

Ovicidal activity of green synthesized ZnO nanoparticle on *Spodoptera litura*

J Varshini premakumari^{1*}, Dr. M Job Gopinath¹, V Duraipandiyar², M Muthupandi³, M Kabilan²

¹ Department of Zoology, Voorhees College, Vellore, Affiliated by Thiruvalluvar University, Vellore, Tamil Nadu, India

² Department of Microbiology, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India

³ Department of Insect Pest Management, Entomology Research Institute, Loyola College, Chennai, Tamil Nadu, India

Abstract

Biologically produced nanoparticles are widely used in various fields, including agriculture and health. Nanotechnology research indicates that green chemistry approaches might provide technologically significant nanomaterials. The advantages of ZnO Nanoparticles are their low cost, safety, and ease of preparation. The current investigation aimed to examine the synthesis and analysis of ZnO NPs derived from leaf extract of *Azadirachta indica*, *Calotropis gigantea*, and *Ricinus communis*. XRD and FESEM were used to characterize the ZnO NPs. The green synthesis approach, which uses fewer chemicals to make nanoparticles than standard procedure, has been proven environmentally beneficial. The ovicidal efficacy of green-produced zinc oxide nanoparticles against *Spodoptera litura* is investigated in this work. The results of this study indicate that ZnO NPs are an effective tool for integrated pest management and have the potential to be used as an alternative to the more lethal pesticides. The ovicidal activity of ZnO NPs is significantly impacted by lowering the hatchability of eggs. It is concluded that ZnO NPs can be biologically synthesized very quickly, easily, affordably, environmentally, and without any side effects.

Keywords: Green synthesis, *Azadirachta indica*, *Calotropis gigantea*, *Ricinus communis*, ZnO NPs, ovicidal activity, *Spodoptera litura*

Introduction

Insect pests cause a massive 14 percent crop loss in World Wide, while plant infections are predicted to cause a loss of up to 13 percent with an annual cost of US \$2,000 billion. For the safe application of insecticides, herbicides, and fertilizers at lower concentrations, nanomaterials are effectively employed. Due to the negative effects that pesticides have on both humans and pollinating insects, nanoparticles are crucial in reducing their toxicity, which in turn helps to increase the effectiveness of pesticides (Gowda, 2013) [3]. To protect plants from pests, fungi, and weeds, pesticides are utilized in agricultural production around the world. These pesticide residues are consequently spread in drinking water, groundwater, and soils (T. Taghizade firozjaee, 2018) [5]. The application of nanotechnology in various areas of agriculture and the food industry through proper monitoring systems, pest and disease detection, easy system of gene and chemical delivery in the crops, nano-pesticides, and encapsulation (S. Routray, 2016) [4]. ZnO nanoparticles are their safety, affordability, and ease of preparation. ZnO has been designated as a GRAS (Generally regarded as safe) metal oxide by the US FDA (Pulit-Prociak *et al.*, 2016). The green synthesis process is more effective, easy, and affordable and it is simple to scale it up to function on a bigger scale. Green synthesis has made it simple to create a variety of nanoparticles, including zinc oxide, silver, gold, palladium, and other metal oxides. The phytochemicals found in plant extracts, such as polyphenols, terpenoids, and polyols are the ones in charge of the bio-reduction of metallic ions. *Spodoptera litura*, the tobacco cutworm, is a defoliating, chewing pest that spreads around the world and feeds on over one hundred host plants. All year around. *Spodoptera litura* causes significant economic loss by damaging wide-leaf plants including legumes, brassicas, and other economically significant crops. (Radhakrishnan & Shanmugam, 2017) [1]. It is a frequent and economically

significant polyphagous pest that attacks a variety of horticultural and field crops. It is predicted to reduce output in over 180 crops globally by 26 to 100% when grown in the field. There was a critical need for new pest management agents with higher activity and less negative effects on the emergence of resistance to conventional synthetic pesticides and growing public concern over the pollution of the environment and health risks posed by synthetic pesticides (Kombiah & K, 2012) [3]. Green synthesized nanoparticles have shown themselves to be easy to utilize, effective, and conducive to growth in the food and agriculture industries. Green-produced zinc oxide nanoparticles' ovicidal efficacy against *Spodoptera litura* is investigated in this work. The present study synthesizes ZnO NPs, a simple and low-toxicity method for controlling agricultural pests. This work describes a simple, economical, and environmentally friendly process for producing zinc oxide NPs from the plant extract of *Azadirachta* leaves. The green synthesis approach, which uses fewer chemicals to make nanoparticles than standard procedure has been proven to be environmentally beneficial. The average size of the nano zinc oxide nanoparticle was determined to be 40-79 nm. ZnO NPs size and structure are confirmed by FESEM examination and XRD analysis was used to analyze the average size of crystallite size of NPs. ZnO NPs showed a promising potential to be employed as an alternative to the more deadly pesticides. The results of this study point to the potential use of ZnO NPs to manage ovicidal activity that is greatly affected by reducing the hatchability of eggs. It has been demonstrated to be an effective tool for integrated pest management.

