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BIOGENIC SYNTHESIS OF ZINC NANOPARTICLES WITH INDIGENOUS PENICILLIUM CITRINUM IBA5VYT AND THEIR EFFECTIVENESS AGAINST COTTON LEAFWORM

Anamika Sharma* and Pragya Kulkarni

Department of Microbiology, Government V. Y. T. PG Autonomous College, Durg - 491001, Chhattisgarh, India

ABSTRACT

This research explored the preliminary insecticidal potential of zinc oxide nanoparticles (ZnO NPs) synthesized through a green chemistry approach against *Spodoptera litura*, a major pest of soybean crops. Among four *Penicillium* isolates tested, P3, P6, P14, and P17, *Penicillium citrinum* IBA5VYT (P17) demonstrated the highest efficacy, achieving mortality rates of 83.34% and 96%. Subsequently, ZnO NPs were biosynthesized using P17, and their properties were characterized via X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and UV spectroscopy. The effectiveness of these ZnO NPs was evaluated at concentrations of 100, 200, 300, 400, and 500 ppm against third and fourth instar larvae of *S. litura*. Notably, complete mortality was observed at 300 ppm and 500 ppm concentrations respectively. The study calculated the LC50 and LT50 values for both *Penicillium citrinum* IBA5VYT and the biogenic ZnO NPs for the larvae. Results indicated that biogenic ZnO NPs exhibit significant pest control efficacy at relatively low concentrations, with improved performance at higher doses. These findings emphasize the potential of biosynthesized ZnO NPs as a viable, eco-friendly alternative to conventional chemical pesticides.

KEYWORDS

Bioinsecticide,ZnO NP, Penicillium citrinum,Spodoptera litura,FTIR, XRD, SEM

Introduction

Spodoptera litura is classified as a polyphagous pest due to its extensive host range, feeding on a diverse array of plant species. Research by Shu *et al.* (2018) highlights that larvae of this pest can consume between 120 and 180 different plant species, demonstrating remarkable dietary adaptability. Additionally, it is also known to affect 389 host species across 40 plant families. This pest poses a significant threat to open field crops in the Asia-Pacific region, leading to substantial losses in economically important crops such as cotton, maize, groundnuts, soybeans, and tobacco, as well as various vegetables. Its impact extends to controlled environments like greenhouses and glasshouses, where it severely damages crops including tomatoes, sweet peppers, and eggplants (Bragard *et al*., 2020).

Penicillium citrinum, initially recognized for its role as a phytopathogen (Gupta & Chauhan 1998; Estrada *et al*., 2017), has recently been studied for its entomopathogenic properties. Research indicates that this fungus holds potential as a biocontrol agent and plant growth enhancer, positioning it as a valuable tool in integrated pest management strategies (Waqas *et al*., 2015; Damasceno *et al*., 2019). Its ability to both control pests and support plant health opens up new avenues for sustainable agricultural practices.

Integrated Pest Management (IPM) is an ecologically sound approach aimed at minimizing agricultural pest impacts through a combination of biological, cultural, physical, and chemical control strategies. It reduces reliance on synthetic pesticides and fosters sustainable farming practicesensuring long-term sustainability by closely monitoring pest populations. Additionally, IPM promotes practices that enhance soil health and biodiversity, contributing to a more resilient agricultural system. It also relies on the integration of multiple strategies for effective and enduring pest management (Rai *et al*., 2012).

Nanotechnology, encompassing both 'Degradative' and 'Inductive' approaches, offers innovative solutions across various fields such as medicine, electronics, and materials science (Rai *et al*., 2012). In agriculture, nanotechnology holds promise for developing novel pest control methods that can complement traditional practices. Researchers are investigating how nanoparticles, including zinc oxide nanoparticles (ZnO NPs), can enhance pest control while remaining environmentally friendly. ZnO NPs have demonstrated antimicrobial properties and potential as fungicides (Seven *et al*., 2004; Levin *et al*., 2007), and their effectiveness against pests like aphids and caterpillars has been noted (Huang *et al*., 2010). Their low toxicity to non-target organisms makes them a promising alternative to conventional pesticides (Arumugam *et al*., 2016). Green synthesis methods, which use natural reducing agents like plant extracts or microorganisms, are increasingly preferred for producing ZnO NPs due to their environmental benefits and sustainability (Mohan *et al*., 2015; Singh *et al*., 2018). This study presents findings on the green synthesis of ZnO NPs using *Penicillium citrinum*, their characterization through standard analytical techniques, and their effectiveness against *Spodoptera litura* (Cotton leafworm) under laboratory conditions.

