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RESEARCH PAPER

Screening the larvicidal activity of ZnO, CuO nanoparticles, and neem seed oil extract against the full armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera, Noctuidae)

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Abstract

Background: The fall armyworm, *Spodoptera frugiperda*, poses a significant threat to over 90 plant species, causing extensive damage by consuming fresh leaves, hindering growth, and ultimately reducing crop yields. Integrated pest management strategies emphasize eco-friendly approaches, minimizing harm to non-target organisms. Among these, biological control methods, including the use of plant extracts and specific microorganisms, have shown considerable efficacy in controlling the fall armyworm. **Methods:** This study assessed the impact of neem seed oil extract and CuO & ZnO nanoparticles on the biological aspects of *Spodoptera frugiperda*. Fourth-instar larvae were exposed to fresh castor leaves treated with neem seed oil extract, obtained through Soxhlet extraction from neem seeds, and nanoparticles prepared via direct precipitation. **Results:** The determined LC_{50} values of neem seed oil extract, ZnO, and CuO NPs against the 4th instar were 500, 520, and 440 ppm, respectively. All treatments had an impact on the durations of larval and pupal stages, resulting in increases of 66% and 15% with neem seed extract, 11.5% and 15% with ZnO, and 21.7% and 23% with CuO. Additionally, pupation rates were reduced by 50% following exposure to neem seed extract and ZnO, and by 45% with CuO nanoparticles. It is also worth noting that the digestive enzymatic activities in the insects were significantly affected following treatment with the LC_{50} values of neem seed extracts and nanoparticles. **Conclusion:** The study demonstrates the potent larvicidal properties of both neem seed extract and nanoparticles against *Spodoptera frugiperda*. All treatments effectively prolonged larval and pupal stages and reduced successful adult emergence at low concentrations. All treatments also showed a negative effect on the enzymatic activity of insects, which indicates their potential as natural insecticides in combating fall armyworm.

Keywords

Biological control, CuO & ZnO NPs, Neem seed extract, *Spodoptera frugiperda*

Introduction

The species identified as *Spodoptera frugiperda*, more widely recognized by its common name, the fall armyworm (FAW), is a type of moth that is a member of the Noctuidae family. The fall armyworm, *Spodoptera frugiperda*, is a significant agricultural pest with a notorious reputation for its destructive impact on a wide range of crops worldwide. This species is indigenous to environments that are characterized by tropical and subtropical climates. In these areas, the combination of temperature, humidity, and natural habitat forms a perfect setting that supports the life cycle and growth

of this particular species. (Montezano et al. 2018). Additionally, it has recently garnered attention due to its rapid spread to Africa, Asia, and Australia, posing a severe threat to food security and agricultural economies in these regions. (Timilsena et al. 2022; Rahul et al. 2023) The species are highly adaptable, thriving in diverse climatic conditions. These caterpillars are known for their voracious appetite and can cause substantial damage to crops. They have the capability to feed on over 85 different plant species, which includes economically significant crops like maize, rice, sorghum, sugarcane, and cotton (FAO 2019). The widespread and intense feeding habits of the larvae highlights an important need for effective strategies to reduce the harmful impact of these species (Kandel and Poudel 2020; Snigdha et al. 2023). FAW larvae caused extensive damage to the maize fresh leaves, resulting in poor growth and a significant reduction in crop yield by 20–50% in Africa which suggest severe impact on livelihoods (Kasoma et al. 2022; Singh et al. 2023).

The widespread use of chemical pesticides to control *Spodoptera frugiperda* has raised environmental and health concerns. Furthermore, the pest's ability to rapidly develop resistance to various pesticides necessitates the continuous development of new control methods, increasing the environmental and economic burden. Ecofriendly insecticides are recognized for their biodegradability and minimal impact on non-target organisms (Nawaz et al. 2021). This enables us to integrate these pesticides within programs aimed at managing pests effectively (Kamaraj et al. 2018; Khursheed et al. 2022; Usama et al. 2023). According to the growing demand for the utilizing safe insecticides, numerous studies have been dedicated to identifying alternative sources of these products. Critically, the active ingredients, obtained from *Azadirachta indica*, commonly known as the Indian neem tree, has become a bio insecticide used in vegetable agriculture (Khursheed et al. 2022). Neem has been extensively studied and found to exhibit insecticidal, antifungal and nematocidal properties (Pankaj et al. 2011).