Materials and Methods

Selected plant parts for Synthesis of Nanoparticle

Green synthesis of Zinc oxide nanoparticle using the leaf of *Calotropis gigantea*. Leaf of *Ricinus communis*. Leaf of *Azadirachta indica*. These plants' part was collected and

cleansed with Distilled water. After that, it was motorized and pestled into little pieces. Separately, known quantities of crushed plant materials were added to the distilled water and then heated. The aqueous extract was then obtained via filtering. (Vidhya *et al.*, 2013) [6].

Preparation of Zinc oxide nanoparticle

Using a stirrer-heater, 50 milliliters of extract from plant leaves separately were heated to between 60 and 80 degrees Celsius to create the nanoparticles. When the temperature hit sixty degrees Celsius, five grams of zinc nitrate hexahydrate were added to the mixture. After that, this combination was maintained until the yellow paste was obtained. After that, this paste was taken in a watch glass and kept in a hot air oven at 100 °C for 1 hour for a drying process to be kept in a ceramic crucible and maintained for two hours at 400 degrees Celsius in an air-heated furnace. A light-colored powder was obtained carefully gathered and preserved for characterization purposes. Vidhya *et al.*, (2013) [6] with slight modifications.

Insect culture

From a groundnut field, egg masses of *S. litura* were gathered. The eggs were surface-applied with a 0.02% sodium hypochlorite solution, dried, and allowed to hatch. Following hatching, the neonates were raised on *Ricinus communis* (*Euphorbiaceae*) leaves until they reached the prepupal stage. For pupation, sterilized soil was supplied at room temperature (27±2°C), with a 14:10 light/dark photoperiod and 75±5% relative humidity in the insectary. The pupae were removed from the earth after pupation and put into the oviposition chamber. After the adult emerged, adult nutrition was given using cotton soaked in a 10% (w/v) sugar solution with a few drops of multivitamins. Within the adult emergence cage was a potted groundnut plant intended for egg-laying. Following hatching, the larvae were given Baskar *et al.*, (2015) [7]. The newly molted third instar larvae were obtained from the stock culture of *Spodoptera litura* larvae, which was maintained in a laboratory setting on artificial food and castor leaves, respectively.

Ovicidal activity

By spraying freshly laid *Spodoptera litura* eggs with a nanoparticle solution, the examined chemicals' ovicidal activity was studied. (Plate – 17) Scales from the *S. litura* egg mass were carefully removed and dosages of 125, 250, and 500 ppm of the test ZnO NPs were sprayed over the eggs. (Plate – 18) Azadirachtin 95 % was used as a positive control mixed with DMSO and bulk zinc nitrate hexahydrate was used as a negative control. After being sprayed, the eggs were transferred to petri dishes lined with wet filter paper and incubated at room temperature. Furthermore, untreated eggs were separated. Three replicas with 10 eggs each were retained for each treatment. The ovicidal activity was calculated using the formula below. (Baskar *et al.*, 2012) [8].

$$\text{Ovicidal activity} = C - T / C \times 100$$

Where C is the percentage of eggs hatched in control and T is the percentage of eggs hatched in treatment.

Results and Discussion:

Characterization of Green synthesized Zinc oxide nanoparticle

The characterization of Green synthesized Zinc oxide nanoparticles was performed using different instruments. The structure of the produced nanoparticles was examined using FESEM.