Materials and Methods

Fungal Isolates

In this study, four Penicillium spp. strains (P3, P6, P14, and P17) were isolated from soil samples collected from insect-infested crop fields in Kabirdham district, Chhattisgarh. The isolates were cultured in pure form on Sabouraud Dextrose Agar (SDA) medium with chloramphenicol and streptomycin at 25°C (Goettel & Inglis, 1997). Both morphological and genetic identification were conducted to characterize these isolates, with a particular focus on determining the potential isolate.

Pathogenic Effects and Analysis

Spodoptera litura larvae, sourced from the Department of Entomology at Indira Gandhi Krishi Vishwavidyalaya Raipur, were reared in vitro for assessing the pathogenic potential of *Penicillium citrinum* on *Spodoptera litura*. Ten larvae each of the third and fourth instar stages were exposed to different spore concentrations of *Penicillium citrinum*, applied via spraying a pore suspension prepared with 0.01% Tween 80. The larvae were then placed on fresh castor leaves in controlled conditions. The control group was treated with a 0.01% Tween 80 solution and sterile water (Namasivayam *et al*., 2015). The experiments were conducted in triplicate, with all test and control insects incubated at 25 ± 2°C and 55% ± 5% relative humidity for 10 days.

Preparation of Biomass Extract

A 5day old broth culture of *Penicillium citrinum* was employed for biomass extraction. The culture was filtered, then mechanically homogenized with distilled water using a mortar and pestle. The biomass extract was centrifuged at 5000 rpm for 5 minutes, and the resulting supernatant was utilized for the synthesis of ZnO nanoparticles (Moormann & Bachand, 2021).

Biosynthesis of Zinc Nanoparticles

Zinc nanoparticles were synthesized by mixing 5 ml of fungal cell supernatant with 25 ml of 0.1 M ZnSO4 solution. The mixture was sonicated for 15 minutes and maintained at a pH of 10. Following sonication, the precipitate was separated by centrifugation (Sharif-al-hoseini*et al.,* 2015). To purify the ZnO nanoparticles, the pellet was washed and redispersed three times with double distilled water. The green-synthesized material was then dried in a hot air oven at 60°C for 5 hours. After drying, the material was annealed at 540°C for 2 hours to produce a white powder, which was then used for further analysis (Moormann & Bachand, 2021).

Characterization of ZnO Nanoparticle

The optical properties of ZnO nanoparticles (NPs) were assessed by recording their UV-VIS absorption spectra in the range of 300-400 nm using a UV-VIS spectrometer. X-ray diffraction (XRD) analysis was conducted with a Rigaku Miniflex II instrument operating at 30 kV and 15 mA, utilizing Cu Kα radiation ($\lambda = 1.5406$ Å) over a 2θ range of 20-80°. Surface morphology and particle size of the ZnO NPs were examined using scanning electron microscopy (SEM) on a JSM-6750F (JEOL model). For Fourier transform infrared (FTIR) spectroscopy, the ZnO NPs powder was combined with potassium bromide (KBr) and analyzed in the 500-4000 cm^{-1} range using a Bruker Optics FT-Tensor 27 (Germany) in diffuse reflectance mode, with a spectral resolution of 4 cm^{-1}.