Addressing this challenge necessitates a multifaceted approach that encompasses the development of resistant crop varieties, the implementation of biological control methods, and the use of environmentally safe chemical pesticides. Within this framework, the substantial benefits and potential applications of nanotechnology cannot be understated. Notably in the realm of agriculture, nanotechnology plays a critical role in pest control, paving the way for a reduced dependence on environmentally detrimental chemical pesticides. (El-bendary and El-Helaly 2013; Hasanin et al. 2022; Rahman et al. 2022; Shabir et al. 2023).

The study objective is to investigate the pesticidal properties of neem seed extract and assess the entomotoxic effects of ZnO and CuO nanoparticles on various larval stages of the fall armyworm, *Spodoptera frugiperda*, specifically focusing on the 2nd and 4th larval instars.

Materials and methods

Insect collection and rearing

The fall armyworm, *S. frugiperda*, was reared under laboratory conditions (28 ± 2 °C, RH 60 ± 5%) using fresh *Ricinus communis* (L.) leaves, following the methodology outlined by Khamis et al. 2023. *S. frugiperda* colonies, collected from various regions of the Bani-Suef governorate (29°04'00"N, 31°05'57"E), and were consistently reared in laboratory conditions for up to three generations. Larvae were provided with castor oil plant leaves in large glass jars until they reached the pupation stage, after 1 week the adults emerged (Adamczyk et al. 1999). Once the adults emerged, they were allowed to mate in glass jars, where they were provided with cotton pieces soaked in a 10% sugar solution as a food source and castor oil plant leaves for oviposition. Egg masses were then maintained in plastic jars until hatching.

Tested bio-insecticide agents

Neem seed oil extract

Mature neem fruits were collected, ground to extract the seeds, and then air-dried for five days to optimize extraction efficacy. The seeds were crushed in a blender (Kenwood Limited, Havant, UK) to obtain neem seed kernel cake, which was subsequently heated for 10 minutes at 55 °C to remove any residual moisture (Paragas and Fiegalan 2018). Soxhlet method was used for Neem seed oil extraction. The solvent used for the oil extraction was a mixture of hexane and ethanol in a 6:4 ratio. Once the neem oil was concentrated, it was transferred to an evaporating dish and stored in a desiccator until needed for use (Oyekanmi et al. 2021). The extraction process lasted approximately 12 to 14 hours.

Analysis of seed oil extract

Chromatographic analysis using GC-MS was conducted with the 7890B GC Systems paired with the 5977A Mass Selective Detector. A capillary column (HP-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 µm film) was utilized, employing helium as the carrier gas at a pressure of 7.07 psi and an injection volume of 1 µl. The analysis began with the column initially set at 60 °C for 3 minutes following injection, then the temperature was increased to 300 °C at a rate of 20 °C per minute and maintained for 5 minutes. Injection was performed at 300 °C in splitless mode. The MS scan range for the analysis was from 50 to 550 atomic mass units (AMU), with electron impact (EI) ionization at 70 eV and a solvent delay of 4.0 minutes. The NIST/EPA/NIH mass spectral library Version 2.2, along with the NIST mass spectral search program, was used to identify constituents through mass fragmentations (Morrison and Smith 1964). Methyl esters, primarily derived

from fatty acids, were synthesized by heating free fatty acids in the presence of anhydrous methanol and a catalyst, specifically boron trifluoride.

Synthesis of nanomaterial

The direct precipitation technique was adopted to synthesize ZnO and CuO NPs (Gatou at al. 2023).

ZnO nanoparticles synthesis

The direct precipitation method was used to synthesize ZnO nanoparticles. Zinc acetate dihydrate was dissolved in 100 ml of distilled water to create a 0.15 M solution. Following the established protocol, sodium hydroxide solution was added dropwise under continuous magnetic stirring for 2 hours. The resulting precipitate was subjected to three rounds of centrifugation at 9000 rpm for 10 minutes each. This was followed by two additional rounds of centrifugation with water and a final round using pure ethanol. The precipitate was then dried for an hour at 100 °C and subsequently annealed for 3 hours at 400 °C (Phiwdanga et al. 2013).

