Plant Archives Vol. 25, No. 1, 2025 pp. 3011-3016 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.1.432

NANO-DEFENCE: A GREEN APPROACH TO COMBAT *SPODOPTERA* SPP.

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(Date of Receiving-18-02-2025; Date of Acceptance-22-04-2025)

ABSTRACT

Pests like armyworm and cutworm, which are very polyphagous, are found all over India. They are known to seriously reduce the yield of a number of farmed crops. Because chemical insecticides have been used so widely, environmental degradation and pesticide resistance are serious problems. Thus, the idea of utilizing the green nano formulations that may help preventing the highly toxic pesticides. This study investigated the synthesis and testing the efficacy of nano formulation of Zinc oxide for the management of *Spodoptera* spp. under laboratory conditions during 2023-24. Laboratory bioassays were performed to assess the effectiveness of zinc oxide nanoparticles against the second instar larvae of *Spodoptera frugiperda* and *Spodoptera litura* using the standard leaf dip method. Zinc oxide nanoparticles were tested at various concentrations, the treatment with 2000 ppm concentration resulted in significantly higher mortality against second instar larvae of *Spodoptera litura* and *Spodoptera frugiperda.* The lethal concentrations response of second instar larvae of *Spodoptera litura* and *Spodoptera frugiperda* was 447.82 and 682.41 ppm after three days of treatment.

Key words : Entomology, Nanotechnology, Green synthesis, zinc oxide nanoparticles, *S. litura, S. frugiperda,* Nano pesticide.

Introduction

Zinc oxide nanoparticles, or ZnO NPs, have become a viable substitute for pesticides because of their special physicochemical characteristics resulting from their nanoscale size. By upsetting the insect cuticle, producing reactive oxygen species (ROS) that cause oxidative stress, interfering with digestive enzymes, and possibly having neurotoxic consequences, these particles exhibit insecticidal action. Zinc oxide nanoparticles (ZnO NPs) have proven effective against a variety of agricultural pests and disease vectors. Compared to conventional pesticides, ZnO NPs have a lower environmental impact, increased insecticidal activity, and a lower risk of resistance development because of their diverse modes of action. To completely evaluate the possible hazards of ZnO NPs to non-target organisms, however, and to improve formulation and delivery techniques for successful pest management measures, more research is required. Furthermore, in order to guarantee the practical application of ZnO NP-based insecticides in agricultural environments, their economic feasibility must be shown.

The economic impact of *Spodoptera* spp., particularly the fall armyworm and cutworm, is substantial, resulting in considerable financial losses for farmers and threatening food security, especially in regions where these pests are prevalent. Their ability to rapidly reproduce and disperse, coupled with their broad host range, makes them a challenging pest to manage. The fall armyworm, for example, is a highly invasive species that has spread rapidly across continents, causing widespread damage to crops. Due to their significant impact on agriculture and food security, effective management strategies for *Spodoptera* spp. are crucial for sustainable crop production and mitigating the economic losses caused by these pests.

This research aims to synthesize zinc oxide nanoparticles (ZnO NPs) using a green method and evaluate their potential as insecticides against the agricultural pest *Spodoptera* spp. The study focused on developing a cost-effective and environmentally friendly synthesis method using plant extracts or other bio-based materials. The synthesized ZnO NPs was characterized to determine their size, morphology, and crystallinity. Bioassays conducted to evaluate the insecticidal activity of ZnO NPs against *Spodoptera* spp. assessing their impact on larval mortality, growth, and development.

Materials and Methods

Study area

The present investigations on synthesis and effect of zinc oxide nanoparticles against *Spodoptera* spp. (Lepidoptera: Noctuidae) were carried out during 2023-24. Synthesis, characterization and stability studies of the prepared nano formulations were carried out at the Centre for Nanotechnology, University of Agricultural Sciences, Raichur. The efficacy studies on *Spodoptera* spp. under laboratory conditions were carried out at the Department of Entomology, College of Agriculture, University of Agricultural Sciences, Raichur.

Preparation of Leaf extract

The spinach leaves (*Spinacia oleracea*) were collected from University of Agricultural Sciences, Raichur campus, for the synthesis of zinc oxide nanoparticles. Further washed with distilled water to remove contamination and dried the spinach leaves in solar tunnel dryer for about 3 hours later which were powdered using pulvilizer. Further Spinach leaves powder (5 g) was added to 100 ml ethanol (98%) and kept the solution for 24 hours. Later solution was filtered using Whatman filter paper No.1 and stored at 4ºC.