Assessment of Insecticidal Efficacy

Ten larvae each of the third and fourth instar stages of *Spodoptera litura* were exposed to different concentrations of ZnO nanoparticles, in triplicate, which were applied by spraying a suspension prepared with distilled water. The larvae were then fed fresh castor leaves and maintained in controlled conditions. The control group was treated with distilled water only. All test and control larvae were incubated at 25 ± 2°C and 55% ± 5% relative humidity for a duration of 3-4 days.Mortality rates were corrected using Abbott’s formula, and probit analysis was performed with SPSS software to calculate LC50 and LT50 value (Abbott1925; Khooshe*et al.,* 2016).

Results&Discussion

Fungal Isolates All*Penicillium* isolates were cultured on modified SDA medium, revealing distinct colony characteristics. P1, P3, P14, and P17 displayed dark green colonies with rough textures; bluish-green to olive green colonies with smooth surfaces; olive green colonies with smooth surfaces; and dark green to olive green colonies with smooth surfaces, respectively. All isolates appeared short, floccose, and flat with a white peripheral zone on the plates (Figures 1a to 1d). Microscopic examination showed that *P. citrinum* colonies formed a basal felt with floccose aerial mycelium and produced conidiophores. These conidiophores were solitary, featuring smooth, thin walls and uneven penicilli bearing conidia and phialides. Conidia were observed in distorted chains, ranging from spherical to subspherical forms (Figures 2a–2d). The morphological features of the isolates matched those previously described for *P. citrinum* by Nguyen *et al*., 2023.

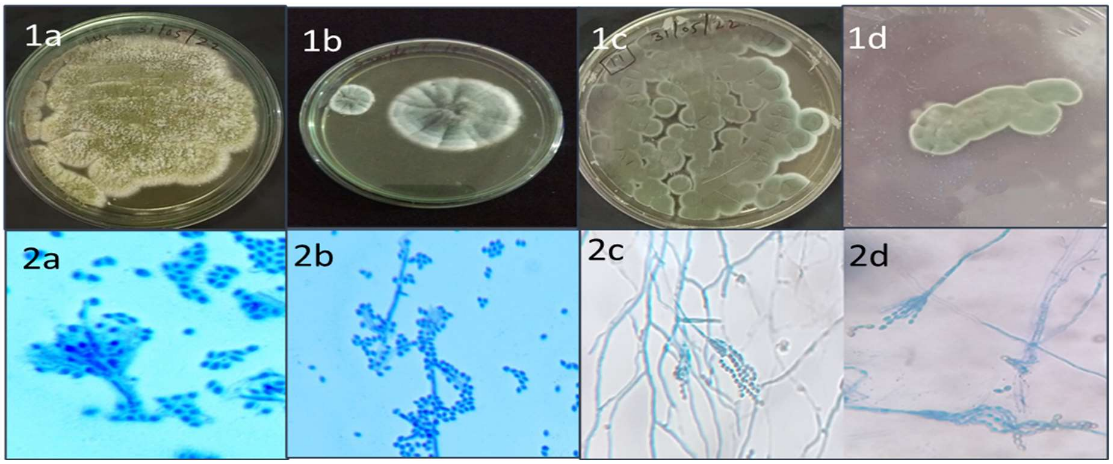

Figure 1. Macroscopic and microscopic images of test Penicillium spp.
(1a & 2a) P3 isolate; (1b & 2b) P6 isolate; (1c & 2c) P14 isolate; (1d & 2d) P17 isolate.

Pathogenic Effects and Analysis

After a ten-day incubation period, larvae were assessed, showing a mortality rate ranging from 30% to 96% in both third and fourth instar stages **(Table - 1)**. Isolate P17 exhibited the highest mortality rates, inducing 96% mortality in 3rd instar larvae and 83.4% in 4th instar larvae of *Spodoptera litura*, and thus emerged as a promising candidate for further investigation.This was followed by isolate P3, which resulted in 70% mortality in 3rd instar and 50% in 4th instar larvae. Isolate P4 showed moderate efficacy with 60% mortality in 3rd instar larvae and 33.4% in 4th instar larvae. The least effective was isolate P1, with mortality rates of 36.7% for 3rd instar larvae and 30% for 4th instar larvae.Early research on *Penicillium citrinum* mainly focused on its phytopathogenic effects (Gupta & Chauhan, 1998 and Estrada *et al*., 2017). However, more recent studies have investigated its potential as an entomopathogen for controlling some agricultural pests (Maketon *et al*., 2014; Wu *et al*., 2022 and Idrees *et al*., 2023).