The chemical reaction for the synthesis reaction was:

$$Zn\ (CH_3COO)_2 + 2(NaOH) \rightarrow ZnO + 2\ (CH_3COONa) + H_2O$$

Synthesis of CuO (NPs)

The simple precipitation method was utilized to synthesize copper oxide nanoparticles from an aqueous medium. This process involved dissolving 100 mM of copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) in 50 mL of distilled water. The mixture was stirred for 30 minutes at room temperature using a magnetic stirrer until a uniform solution was obtained. A 100 mM NaOH solution was prepared by dissolving it in distilled water. With continuous stirring at room temperature, the NaOH solution was added dropwise to the $CuSO_4$ solution. The resulting precipitate was then washed by centrifugation for 15 minutes at 6000 rpm, first with deionized water and then with 70% ethanol. The final product was dried in a hot air oven at 60 °C for 24 hours and calcined for 4 hours at 400 °C to obtain the powdered form (Mir et al. 2020).

The chemical reaction for the synthesis reaction was:

$$CuSO_4{\cdot}5H_2O + 2(NaOH) \rightarrow Cu\ (OH)_2 + Na_2SO_4 + 5(H_2O)$$

Bioassay and sublethal calculations

Preparation of castor oil plant leaves involved immersing them in solutions of various bioinsecticide concentrations (1000, 500, 250, 125, and 62.5 ppm). All the three compounds were used in separate treatment. Control leaves remained untreated. Each treatment consisted of three replicates, with each containing ten larvae (either 2nd or 4th instar) of *S. frugiperda*. The bioassays were conducted at 25 ± 2 °C and 65 ± 5% RH, with insect mortality monitored and statistically analyzed using Ldp Line software for Probit analysis; an online software for calculating the probit analyses according to Finney (1971), which is used to represent the relation between influential and respondent in toxicological studies. The second instar larvae were treated six days post-hatching, and the fourth instar larvae received treatment 12 days after hatching. The experiment was conducted over a complete life cycle, concluding at the point of egg hatching. Castor oil leaves were immersed in each concentration for five minutes, then air-dried. The larvae were allowed to feed on these leaves for 48 hours before the leaves were replaced with untreated ones. Excess solution from the treatment process was carefully discarded.

Biological aspects investigation

The impact of the LC50 of each bioinsecticide agent on *S. frugiperda*'s biological activities, including larval and pupal durations, pupation, and adult emergence percentages, as well as pupal and moth malformations, were studied. Ten 4th instar larvae were used in bioassay tests. Fresh castor leaves treated with the extract were fed to the larvae, and experiments were replicated three times. Controls involved untreated leaves (Tulashie et al. 2021).

Determination of carbohydrate hydrolyzing enzymes activities

This study aimed to evaluate the impact of the tested materials on the digestive system's physiological functions in affected larvae. The enzymatic activity was measured following the procedure of Ishaaya and Swirski 1976 which involved quantifying glucose produced from starch and sucrose digestion using 3, 5-dinitrosalicylic acid reagent. Specific reaction mixtures were prepared for each enzyme, and the enzymatic activity was expressed as µg glucose released/min/g body weight. The activities of three carbohydrate-hydrolyzing enzymes—α-amylase, invertase, and trehalase were assessed. Three treated larvae were weighed after they reached the 6th instar, then placed in Eppendorf tubes in the freezer, then the samples were sent to the laboratory for analysis. For the enzyme activity assay, we utilized extracts obtained as follows: All analyses were conducted at the Microchemical Analysis Lab (MCAL) within the Plant Protection Research Institute (PPRI) of the Agricultural Research Center (ARC) in Giza, Egypt. Three larvae treated to reach the 6th instar for each specific enzyme were placed in Eppendorf tubes and stored in the freezer. Subsequently, these samples were dispatched to the laboratory for analysis.

Statistical analysis

The overall percentage of larval mortality was determined and corrected using the Abbott formula (Abbott 1925). Probit analysis estimated the LC50 values for the three treatments. At these LC_{50} values, various biological effects were assessed. Data were analyzed using Excel to calculate *P* value, *F*-value, and L.S.D at 0.05 degrees of freedom. Enzymatic activity data were subjected to ANOVA and Tukey's test for mean comparison ($P \leq 0.01$) using the SISVAR application (Ferreira 2011).