Synthesis of zinc oxide nanoparticles

The leaves extract (50 ml) was boiled at 60°C alongside zinc nitrate solution (1 mM) was added to the extract at 60°C. Boiled the solution until the colour changed from dark green to pale yellow indicating the formation of ZnO nanoparticle and further subjected for characterization studies

Particle Size of nanoparticles

Particle size analysis of the synthesized nanoparticle was conducted using a zetasizer (Malvern, ZETA Sizer, nano 383 issue 5.0, England). Prior to measurement, all samples were diluted to 10 per cent concentration with deionized water to prevent multiple scattering effects. The average particle diameter (nm) was determined by averaging three measurements, and the polydispersity index (PDI) was calculated to assess the uniformity of particle size within the nanoemulsion (Das *et al.,* 2014).

Absorbance analysis of nanoparticles

UV-Visible spectrophotometry is a technique used to measure the extinction (light scattering and absorption) as light passes through a sample. Nanoparticles possess unique optical properties that are influenced by their size, shape, concentration, agglomeration state, and the refractive index near their surface.

Crystallinity characterization of the nanoparticles

X-ray diffraction (XRD) is a quick analytical method primarily used to identify the crystalline phases present in synthesized nanoparticles. The XRD instrument (Rigaku corporation, Ultima IV, Tokyo, Japan) is employed to measure various characteristics of biosynthesized nanoparticles.

Surface morphology of the nanoparticles

Scanning electron microscopy (SEM) is a technique used to visualize the surface morphology of a sample by scanning it with a high-energy electron beam in a vacuum chamber. When the electron beam interacts with the sample's atoms, secondary and backscattered electrons are emitted, providing information about the surface's structure.

Chemical structure and molecular interactions of nanoparticles

Raman spectroscopy is a non-destructive analytical technique used to provide detailed information about the chemical structure, phase, and molecular interactions within a nanomaterial. It is based on the inelastic scattering of light, known as Raman scattering, which occurs when a molecule interacts with a photon from a high-intensity laser source.

Bioassays of Zinc oxide nanoparticles against *Spodoptera* spp.

The effectiveness of zinc oxide nanoparticles against *Spodoptera litura* and *Spodoptera frugiperda* is often assessed using the leaf dip bioassay method. For determining the LC_{50}, the doses that showed promise in early research and caused fatality rates ranging from 20 to 80 percent were chosen. The synthesized Zinc oxide nanoparticles at five different concentrations was evaluated in these bioassays, and its effects were compared with a commercial formulation. That is, the mortality rate at these concentrations was compared to the amount at which the natural pesticide spinosad, at a recommended dosage of 0.12 ml/L, achieved. The purpose of this comparative analysis was to evaluate the zinc oxide nanoparticle formulation's effectiveness in comparison to the recognized chemical pesticide.

Data analysis

The per cent mortality was calculated as per Abbott's formula (Abbott, 1925) and subjected to probit analysis using the statistical program SPSS to obtain toxicity parameters like LC_{50} and LD_{50}.

The formula to calculate per cent mortality is as follows and the mortality rate was be recorded 1, 3 and 5 days after treatment.

$$\text{Per cent larval mortality} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

Statistical analysis

The collected data were statistically analysed using ANOVA with a completely randomized design. Following this, Duncan's Multiple Range Test (DMRT) was applied to the mean values of each treatment group. The toxicity parameter, specifically the median lethal concentration (LC_{50}), was determined using SPSS v.16 software.

Results

Particle size of nanoparticles

The average particle diameter of the zinc oxide nanoparticles was found to be 23.20 nm, as shown in Fig. 1, using a zetasizer to analyze their intensity distribution. This characterization paved the way for further analysis and applications of the nanoparticles.