Table 1- Mortality percentage in 3rd and 4th Instar Larvae of *Spodoptera litura*

Isolate	**% Mortality rate**	
	3rd instar	**4th instar**
P1	36.7± 1.93	30± 1.93

P3	70±1.93	50±2.94
P14	60±3.86	33.4± 0.95
P17	**96± 1.93**	**83.4 ± 1.90**

Molecular Characterization of Potential Isolate (P17)

The presence of green fungal growth on the deceased larvae served as an initial confirmation. The infected larvae were then re-cultured on Sabouraud Dextrose Agar (SDA), and the resulting fungal culture was purified and preserved. Isolate P17 was identified as *Penicillium citrinum* IBA5VYT with accession number PP721327 through molecular characterization. It showed a 96.97% similarity to the *Penicillium citrinum* strain Xia16, particularly in the small subunit ribosomal RNA and internal transcribed spacer regions, with accession number OR346130.1.

Biosynthesis of Zinc nanoparticles

The biosynthesis of ZnO nanoparticles (NPs) by *Penicillium citrinum* was confirmed by observing the formation of a transparent to white precipitate in the mixture of ZnSO4 and *P. citrinum* extract. This change in appearance indicates the successful synthesis of ZnO NPs.

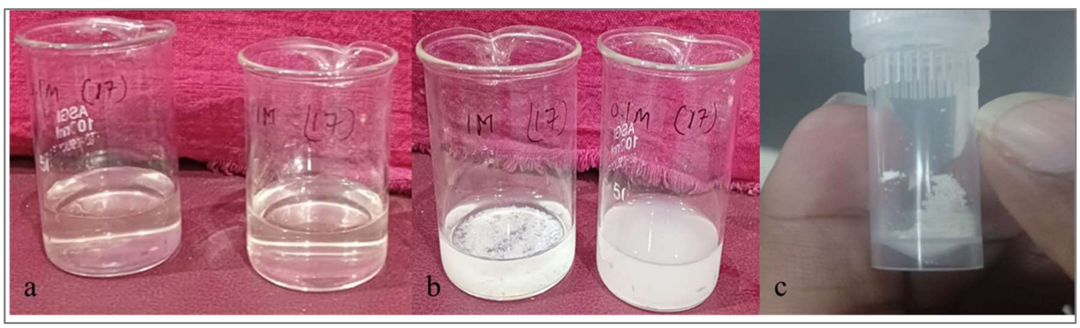

Figure 2: Biosynthesis of ZnO Nanoparticle by sonication method **a)** ZnSO4 + *Penicillium citrinum* extract **b)** after sonication white precepted of ZnO NPs **c)**ZnO NPs crystalline powder after centrifugation.

Bioassay of Biogenic ZnO Nanoparticle

Third and fourth instar larvae were separately treated with biogenic ZnO nanoparticles and the effective *P. citrinum* isolate for 7 days **(Table - 2)**. For *P. citrinum*, the LC50 and LT50 values were 4.5 × 10□ spores/ml and 4.2×10^5 spores/ml, and 121 hours and 134 hours for third and fourth instar larvae, respectively. In comparison, the biogenic ZnO nanoparticles had LC50 and LT50 values of 106 ppm and 140 ppm, with 30.46 hours and 50.23 hours for third and fourth instar larvae, respectively. These results demonstrate that ZnO nanoparticles are more effective than *P. citrinum* in controlling *Spodoptera litura*. The data also show a clear correlation between ZnO NP concentration and mortality rate, with higher concentrations leading to increased mortality and shorter LT50 values, consistent with findings by Thakur *et al.* (2022) for third instar larvae. Various studies as depicted in **Table – 3**, have highlighted the insecticidal properties of ZnO nanoparticles against a range of crop pests, underscoring their potential as effective bioinsecticides in pest management.