FESEM Analysis

Figure - 1 shows the FESEM analysis of ZnO nanoparticles as well as several aggregates. *Calotropis gigantea* leaf (Fig.1a) showed the ZnO NPs size of 95nm, 89 nm, 102 nm, and 120 nm in size. *Ricinus communis* leaf (Fig.1b) showed the ZnO NPs size of 55 nm, 70 nm, and 106 nm. *Azadirachta indica* leaf (Fig.1c) showed the ZnO NPs size of 40.04 nm, 40.24 nm, 69.22 nm, 70.42 nm, and 79.04 nm. FESEM images show that Nanoparticles are spherically shaped in the leaf and latex, these results are coincided with the study of Vidhya *et al* (2013) [6].

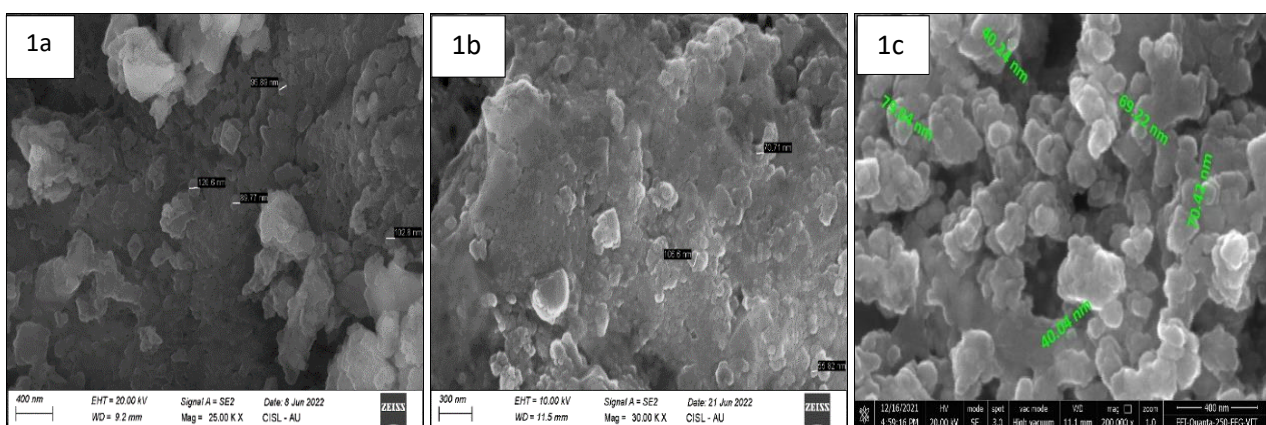


Fig 1

- 1a) FESEM analysis of *C. gigantea* synthesized ZnO NPs
 1b) FESEM analysis of *R. communis* synthesized ZnO NPs
 1c) FESEM analysis of *A. indica* synthesized ZnO NPs

X-Ray Diffraction

(Figure – 2) shows Peaks on the X-ray diffraction graph were exclusively caused by the X-ray diffractometer, which

was used to confirm the existence of ZnO. The ZnO NPs synthesized by the *Calotropis gigantea* (Fig.2a) showed strong diffraction peaks at 31.8, 34.4, 36.3, 47.6, 56.6, 62.9, 66.4, 68.0, 69.1, 72.6, 77.0 and 81.5 degrees of 2 θ. The green synthesized ZnO NPs diffraction peaks corresponded to (100), (002), (101), (102), (110), (103), (200), (201), (004), (202), (104) crystal planes. Using the Scherrer

equation, the average crystallite sizes of ZnO NPs were calculated at 19nm from the width of the dominating peaks. (Fig.2b) ZnO NPs synthesized by *Ricinus communis* had strong diffraction peaks at 31.9, 34.6, 36.4, 47.7, 56.7, 63.0, 66.5, 68.1, 69.2, 72.7, 77.1, 79.2, 81.5 and 89.7 degrees of 2θ . The synthesized ZnO NPs diffraction peaks corresponded to (100), (002), (101), (102), (110), (200), (201), (004), (104), and (203) crystal planes. Using the Scherrer equation, the average crystallite sizes of ZnO NPs were calculated it showed 27nm from the width of the dominating peaks. (Fig.2c) *Azadirachta indica* produced ZnO NPs with significant diffraction peaks at 31.8, 34.4, 36.3, 41.1, 47.6, 56.6, 62.9, 66.4, 68.0, 69.1, 72.6, 77.0, 81.4 and 81.7 degrees of 2θ . The green synthesized ZnO NPs diffraction peaks corresponded to (100), (002), (101), (102),