Result and discussion

GC-MS analysis of Neem seed oil extract

In the GC-MS analysis, as presented in Table 1 and Fig. 1, 44 compounds were identified in the neem seed oil extract. These compounds, predominantly volatile, were classified into various chemical classes, including esters, hydrocarbons, phenolics, fatty acids, amines, and sulfur compounds. The most prominent compounds identified were esters and fatty acids. Many of the active compounds

Table 1. GC-MS (Gas chromatography-mass spectrometry) results for Neem Seed Oil Extract.

Peak	Ret. Time	Percentage %	Compound
1	5.1625	0.0913	Octamethyl cyclotetrasiloxane
2	6.7303	0.0105	Decamethyl cyclopentasiloxane
3	9.1564	0.0292	4-(Isopropyl)-1,5-cyclohexadiene-1-methanol
4	9.4482	0.0521	4-(2,6,6-Trimethyl-cyclohexa-1,3-dienyl)-pent-3-en-2-one
5	9.5855	0.1577	Methyl dodecanoate
6	9.906	0.0924	1,1,1,2,2,5,5,6,6-nonafluorooctane
7	10.0719	0.0473	5-cyano-6-ethoxy-2-methyl-4-(4-pyridyl)- 4*H*-pyrane-3-carboxamide
8	10.3695	0.0797	3-cyclohexene-1-methanol
9	10.564	0.0796	Cis-*p*-mentha-2,5-dien-7-ol
10	10.7528	**1.0361**	Methyl tetradecanoate
11	11.3536	0.3278	(6-bromo-3-cyclohexen-1-yl)- Propanedinitrile
12	11.4967	0.2377	4-(1-methylethyl)-1,5-cyclohexadiene-1-methanol
13	11.84	**15.0196**	Methyl 14-methylpentadecanoate
14	12.0574	**8.1732**	Methyl 14-methylpentadecanoate
15	12.355	0.4739	Methyl 14-methylhexadecanoate
16	12.4179	0.1994	Methyl 12-methyltetradecanoate
17	12.4637	0.4003	Methyl 14-methylhexadecanoate
18	12.6754	**17.7139**	(Z)-9-octadecenoic acid, methyl ester (Methyl oleate)
19	13.0988	**18.2909**	Methyl 6-octadecenoate
20	13.2648	**1.1944**	Methyl 9-*cis*,11-*trans*-octadecadienoate
21	13.4994	**4.0900**	13-tetradecen-1-ol acetate
22	13.6653	**6.5171**	*cis*-13-Eicosenoic acid, methyl ester (Methyl cis-13-eicosenoate)
23	13.734	**5.1478**	Methyl eicosanoate
24	13.9228	0.4401	*N*-butyl-, *cis*-4-Cyclohexene-1,2-dicarboximide
25	14.1116	0.3467	2-butyl-4,5,6,7-tetrahydro-*1H*-Isoindole-1,3(*2H*)-dione
26	14.1631	0.5632	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one
27	14.3005	0.2754	2-butyl-4,5,6,7-tetrahydro-*1H*-Isoindole-1,3(*2H*)-dione
28	14.3977	0.939	Methyl (Z)-13-docosenoate
29	14.4721	**2.2427**	20-methyl-heneicosanoate
30	14.5866	0.1268	2-butyl-4,5,6,7-tetrahydro-*1H*-Isoindole-1,3(2H)-dione
31	14.6724	0.2692	2-butyl-4,5,6,7-tetrahydro-*1H*-Isoindole-1,3(2H)-dione
32	14.8383	0.771	*N*-butyl-*cis*-4-Cyclohexene-1,2-dicarboximide
33	15.0443	0.906	3-methyl-heneicosane
34	15.2045	**2.3041**	Methyl tetracosanoate
35	15.422	0.7553	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one
36	15.565	**2.389**	Squalene
37	15.7939	**1.9485**	Eicosane
38	16.0056	**1.1553**	1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl Heptasiloxane
39	16.2002	0.8345	*N*-Methyl-1-adamantaneacetamide
40	16.6865	**1.3526**	1-iodo octadecane
41	16.9383	0.4541	2-methyl-3-phenyl-3*H*-indole
42	17.1786	0.386	1-methyl-4-phenyl-5-thioxo-1,2,4-triazolidin-3-one
43	18.2486	**1.673**	2-Myristynoyl-glycinamide
44	18.981	0.4052	*N*-butyl-*cis*-4-Cyclohexene-1,2-dicarboximide