Absorbance analysis of nanoparticles

Zinc oxide nanoparticles were analyzed using UV-Visible spectrophotometry. The transformation of zinc ions into zinc oxide nanoparticles was evident through a colour shift from dark green to pale yellow. This colour change is linked to the surface plasmon resonance (SPR)

			Size (d.n...	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	23.20	Peak 1:	29.98	97.9	13.70
PdI:	0.266	Peak 2:	4203	2.1	1002
Intercept:	0.896	Peak 3:	0.000	0.0	0.000
Result quality	Good				

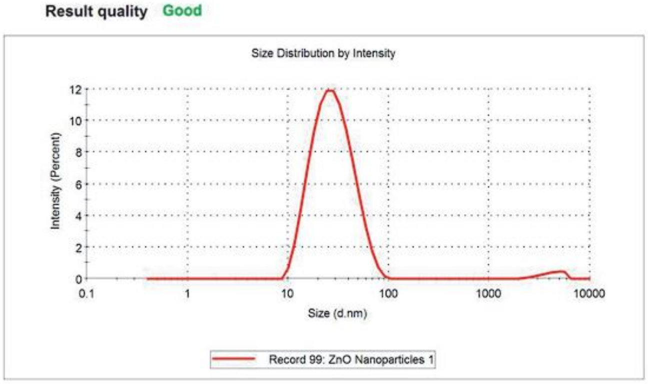

Fig. 1 : Average particle size and polydispersity index of synthesized green zinc oxide nanoparticles.

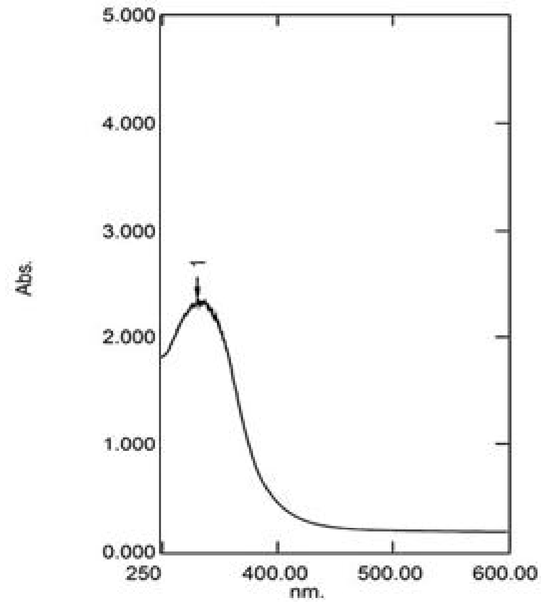

Fig. 2 : UV- Visible spectrum analysis of green zinc oxide nanoparticles.

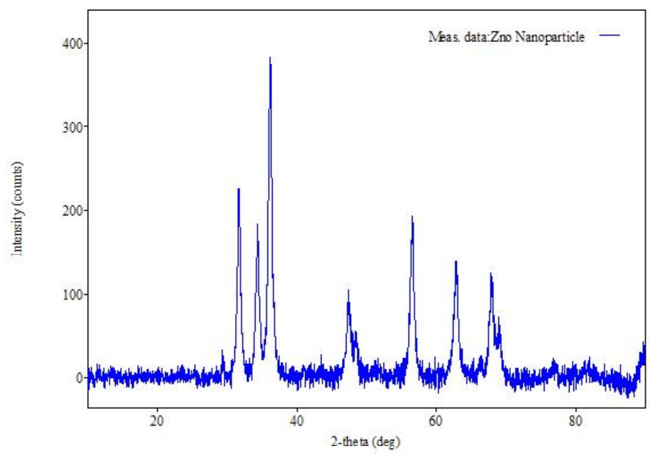

Fig. 3 : X- ray diffraction of green zinc oxide nanoparticles.

phenomenon, where free electrons in metal nanoparticles like zinc oxide collectively oscillate in response to light waves, leading to the SPR absorption band. The zinc oxide nanoparticles showed a clear absorption peak at approximately 337 nm (Fig. 2).

Crystallinity characterization of the nanoparticles

X-ray diffraction (XRD) analysis of the biosynthesized zinc oxide nanoparticles showed a (Fig. 3) broad peak between 31 to 67 degrees, indicating a lack of long-range order in the nanoparticles. However, distinct peaks were observed at specific angles (31.67°, 34.42°, 36.25°, 47.38°, 56.60°, 62.94° and 67.86°), confirming a crystalline structure consistent with the wurtzite form of ZnO.

Surface morphology of the nanoparticles

Scanning electron microscopy (SEM) analysis revealed well-formed, spherical zinc oxide nanoparticles

Table 1 : Toxicity of green zinc oxide nanoparticles against larvae of *Spodoptera litura*.

