observed. ZnO nanoparticles at 750 ppm and 550 ppm exhibited comparatively higher mortality than lower doses, with mortality increasing gradually over the exposure period. At 750 ppm, mortality increased from 12.50% on day 1 to 22.50% by day 10. ZnO nanoparticles at 1050 ppm showed moderate mortality during the initial exposure period; however, no mortality was recorded on day 10, suggesting reduced persistence of insecticidal activity at this concentration. Lower concentrations (150–450 ppm) resulted in relatively low mortality, although a gradual increase was observed with prolonged exposure. A constant background mortality of 12.50% was recorded in the untreated control, which may be attributed to natural causes rather than treatment effects. The insecticidal activity of ZnO nanoparticles may be associated with abrasion and sorption of the insect cuticular wax layer, disruption of the protective barrier, and induction of cellular stress following nanoparticle penetration through spiracles or cuticular openings [9].

**Table 1:** Mortality (%) of *Callosobruchus maculatus* adults at different concentrations of biosynthesized ZnO nanoparticles over a 10-day exposure period

Treatment (ppm)	Day 1 (%)	Day 3 (%)	Day 5 (%)	Day 7 (%)	Day 10 (%)
150	12.50	1.25	4.62	6.25	12.00
300	12.50	1.75	5.00	7.12	13.00
450	12.50	2.12	5.87	8.37	15.50
550	12.50	2.50	6.75	11.20	19.50
750	12.50	3.37	7.50	15.50	22.50
1050	12.50	5.00	10.50	19.20	0.00
Control	12.50	12.50	12.50	12.50	12.50

### Population buildup of *Callosobruchus maculatus*

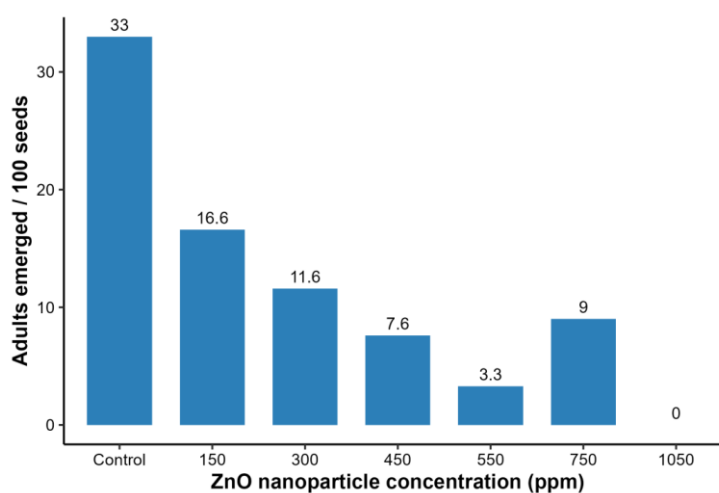
The effect of ZnO nanoparticles on population buildup was assessed after 30 days of storage, and the results are summarized in Table 2. A marked reduction in adult emergence was observed in nanoparticle-treated seeds compared with the untreated control. Adult population buildup ranged from 0.00 to 33.00 adults per 100 g of seeds. ZnO nanoparticles at 1050 ppm resulted in complete suppression of adult emergence (0.00 adults/100 g). Substantial reductions were also observed at 550 ppm (3.30 adults/100 g) and 750 ppm (9.00 adults/100 g). In contrast, the untreated control recorded the highest adult population (33.00 adults/100 g of seeds) (Figure7). The reduction in adult emergence may be attributed to adverse effects on egg viability, larval development, pupal survival, and interference with oviposition behavior

**Table 2:** Effect of biosynthesized ZnO nanoparticles on population buildup, seed damage, seed weight loss, and seed germination of *Callosobruchus maculatus* after 30 days of storage