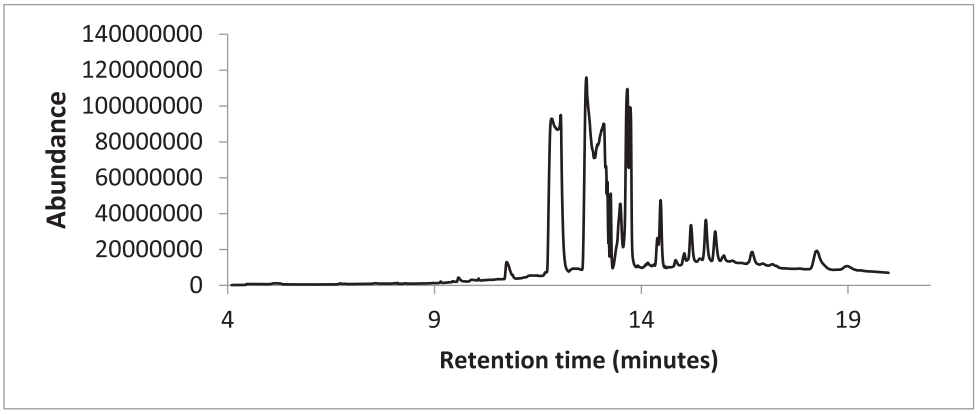

Figure 1. GC-MS (Gas chromatography-mass spectrometry) results for Neem Seed Oil Extract.

in neem extract are known for their diverse biological activities, notably pesticidal and cytotoxic properties (Tulashie et al. 2021). Some other neem derivatives were identified by Adusei and Azupio (2022), which are recognized as the primary active ingredient in neem extract, responsible for its antifeedant and toxic effects on insects.

Nanoparticles characterization

Following incubation at 37 °C for 60 minutes, 0.8 ml of the reagent was added to the test tubes, which were then heated for 5 minutes at 100 °C, cooled, and the produced nanoparticles were characterized using two methods (Yan et al. 2022). X-ray diffraction analysis was performed at room temperature (29 °C) using CuKα radiation, spanning a theta Data were analyzed using Excel to calculate *P* value, *F*-value, and L.S.D at 0.05- or 0.01-degrees range of 20 to 80 degrees (Figs 2, 3). For nanoparticle size assessment, transmission electron microscopy (TEM) was employed, operating at an 80 kV accelerating voltage. The TEM images presented in the figures (Figs 4, 5) illustrated a bimodal profile of the particle size distribution, the aggregation occurred in all synthesized nanoparticles. The size of CuO (NPs) was around 47 nm in diameter and 32 nm for ZnO (NPs).

Laboratory toxicity bioassay

The toxicity of five concentrations (1000, 500, 250, 125, and 62.5 ppm) of each bioinsecticide agent, along with a con-

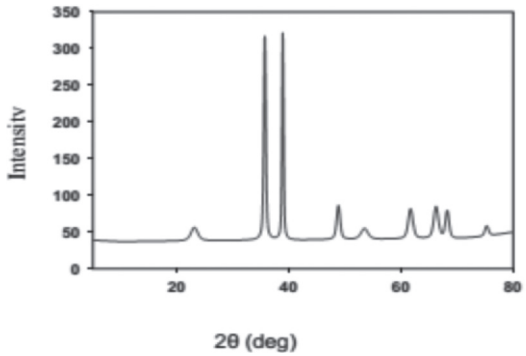

Figure 2. XRD of ZnO nanoparticles.

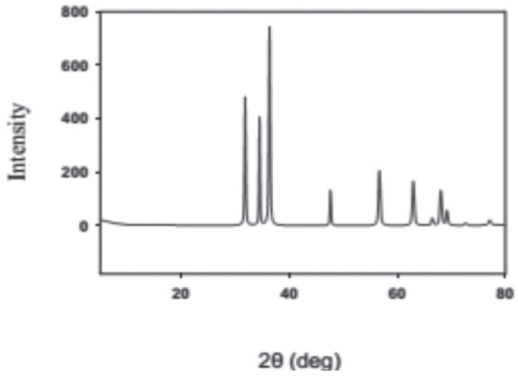

Figure 3. XRD of CuO nanoparticles.