Table - 2*S.litura* mortality by biogenic ZnO nanoparticles and *P. citrinum*

Treatment	Larvae stages	LC50	LT50	χ^2	Reg. Equation
P.citricum	Third instar	4.5×10^4	121 hrs	.477	Y= 0.82x+(-3.819)
	Fourth instar	4.2×10^5	134 hrs	1.251	Y= 0.75x+(-4.245)
Biogenic ZnO NPs	Third instar	106 ppm	30.46 hrs	9.29	Y= 4.93x +(-2.307)
	Fourth instar	140 ppm	50.23 hrs	7.81	Y= 5.37x +(-4.134)

Note: Probit analysis was carried out at p = 0.05 and 95% CI.

Table - 3Reports on insecticidal activity of biogenic Zinc Nanoparticles against crop pests

S. No.	Crop pest	Reference
1.	*Aphis nerii* (Oleander aphid)	Rouhani et al. 2012
2.	*Sitophilus oryzae* (Rice weevil)	Nasr et al. 2015
3.	*Callosobruchus maculatus* (Pulse beetle	Malaikozhundan &Vinodhini 2018
4.	*Bactericeracockerelli* Sulc.	Gutiérrez-Ramírez et al. 2021
5.	*Puto barberi* (mealybug)	Agredo et al. 2024

Characterization of ZnO nanoparticle

UV- Visible spectroscopy

The UV-visible spectrophotometer recorded the optical absorbance spectrum of ZnO nanoparticles across the 200-600 nm range, revealing a distinct absorption peak at 340 nm**(Figure 3)**. This peak confirms the successful synthesis of biogenic ZnO nanoparticles and highlights its potential as a novel and eco-friendly approach. The broad peak at 340 nm, indicative of absorption in the 290-360 nm range, suggests that the shift from 380 nm to 340 nm reflects a reduction in particle size from bulk material to nanoparticles (Fakhari *et al*., 2019).

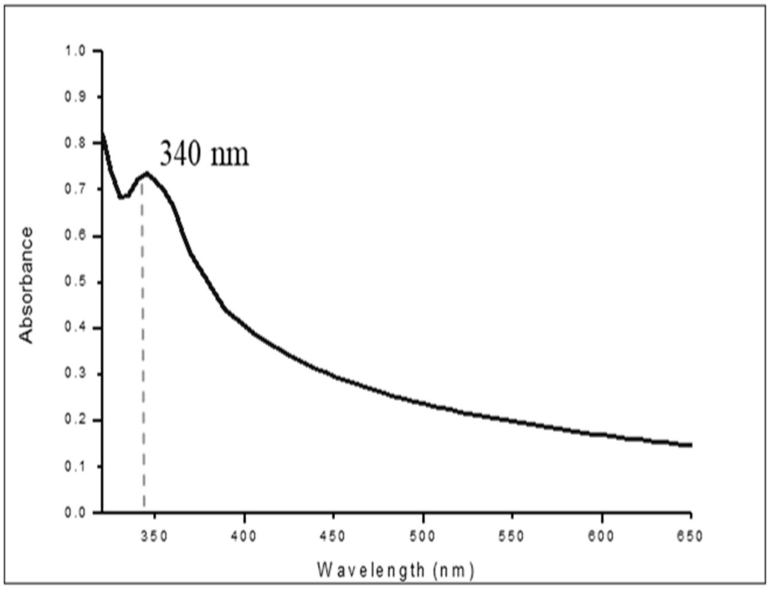

Figure 3: UV-Visible spectrum of biogenic ZnO Nanoparticles.