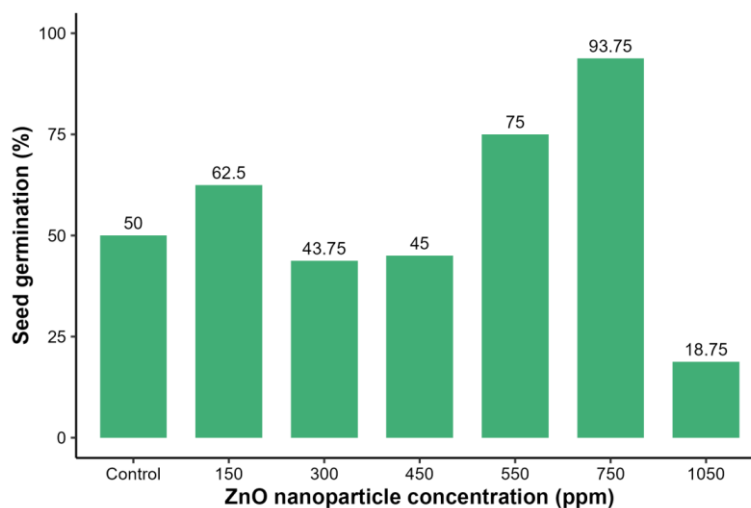
Treatment details Conc. of ZnO NPs (ppm)	Adults emerged (No./100 seeds)	Seed damage (%)	Seed weight loss (%)	Seed germination (%)
150	16.60	63.30	6.00	62.50
300	11.60	60.30	5.25	43.75
450	7.60	54.00	3.25	45.00
550	3.30	44.30	2.75	75.00
750	9.00	30.30	2.25	93.75
1050	0.00	7.30	1.50	18.75
Control	33.00	66.00	7.00	50.00

### Seed damage and seed weight loss

Seed damage and seed weight loss caused by *C. maculatus* were significantly reduced in ZnO NP-treated seeds in a dose-dependent manner (Table 2). The lowest seed damage (7.30%) and minimal seed weight loss (1.50%) were recorded at 1050 ppm. Treatments at 750 ppm and 550 ppm also showed substantial reductions compared with the control. In contrast, the untreated control exhibited severe seed damage (66.00%) and the highest weight loss (7.00%), reflecting extensive feeding and internal seed degradation. The reduction in damage and weight loss observed in treated seeds may be attributed to suppression of adult feeding, inhibition of larval development, and reduced seed perforation caused by ZnO nanoparticles.



**Figure 7.** Effect of biosynthesized ZnO nanoparticles on population buildup of *Callosobruchus maculatus*. Bar graph showing the number of adult *Callosobruchus maculatus* emerged per 100 cowpea seeds after 30 days of storage following treatment with different concentrations of biosynthesized zinc oxide nanoparticles (ZnO NPs). Adult emergence decreased progressively with increasing nanoparticle concentration, with complete suppression observed at 1050 ppm. Values represent mean observations from three replicates.



**Figure 8.** Effect of biosynthesized ZnO nanoparticles on seed germination of cowpea. Bar graph illustrating the percentage seed germination of cowpea after 30 days of storage following treatment with different concentrations of biosynthesized zinc oxide nanoparticles (ZnO NPs). Seed germination increased with nanoparticle concentration up to 750 ppm, which showed the highest germination rate, indicating effective pest control without adverse effects on seed viability. A decline in germination was observed at 1050 ppm, suggesting mild phytotoxic effects at higher concentrations. Values represent mean observations from three replicates.

#### Effect on seed germination

Seed germination varied with ZnO nanoparticle concentration, indicating a concentration-dependent response (Table 2). The highest germination percentage (93.75%) was recorded at 750 ppm, demonstrating effective pest control without adverse effects on seed viability (Figure 8). At 1050 ppm, a slight reduction in germination was observed, suggesting mild phytotoxic effects at higher nanoparticle concentrations; however, germination remained within acceptable limits. In contrast, the untreated control exhibited lower germination due to severe infestation and internal seed damage caused by *C. maculatus*. These results highlight the importance of dose optimization to balance pesticidal efficacy and seed quality. Collectively, the results demonstrate that *Hyptis suaveolens*-mediated ZnO nanoparticles are structurally stable, chemically pure, and biologically active. The plant extract plays a crucial role in nanoparticle formation and stabilization, while the synthesized ZnO nanoparticles exhibit significant antibacterial and pesticidal activity. At optimized concentrations, particularly 750 ppm, these biogenic nanoparticles provide effective pest control without compromising seed viability, highlighting their potential as eco-friendly alternatives for post-harvest pest management and microbial control.

#### Conclusion

This study demonstrates the green synthesis of zinc oxide nanoparticles (ZnO NPs) using *Hyptis suaveolens* leaf extract, enabling an eco-friendly and efficient fabrication process. The synthesized nanoparticles were crystalline,

pure, and phytochemically stabilized, as confirmed by physicochemical analyses. The ZnO NPs exhibited significant antibacterial activity, particularly against Gram-positive bacteria, along with effective pesticidal action against *Callosobruchus maculatus*. Notably, 750 ppm provided an optimal balance between pest control and seed viability. Overall, these biogenic ZnO nanoparticles show strong potential as sustainable alternatives for microbial control and post-harvest pest management.

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