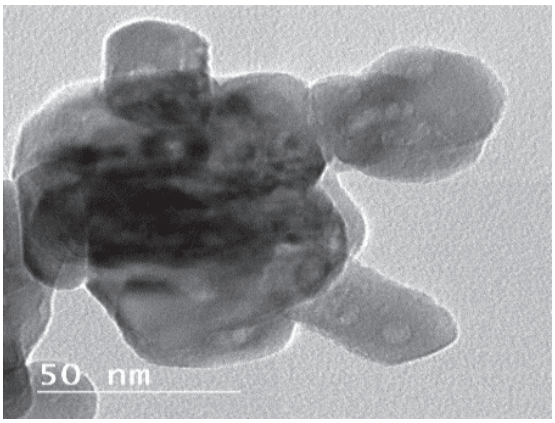

Figure 4. TEM image of ZnO nanoparticles.

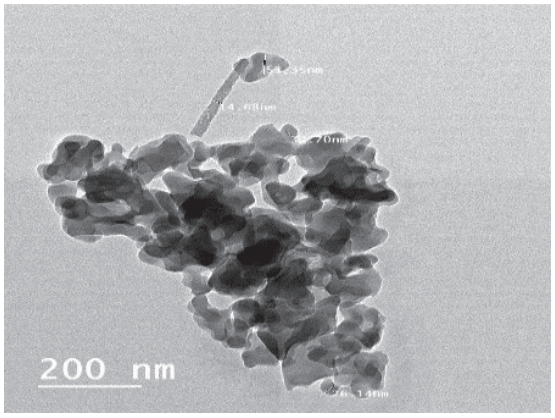

Figure 5. TEM image of CuO nanoparticles.

trol, was evaluated on the 2nd and 4th instar of the fall armyworm, *S. frugiperda*. Larvae mortality was first observed after 48 hours and then recorded daily. The mortality rate increased proportionally with higher extract concentrations and longer exposure periods. CuO nanoparticles showed greater efficacy, resulting in an 80% mortality rate at 250 ppm, compared to the 40% mortality rate achieved by ZnO and neem seed extract at the same concentration for the 2nd instar. Additionally, all treatments led to 100% mortality at 1000 ppm after the exposure period. For the 4th instar, lethal toxicity values were also determined for the three compounds. Larval mortality exceeded 70% for all treatments at a concentration of 500 ppm, while at 1000 ppm, mortality reached 100% for both nanomaterials and 80% for neem seed extract. The LC_{50} values, indicating total mortality, are presented in Tables 2, 3. For the 2nd instar, the values were 233 ppm for neem seed extract, 125 ppm for CuO, and 130 ppm for ZnO. The LC_{50} values for the 4th instar were 500 ppm for neem seed extract, 440 ppm for CuO, and 520 ppm for ZnO, respectively. The results make it evident that the fourth instars have higher deadly amounts. This is because the larvae require higher concentrations to reach the LC values as they become older since they become more resistant to the harmful toxin.

Table 2. Susceptibility of *Spodoptera frugiperda* 2nd instar Larvae.

Treated compound	LC_{50} (ppm)	Confidential limit (95%)		Slope ± S.E.
		Lower	Upper	
Neem seed extract	273	200	375	1.36 ± 0.25
CuO	125	113	191	1.87 ± 0.26
ZnO	130	117	202	2.16 ± 0.36

Table 3. Susceptibility of *Spodoptera frugiperda* 4th instar Larvae.

Treated compound	LC_{50} (ppm)	Confidential limit (95%)		Slope ± S.E.
		Lower	Upper	
Neem seed extract	500	700	370	4.81 ± 0.31
CuO	440	500	260	4.01 ± 0.28
ZnO	520	750	400	5.79 ± 0.41