	DAT	LC_{50} values (ppm)	Slope function (± SD)	Chi-square	95% confidence limit	
					Lower	Upper
Green zinc oxide nanoformulation	1	1575.16	0.60± 1.94	0.20	807.38	4583.28
	3	447.82	1.16± 3.07	0.30	180.37	682.79
	5	117.66	1.58± 3.27	5.54	14.77	225.79
Metal zinc oxide nanoparticle	1	1143.60	0.59± 1.83	0.19	-	-
	3	473.51	1.24± 3.21	0.20	145.88	571.64
	5	138.03	2.22± 4.77	7.41	-	-

Table 2 : Toxicity of green zinc oxide nanoparticles against larvae of *Spodoptera frugiperda*.

	DAT	LC_{50} values (ppm)	Slope function (± SD)	Chi-square	95% confidence limit	
					Lower	Upper
Green zinc oxide nanoparticle	1	2015.10	0.71± 2.36	0.41	1022.97	2975.10
	3	682.41	1.07± 3.03	0.52	349.35	1096.90
	5	94.90	1.21± 2.39	1.03	2.54	221.01
Metal zinc oxide nanoparticle	1	1292.59	0.80± 2.49	0.21	707.04	953.83
	3	555.09	1.14± 3.14	1.14	264.33	842.03
	5	128.30	1.90± 4.01	3.08	23.87	225.25

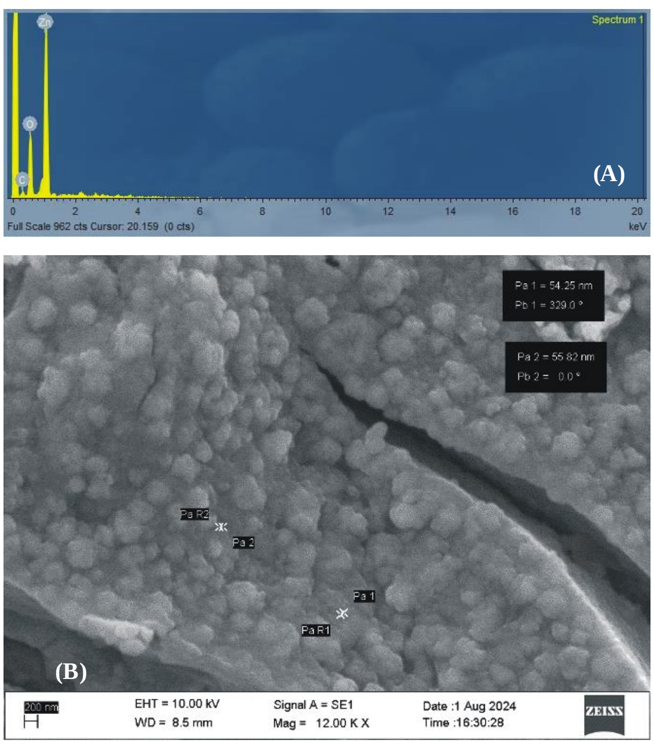

Fig. 4 : Elemental analysis of zinc oxide nanoparticles revealed by (A) SEM-EDS and (B) SEM.

(Fig. 4). The absence of any impurity peaks in the analysis confirms the high purity of the sample. Understanding the morphology of these biosynthesized nanoparticles is crucial for predicting their properties and potential applications. The prominent peaks for zinc (Zn) and oxygen (O) are clearly visible, indicating the primary elemental composition of the sample. The presence of a carbon (C) peak is likely due to surface contamination or the use of a carbon-based support during the analysis.

Chemical structure and molecular interactions of nanoparticles

Raman spectroscopy, a powerful analytical technique that identifies and characterizes materials by their unique molecular vibrations, was employed to analyze a sample. This method involves shining a laser on a sample and carefully analyzing the scattered light, which reveals a spectrum that acts like a distinct molecular fingerprint. The Raman spectrum confirms the presence of zinc oxide nanoparticles with a wurtzite hexagonal crystal structure, evidenced by the characteristic peaks at 1049 cm^{-1} (E2 high phonon mode) and 141 cm^{-1} (E2 low phonon mode) (Fig. 5).

Bioassays of Zinc oxide nanoparticles against *Spodoptera* spp.