FTIRand XRD analysis of ZnO NPs

Fourier Transform Infrared (FTIR) spectroscopy was employed to identify the functional groups present in the ZnO nanoparticles (ZnO NPs). The FTIR spectrum exhibited prominent bands at 3334 cm^{-1} and 1630 cm^{-1}, alongside weaker bands at 2359 cm^{-1}, 2063 cm^{-1}, 1505 cm^{-1}, 1300 cm^{-1}, 671 cm^{-1}, and 634 cm^{-1}. These bands correspond to N-H stretching (3334 cm^{-1}), C-O stretching (2359 cm^{-1}), C=C=N stretching (2063 cm^{-1}), C=C stretching (1630 cm^{-1}), C-H bending (1300 cm^{-1}), and M-O-M stretching vibrations (671 cm^{-1} and 634 cm^{-1}) **(Figure 4)**. Jalsovszky *et al*., (1995) also indicated that the amide I band (N-H bond) is an important marker for detecting alterations in the secondary structure of proteins.FTIR analysis further reveals robust binding of amine groups to the biogenic ZnO nanoparticles (NPs). Additionally, the presence of various functional groups—such as carbonyl, carbon-nitrogen, alkene, carbon-hydrogen, and metal-oxygen bonds—contributes to both the formation and stabilization of the ZnO NPs.

The X-ray diffraction (XRD) analysis of biogenic ZnO nanoparticles (NPs) synthesized by *P. citrinum* demonstrates a crystalline hexagonal structure, with an average particle size estimated to be between 19 nm and 22 nm. Ramesh *et al*., (2021) also reported similar dimensions for their biocompatible zinc oxide nanoparticlescalculated by Scherrer equation.The crystalline structure was further revealed eight distinct peaks at 31.9°, 34.5°, 36.4°, 47.7°, 56.8°, 63.1°, 66.7°, and 68.1° 2θ **(Figure 5)**. The corresponding Miller indices for these peaks were identified as (100), (002), (101), (102), (110), (103), (200), (112), and (201), thereby confirming the crystalline structure of the ZnO nanoparticles (Moharram *et al*., 2014; Fakhari *et al*., 2019).