Biological aspects

The effects of sub-lethal concentrations of neem seed extract, CuO, and ZnO nanoparticles on the biological aspects of the 4th instar larvae of *S. frugiperda* are detailed in Tables 4, 5, and Figs 6, 7. The data indicates a significant extension in both larval and pupal periods compared to the control. Specifically, the average larval duration was 21.50 days for neem seed extract, 15.70 days for CuO, and 14.4 days for ZnO nanoparticles, compared to 12.9 days in the control. The mean pupal durations were 15 days for neem seed extract and ZnO, and 16 days for CuO, against 13 days in the control. Table 4 presents the pupation and adult emergence percentages for *S. frugiperda* treated as 4th instars. Pupation rates decreased by 50% for both neem seed extract and ZnO, and by 45% with CuO, a significant reduction from the control treatment. Adult emergence rates for neem seed extract, CuO, and ZnO nanoparticles were 90%, 72.7%, and 80%, respectively. Fecundity and fertility of the treated 4th instar larvae, presented in Table 5, were expressed as the average number of eggs per female, mean egg hatch number, and hatchability percentage. All parameters showed a significant decrease. The average number of eggs was reduced by 65%, 91%, and 81% with ZnO, CuO nanoparticles, and neem seed extract, respectively. Additionally, hatchability percentages were 71%, 75%, and 88% for ZnO, CuO nanoparticles, and neem seed extract, respectively, compared to 95% in the control treatment.

Previous studies, which align with the current study's findings, have reported that neem extract's effects –including repellence, developmental delays, reduced fecundity and fertility, and behavioral changes leading potentially to death –are attributable to neem derivatives found in seed oil extract. This compound induces physiological and behavioral alterations in the pest (Prates et al. 2003; Roel et al. 2010; Shukla et al. 2015). Similarly, Lavicoli et al. (2019) and El-Bendary and El-Helaly (2013) found that nanoparticles affected fecundity and fertility in *S. frugiperda*. Although the precise methods by which the nanoparticles produced these effects were not fully clarified in these investigations, it was generally agreed that particles might obstruct biological functions essential to reproduction. Similar results were obtained by Pittarate et al. 2023 who stated that nanopar-

Table 4. Duration of the larval and pupal stages; percentage of pupation and adult emergence of *S. frugiperda* treated with ZnO, CuO nanoparticles and Neem seed oil extract.

Treatment	Mean larval duration (days ± SE)	Mean pupal stage (days ± SE)	% Pupation	% Adult emergence
ZnO	$14.40^{bc} \pm 0.59$	$15^{a} \pm 0.63$	50	80.0
CuO	$15.70^{b} \pm 0.61$	$16^{a} \pm 0.51$	55	72.7
Neem seed extract	$21.50^{a} \pm 0.93$	$15^{a} \pm 0.63$	50	90.0
Control	$12.90^{d} \pm 0.83$	$13^{b} \pm 0.59$	100	95.0
F value	5.65*	4.75*		
LSD	1.945	1.883		

Means with the same letters have no significant differences; *: significant ($p < 0.01$).

Table 5. Fecundity and fertility of *S. frugiperda* treated with ZnO, CuO nanoparticles and Neem seed oil extract.

Treatment	Mean adult life span (days ± SE)	Mean number of eggs/ female (days ± SE)	Mean number of egg hatch ± SE	Hatchability %
ZnO	$7.76^{a} \pm 0.899$	$155^{b} \pm 7.402$	$110^{b} \pm 10.33$	70.97
CuO	$7.76^{a} \pm 0.899$	$40^{d} \pm 1.71$	$30^{c} \pm 1.698$	75.0
Neem seed extract	$9.0^{b} \pm 0.588$	$85^{c} \pm 3.396$	$75^{bc} \pm 4.71$	88.24
Control	$8.5^{b} \pm 1.891$	$450^{a} \pm 29.41$	100	95
F value	0.4196*	1.4036***	166.896***	
LSD	1.087	5.0788	48.463	

Means with the same letters have no significant difference; *** highly significant ($p < 0.001$).

Figure 6. Malformed 6th instar larvae treated as 2nd instar with: a: Control; b: CuO; c: ZnO; d: Neem seed extract.