Zinc oxide nanoparticles tested at various concentrations, the treatment with 2000 ppm concentration resulted in significantly higher mortality

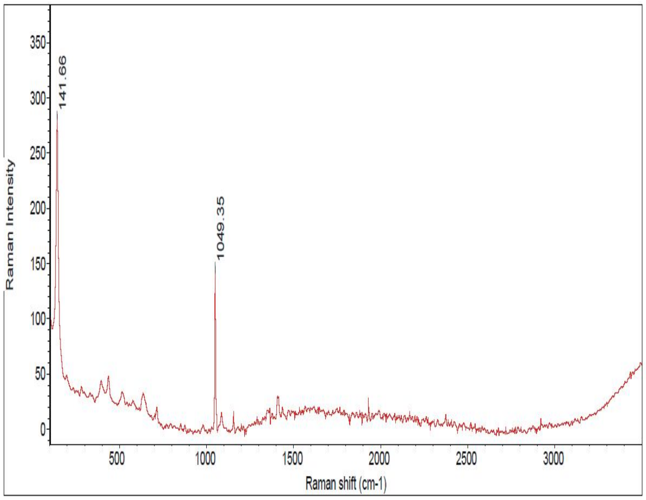

Fig. 5 : Raman spectral analysis of green zinc oxide nanoparticles.

against second instar larvae of *Spodoptera litura* (Table 1) and *Spodoptera frugiperda* (Table 2). The lethal concentrations response of second instar larvae of *Spodoptera litura* and *Spodoptera frugiperda* was 447.82 and 682.41 ppm after three days of treatment.

Discussion

Zinc oxide nanoparticles were synthesized using spinach leaf extract and zinc nitrate hexahydrate solution as a precursor. The nanoparticle formation reaction began after the addition of 1mM zinc nitrate hexahydrate solution, indicated by a colour change from dark green to pale yellow. The size of the biosynthesized zinc oxide nanoparticles was further reduced through centrifugation (5000 rpm for 15 min.) and ultrasonication (10 min at 25°C).

This colour change is linked to the surface plasmon resonance (SPR) phenomenon, where free electrons in metal nanoparticles like zinc oxide collectively oscillate in response to light waves, leading to the SPR absorption band. The zinc oxide nanoparticles showed a clear absorption peak at approximately 337 nm which aligns with Singh *et al*. (2011), who reported a peak at 368 nm.

However, distinct peaks were observed at specific angles (31.67°, 34.42°, 36.25°, 47.38°, 56.60°, 62.94° and 67.86°), confirming a crystalline structure consistent with the wurtzite form of ZnO. This crystallinity can impact properties like surface area and reactivity, which are important for various applications (Goutam *et al*., 2017).

The prominent peaks for zinc (Zn) and oxygen (O) are clearly visible, indicating the primary elemental composition of the sample. The presence of a carbon (C) peak is likely due to surface contamination or the use of a carbon-based support during the analysis. The relative heights of the Zn and O peaks suggest a stoichiometry close to 1:1, which is expected for zinc oxide (ZnO). Overall, the EDS spectrum provides strong evidence for the successful synthesis of zinc oxide nanoparticles. These observations align with the findings of Goutam *et al*. (2017), who also reported similar characteristics in their study.

The observed spectral peak can be attributed to the presence of zinc nitrate hexahydrate. This compound plays a crucial role in the synthesis process of zinc oxide nanoparticles, serving as a precursor material. During the synthesis, zinc nitrate hexahydrate undergoes chemical transformations, ultimately leading to the formation of zinc oxide nanoparticles. The spectral peak, therefore, acts as a fingerprint, confirming the involvement of zinc nitrate hexahydrate in the nanoparticle synthesis (Satyaprakash *et al*., 2008).

In stark contrast, the LC_{50} of the standard metal zinc oxide nanoparticle formulation remained notably higher at 138.03 ppm, with a Chi-square value for heterogeneity of 7.41 for *S. litura* and the LC_{50} of the standard metal zinc oxide nanoparticles remained significantly higher at 128.30 ppm, with a Chi-square value for heterogeneity of 3.08 (Guan and Feng, 2008; Rouhani *et al*., 2011; Samih *et al*., 2011). This observation reinforces the superior and sustained effectiveness of the green zinc oxide nanoparticle formulation, showcasing its enhanced toxicity and potential as a more effective alternative to conventional metal-based pesticides for controlling *Spodoptera* larvae.

The consistent decrease in LC_{50} over time suggests that green zinc oxide nanoparticles may exert a delayed but increasingly potent insecticidal effect (Osman *et al*., 2015). This unique characteristic may be attributed to the nanoparticles gradual accumulation or activation within the target organism, leading to enhanced disruption of physiological processes and ultimately higher mortality rates.

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