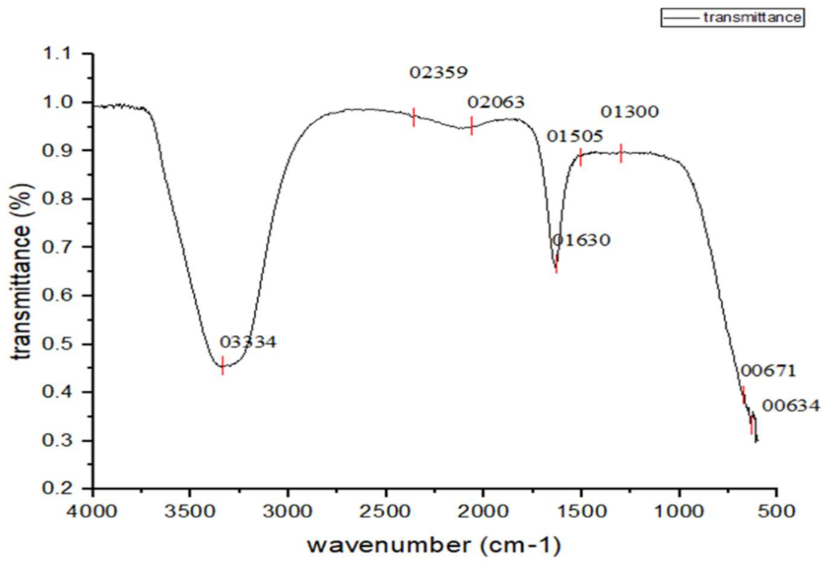

Figure 4: FTIR spectrum of ZnO nanoparticle labelled with different peak

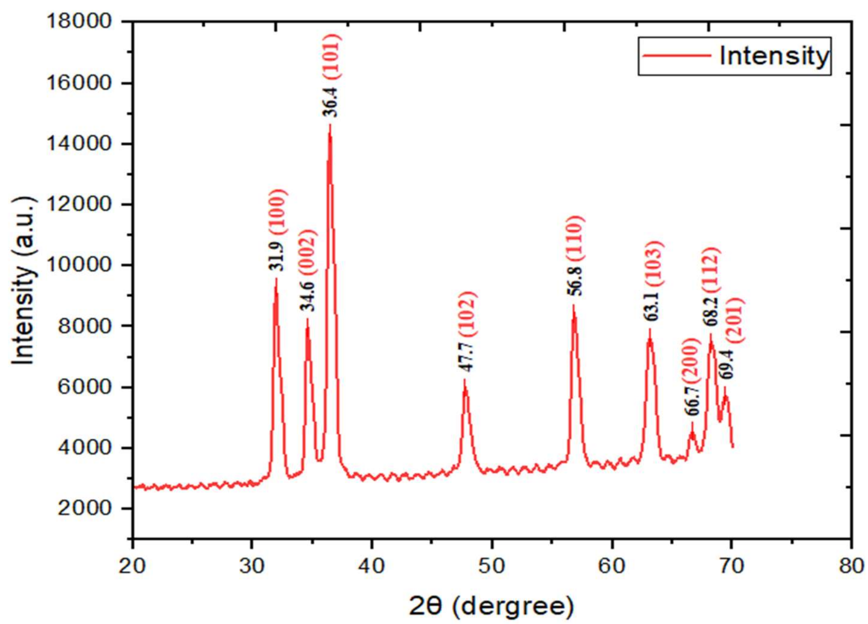

Figure 5: XRD pattern of Zinc oxide nanoparticle

SEM analysis of ZnO NPs

The surface morphology and structure of the ZnO nanoparticles were analyzed using Scanning Electron Microscopy (SEM). The SEM images reveal that the ZnO nanoparticles predominantly exhibit spherical to hexagonal shapes and tend to form aggregates into clusters **(Figure 6)**. These findings align with earlier research by Saravanan *et al.* (2018), Soto-Robles *et al.* (2019), and Ramesh *et al.* (2021), who also observed spherical to hexagonal ZnO nanoparticles clustering together.

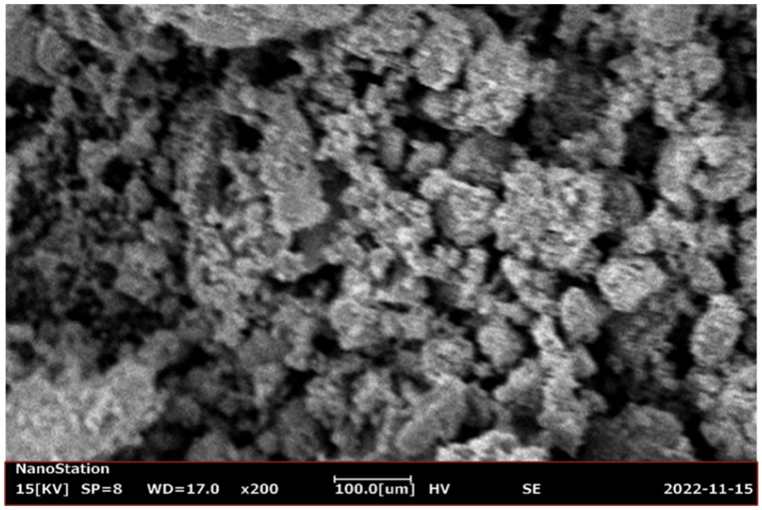

Figure 6: Scanning electron microscopic image of Zinc oxide nanoparticle.

Conclusion

This study evaluates the effectiveness of biogenically produced ZnO nanoparticles in controlling early-stage larvae of *Spodoptera litura* and explores their potential application in pest management strategies for field trials.

The biocontrol potential of biogenic ZnO nanoparticles derived from indigenous *P. citrinum* (P17) against *S. litura* demonstrated effectiveness in bioassays with both fungal spores and biosynthesized ZnO nanoparticles. Characterization through FTIR, XRD and SEM,confirmed the successful synthesis of ZnO nanoparticles induced through *P. citrinum*. The study offers new insights into the potential of using zinc nanoparticles produced by the indigenous entomopathogenic fungus *P. citrinum*and is the first to demonstrate their bio efficacy against *S. litura*.

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