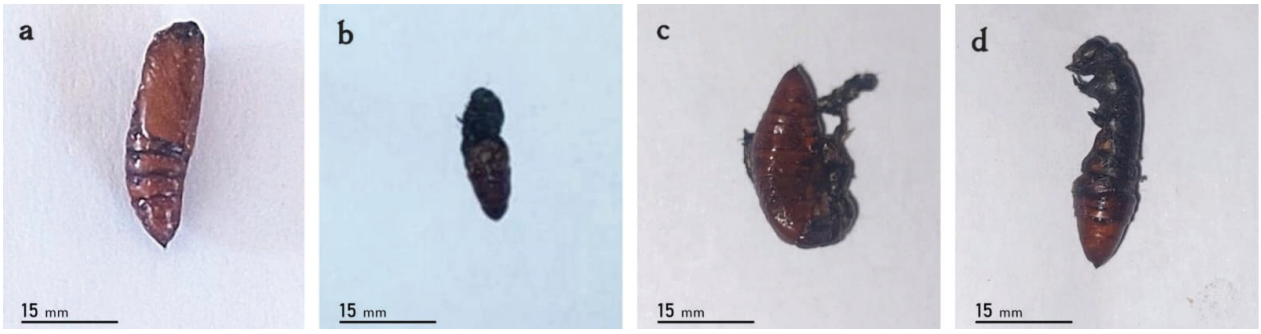

Figure 7. Malformed pupae treated as 2nd instars with: a: Control; b: CuO; c: ZnO; d: Neem seed extract.

ticles suppress substrate migration through the cellular membrane. This suppression may cause problems for several cellular functions, including respiration and metabolism. The insects' general health is impacted, which makes it more difficult for them to develop, reproduce, and survive.

Enzymatic activity

The digestive effects of ZnO, CuO nanoparticles, and neem seed extracts on the fourth instar larvae of *S. frugiperda* are detailed in Table 6. The ingestion of 520 ppm of ZnO, 440 ppm of CuO nanoparticles, and 500 ppm of neem seed oil extracts significantly impacted the digestive enzymatic activity of *S. frugiperda*. The data indicated that α-amylase activity levels increased in treated larvae by 6%, 18%, and 34% for ZnO, CuO nanoparticles, and neem seed extracts, respectively. Furthermore, trehalase activity decreased by 3%, 8%, and 12% after treatment with neem seed extract, ZnO, and CuO nanoparticles, respectively. Invertase enzyme activity levels were observed at 154.1 µg, 120.36 µg, and 159.36 µg of glucose released per minute per gram of body weight for ZnO, CuO nanoparticles, and neem seed extract, respectively, compared to the control treatment, which showed 153.33 µg of glucose released. These findings align with other research on the biological aspects of *S. frugiperda*, highlighting a decrease in trehalase enzyme activity, which plays a critical role in energy regulation and glucose generation through trehalose catabolism (Shukla et al. 2015; Rahman et al. 2022). Furthermore, the neem seed extract influences the digestive enzymatic activity of the *S. frugiperda* larvae, attributed to the effect of the active ingredients in the neem seed extract (Alzohairy. 2016; Foudal et al. 2022). Both plant extracts and nanoparticles represent an Eco-friendly method, so they can be used as an alternative to chemical pesticides that pose a threat to living organisms and environment (Tulashie et al. Shabir et al. 2023).

Table 6. Specific activity of the selected enzymes of *S. frugiperda* (µg /min/ mg protein).

Treatment	Amylase ± SE	Invertase ± SE	Trehalase ± SE
ZnO	$31.81^{c} \pm 0.17$	$154.1^{b} \pm 0.61$	$210.06^{c} \pm 0.58$
CuO	$35.26^{b} \pm 0.17$	$120.36^{d} \pm 0.24$	$200.86^{d} \pm 0.58$
Neem	$45.23^{a} \pm 0.17$	$169.36^{a} \pm 0.40$	$231.53^{a} \pm 0.58$
Control	$29.9^{d} \pm 0.11$	$153.33^{c} \pm 1.36$	$218.5^{b} \pm 1.38$
F value	217.34***	489.15***	577.45***
L.S.D	0.848	5.515	6.549

Means with the same letter are not significantly different ($p \leq 0.05$).
**Highly significant, ***Very highly significant

Conclusion

This study has demonstrated the application of neem seed extract and nanoparticles as an eco-friendly insecticide in managing *Spodoptera frugiperda*. The treatments exhibited effective larvicidal properties, particularly on the 4th instar, by extending larval and pupal durations and inhibiting successful adult emergence at low concentrations. Additionally, the enzymatic activity of the insect was notably altered following the application of the lethal concentration values of neem seed extracts and nanoparticles. Therefore, this research provides basic data to conduct future research on pest control using nanomaterial and plant extracts, which are considered as safe and environmentally friendly. This approach is consistent with the goals of sustainable agriculture and environmentally safe biological control of pests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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