(110), (103), (200), (112), (201), (004), (202), (104), (104). The average crystallite sizes of ZnO NPs were determined using the Scherrer equation, and the results indicated that they were 27nm from the width of the dominating peaks. The similar strong diffraction peaks in the pattern at $2\theta = 31.67, 34.43, 36.27, 47.53, 56.49, 62.93, 66.37, 67.97, 69.12, 72.81, \text{ and } 75.95$ were indexed for the planes of ZnO hexagonal (100), (022), (101), (102), (110), (103), (200), (112), (202), (004) and (202) respectively were found in the synthesis of ZnO NPs using *Calotropis procera* reported by Gawade *et al.*, (2017). Similarly, the main peak corresponds to 2θ values of 31.74, 34.41, and 36.23. the graph peaks and the literature report (JCPDS file NO. 5.0556) agree very clearly with Vidhya *et al.*, (2013) [6].

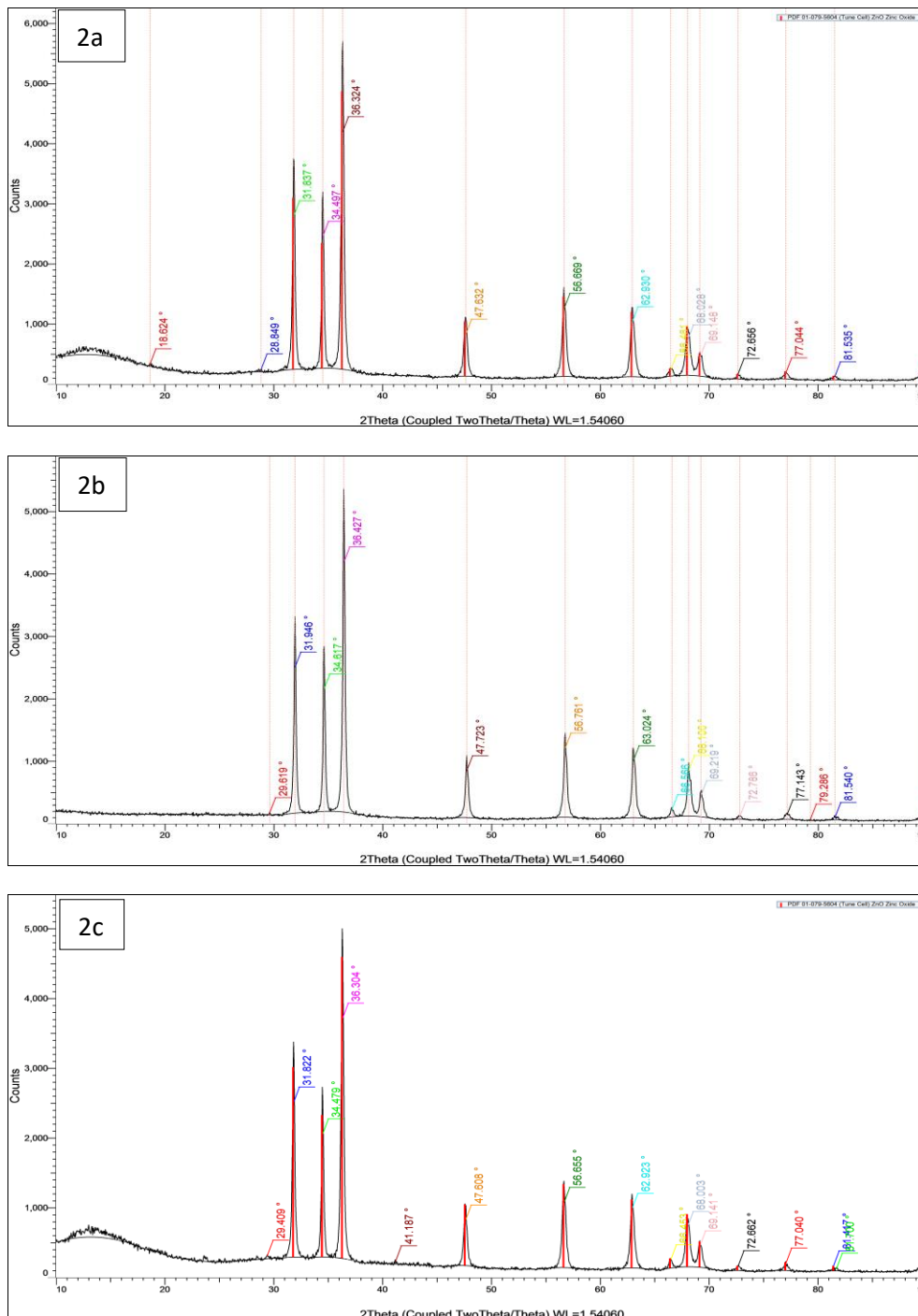


Fig 2

- a) XRD Spectra of synthesized ZnO NPs using *C. gigantea* (leaf)
- b) XRD Spectra of synthesized ZnO NPs using *R. communis* (leaf)
- c) XRD Spectra of synthesized ZnO NPs using *A. indica* (leaf)

Ovicidal activity of *Spodoptera litura*

Ovicidal activity of zinc oxide nanoparticle synthesized by the *Calotropis gigantea*, *Ricinus communis*, *Azadirachta indica* leaf extract. All the synthesized ZnO NPs showed ovicidal activity at all the concentrations while the maximum activity of 86 % was noticed at 500 ppm of all 3 samples of ZnO NPs on the 6th day of treatment and 96 % mortality was shown in *Calotropis gigantea* synthesized ZnO NPs and 93 % of mortality was recorded in *Ricinus communis*, *Azadirachta indica* synthesized ZnO NPs in 7th day of treatment. The percentage of mortality was calculated

by Abbot’s formula. LC 50 and LC 90 were calculated. This was statistically significant when comparing these synthesized ZnO NPs with the Control samples. However, there is no statistical difference when comparing the samples.

In Table 1 Treatment R1 shows the lowest LC50 (2.255) value, indicating higher toxicity compared to other treatments. However, it has a relatively high LC90 (299.677), suggesting that a higher concentration is needed to achieve 90% mortality. Treatment AZ has the highest LC50 (123.167) and LC 90 (466.827) values, indicating lower toxicity compared to the other treatments. The slopes and intercepts vary across treatments, indicating differences. Treatment C2 has the lowest χ^2 value (0.019), indicating a better fit compared to the other treatments. Treatment Az appears to be the most toxic to *Spodoptera* eggs based on the LC 50 value, while Treatment R1 shows the lowest toxicity. Figure 3

Table 1: LC 50 and LC 90 of Ovicidal activity of *Spodoptera litura*

Spodoptera eggs	LC ₅₀	95% Confidence Limit		LC ₉₀	95% Confidence Limit		Slope ± SE	Intercept ± SE	χ^2
		LL	UL		LL	UL			
C2	38.226	0.000	93.944	238.194	107.857	162106.2	1.61±0.79	2.44±1.85	0.019
R1	2.255	-	-	299.677	-	-	0.60±0.72	4.78±1.71	0.288
AZ	123.167	51.651	171.546	466.827	319.875	1427.772	2.21±0.64	0.37±1.50	0.710

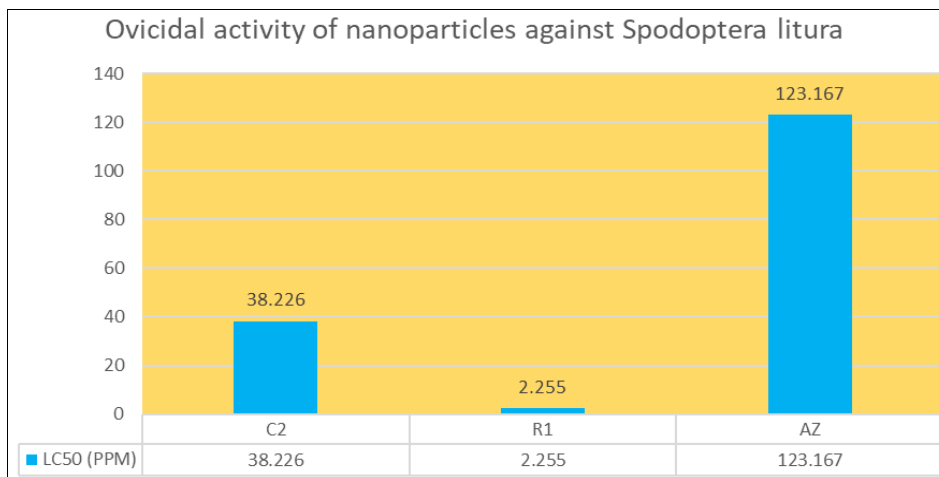


Fig 3: Ovicidal activity of *Spodoptera litura*

Table 2 shows Treatments C2 and R1 seem to have relatively higher mean ovicidal activity compared to treatment AZ. The control groups (both positive and negative) have a mean ovicidal activity of 0.0000, indicating no effect on egg mortality. The total mean ovicidal activity across all treatments is 13.5000. The obtained F value is 23.911. they achieved a significance value (p-value) is .000. The P-value is compared with 0.05. on comparing it is found that the obtained p value is less than 0.05. thus, there is a statistically significant difference between group means. In post hoc test shows in all three cases, the significant (p) values are greater than the (p<0.05) alpha value, it is evident that there is no statistically significant difference between R1, C2, and AZ.

In Table 3 the Tukey HSD value calculated for the experiment is 23.3333 for subset C2, 24.6667 for subset R1, and 18.6667 for subset AZ. These values represent the critical threshold for determining significant differences between group means. The table lists the subsets for alpha=0.05, indicating which groups have means that are

not significantly different from each other at the 0.05 significance level in all the cases. The significance level, .062, indicates the overall probability of making a Type I error (rejecting a true null hypothesis) when performing multiple pairwise comparisons.

The height of each bar or the position of each point along this axis indicates the magnitude of the mean for each group. From the graph, it is evident that C2 has a high magnitude and AZ has a low magnitude.

Baskar *et al.*, (2012) [8] published research evaluating the effectiveness of *Atalantia monophylla* (*A. monophylla*) leaf against *S. litura* eggs in various solvent crude extracts and fractions. At 5.0 % concentration, hexane crude extract demonstrated the highest ovicidal activity of 61.94 % with a correlation value of r²=0.81 and the lowest LC 50 value of 3.06 %. A significant and elevated correlation (r²=0.81%) was observed in the linear regression analysis between the concentration and ovicidal activity of hexane crude extracts and their active fraction. A similar work was carried out using different extracts of *C. excavate* which showed

significant results of larvicidal and ovicidal activities against *A. aegypti*, *An. Stephensi*, *C. quinquefasciatus* and *S. litura* by Thangarasu, Kaliyamoorthy, & Kuppusamy, (2014) [9]. The above study proved that the maximum mortality of ovicidal activity of *S. litura* was 61.94 % using *A.*

monophyla leaf extract but here in the present study of green synthesized ZnO NPs proved that 93-96 % of increased mortality above all references. It demonstrated that employing ZnO NPs for *S. litura's* ovicidal activity is more beneficial than using crude extract.

Table 2: Effect of Green Synthesized ZnO NPs on Ovicidal Activity of *Spodoptera litura* – On 6th-day Treatment Comparison with Controls

Descriptives								
Ovicidal activity (6th)								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
C2	3	23.3333	4.61880	2.66667	11.8596	34.8071	18.00	26.00
R1	3	24.6667	1.15470	.66667	21.7982	27.5351	24.00	26.00
Az	3	18.6667	7.02377	4.05518	1.2187	36.1147	12.00	26.00
Control	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Positive control	3	14.3333	4.50925	2.60342	3.1317	25.5349	10.00	19.00
Negative control	3	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	18	13.5000	10.91276	2.57216	8.0732	18.9268	.00	26.00
ANOVA								
Ovicidal activity (6th)								
		Sum of Squares	df	Mean Square	F	Sig.		
Between Groups		1839.833	5	367.967	23.911	.000		
Within Groups		184.667	12	15.389				
Total		2024.500	17					

Table 3: Effect of Green Synthesized ZnO NPs on Ovicidal Activity of *Spodoptera litura* (Comparison with Controls) - Post Hoc Tests

Multiple Comparisons						
Dependent Variable: Ovicidal activity (6th)						
Tukey HSD						
(I) Factor	(J) Factor	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C2	R1	-1.33333	3.20301	.998	-12.0920	9.4253
	Az	4.66667	3.20301	.695	-6.0920	15.4253
	Control	23.33333*	3.20301	.000	12.5747	34.0920
	Positive control	9.00000	3.20301	.123	-1.7586	19.7586
	Negative control	23.33333*	3.20301	.000	12.5747	34.0920
R1	C2	1.33333	3.20301	.998	-9.4253	12.0920
	Az	6.00000	3.20301	.460	-4.7586	16.7586
	Control	24.66667*	3.20301	.000	13.9080	35.4253
	Positive control	10.33333	3.20301	.062	-4.253	21.0920
	Negative control	24.66667*	3.20301	.000	13.9080	35.4253
Az	C2	-4.66667	3.20301	.695	-15.4253	6.0920
	R1	-6.00000	3.20301	.460	-16.7586	4.7586
	Control	18.66667*	3.20301	.001	7.9080	29.4253
	Positive control	4.33333	3.20301	.752	-6.4253	15.0920
	Negative control	18.66667*	3.20301	.001	7.9080	29.4253
Control	C2	-23.33333*	3.20301	.000	-34.0920	-12.5747
	R1	-24.66667*	3.20301	.000	-35.4253	-13.9080
	Az	-18.66667*	3.20301	.001	-29.4253	-7.9080
	Positive control	-14.33333*	3.20301	.008	-25.0920	-3.5747
	Negative control	.00000	3.20301	1.000	-10.7586	10.7586
Positive control	C2	-9.00000	3.20301	.123	-19.7586	1.7586
	R1	-10.33333	3.20301	.062	-21.0920	.4253
	Az	-4.33333	3.20301	.752	-15.0920	6.4253
	Control	14.33333*	3.20301	.008	3.5747	25.0920
	Negative control	14.33333*	3.20301	.008	3.5747	25.0920
Negative control	C2	-23.33333*	3.20301	.000	-34.0920	-12.5747
	R1	-24.66667*	3.20301	.000	-35.4253	-13.9080
	Az	-18.66667*	3.20301	.001	-29.4253	-7.9080
	Control	.00000	3.20301	1.000	-10.7586	10.7586
	Positive control	-14.33333*	3.20301	.008	-25.0920	-3.5747
*. The mean difference is significant at the 0.05 level.						
Ovicidal activity (6th)						
Tukey HSD						
Factor	N	Subset for alpha = 0.05				

		1	2
Control	3	.0000	
Negative control	3	.0000	
Positive control	3		14.3333
Az	3		18.6667
C2	3		23.3333
R1	3		24.6667
Sig.		1.000	.062
Means for groups in homogeneous subsets are displayed.			
a. Uses Harmonic Mean Sample Size = 3.000.			

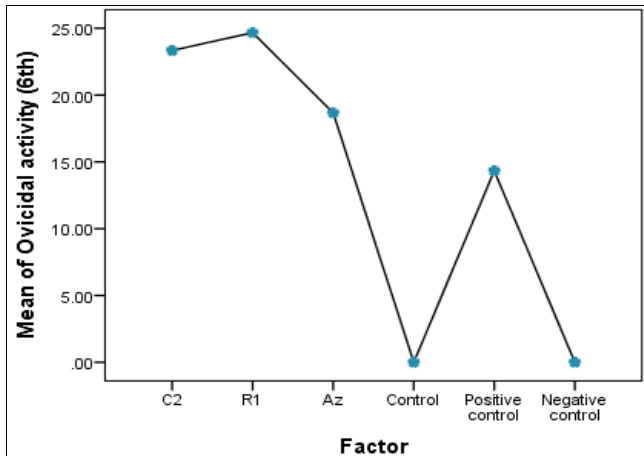


Fig 4: Way Analysis of Ovicidal activity of *Spodoptera litura* (On the 6th day of treatment) Between the samples Means Plots

Conclusion

Green synthesized Nanoparticles are efficiently used in the agriculture and food sector as proven to be simple, efficient, and friendly to development. The present study examines the ovicidal activity of green synthesized zinc oxide nanoparticles on *Spodoptera litura*. In the current study, ZnO NPs are synthesized, which is a low-toxicity and straightforward approach for managing agricultural pests. The FESEM analysis confirms the formation of ZnO NPs with spherical size. The Wurtzite structure and crystalline nature of ZnO NPs are suggested by the narrow peaks that develop with a Bragg's angle of $2\theta = 120^\circ$, as confirmed by the XRD investigation. The Abbotts formula was used to quantify the mortality in ovicidal activity and nematicidal activity. Additionally, LC 50 and LC 90 were computed. Statistical analysis using ANOVA was performed. The mortality was recorded under laboratory conditions. The findings of this study suggest that ZnO NPs may be used to suppress *S. litura* and significantly reduce the population in the environment by regulating the hatchability of their eggs. Furthermore, NPs are being utilized in agriculture more and more to improve plant health, growth, and food security as nanotechnology develops.

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