

Insecticidal efficacy of synthesized ZnO nanoparticles using brown algae *Polycladia myrica* extract against *Callosobruchus maculatus*

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Keywords

Cowpea weevil, *Polycladia myrica*, Insect pest control, Nanotechnology

Received: 10 April 2026

Revised: 21 July 2026

Accepted: 23 July 2026

Available online: 27 July 2026

Abstract

The environmental and human health risks of chemical insecticides have prompted extensive searches for alternative methods to protect stored products. Recently, nanoparticles have been considered as promising alternatives to synthetic chemical products. In this study, ZnO nanoparticles (NPs) were synthesized using *Polycladia myrica* algae extract, and characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), and Field emission scanning electron microscopy (FE-SEM). Three types of ZnO NPs, ZnO-A, ZnO-B, and ZnO-C, were synthesized using algae extract by two different methods. Their insecticidal activity was evaluated and compared with the chemically synthesized ZnO-D NPs against the cowpea weevil, *Callosobruchus maculatus* on stored cowpeas. Biosynthesized ZnO-A, ZnO-B, and ZnO-C NPs exhibited higher activity against *C. maculatus* when compared with ZnO-D NPs. Porous ZnO-A NPs with the smallest particle size (24.3 nm) were determined to be the most toxic nanoparticles, causing the highest mortality rate of *C. maculatus* adults after five days of exposure, while ZnO-D NPs were the least effective for *C. maculatus* control. A significant oviposition inhibition (35.1 to 44.9%) and progeny reduction (35.7 to 45.6%) was also observed for insects exposed to ZnO-A NPs-treated cowpeas at 500 to 2000 mg kg⁻¹. The average weight loss of cowpea seeds treated with ZnO-A, ZnO-B, and ZnO-C NPs at 2000 mg kg⁻¹ was 23.8, 28.1, and 33.5%, respectively, with significant difference compared to untreated control (52.2%). Our results suggest that biosynthesized ZnO NPs can be considered as a viable alternative for controlling *C. maculatus* in stored cowpeas.

Cite this article:

Sohrabi F, Solati Z, Bagheri F, Mirhosseini MA, Ziaee M, Pervez A, 2026. Insecticidal efficacy of synthesized ZnO nanoparticles using brown algae *Polycladia myrica* extract against *Callosobruchus maculatus*. *Appl Res Plant Prot* 15 (2): 107–120.

<https://dx.doi.org/10.22034/ARPP.2026.22040>



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Introduction

The cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae: Bruchinae), is a significant storage pest of legumes, particularly within the Fabaceae family, in many tropical and sub-tropical countries (Deshpande *et al.* 2011; Yusuf *et al.* 2011). The females lay eggs on the surface of seeds, allowing its neonate larvae to penetrate and feed on the seeds, leading to substantial damage (Tengey *et al.* 2023). Larvae can feed on seeds and not only reduce the weight of seeds but also diminishes nutritional value, thereby causing economic damage (Murdock *et al.* 2003; Gbaye *et al.* 2011). Synthetic insecticides and fumigants are commonly used to control insect pests of stored products (Rajendran 2020; Magano *et al.* 2021). However, the widespread use of chemical insecticides adversely affects the environment, human health, and non-target organisms (Wakil *et al.* 2013; Khan 2021). Moreover, overuse of synthetic insecticides and fumigants has led to the emergence of resistance in *C. maculatus* and numerous stored grain insects. The undesirable effects of synthetic chemicals have prompted extensive searches for alternative methods (Gbaye *et al.* 2016; Attia *et al.* 2020; Fouad & Abotaleb 2021; Wakil *et al.* 2021).

In recent years, nanotechnology has emerged as one of the new potential methods for pest management (Sinha *et al.* 2017; Shekhar *et al.* 2021; Deka *et al.* 2021; Usman *et al.* 2022). Various biological applications and original research used nanoparticles (NPs) due to a variety of physical and chemical properties (Noel *et al.* 2007; Ragaei & Sabry 2014; Arumugam *et al.* 2016; Valsalam *et al.* 2019; Azimpanah *et al.* 2022). The efficacy of NPs in managing insect pests can be attributed to different properties including particle size, structure, and morphology (Singh *et al.* 2011). They easily spread on treated surfaces due to their small size and large surface-to-volume ratio and therefore enhanced bioactivity compared to conventional insecticides (Jasrotia *et al.* 2022). Several formulations of NP-based pesticides, such as nanoemulsions, nanosuspensions, and solid-based nanopesticides, have been developed and utilized

for crop protection (Elsharkawy 2020; Jasrotia *et al.* 2022).

Green synthesis of NPs using biological entities such as plants, bacteria, fungi, and algae has lately been developed (Zhao *et al.* 2018; Khanna *et al.* 2019; Azimpanah *et al.* 2022; Pandit *et al.* 2022). Green nanoparticles are considered eco-friendly alternatives due to their lower toxicity levels and have less harmful effects on the environment, human health, and non-target organisms (Ying *et al.* 2022; Vijayaram *et al.* 2023). Previous studies have documented the insecticidal activity of metal oxide NPs, synthesized both by chemical and biological methods, for controlling stored-product insect pests (Sabbour 2012; Vijaya Sankar & Abideen 2015; Diagne *et al.* 2019; Das *et al.* 2019; Badawy *et al.* 2021). Among these, biosynthesized zinc oxide (ZnO) NPs are effective against storage pests, especially the ones prepared from spinach (*Spinacia oleracea* L.) leaves exhibited potential toxicity at the doses as low as 175 ppm against *C. maculatus* in green gram (Lakshmi *et al.* 2020). Similarly, ZnO NPs prepared from *Eucalyptus globulus* L. extracts were effective against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) (Siddique *et al.* 2021). Furthermore, ZnO NPs are recognized for their antimicrobial properties (Patra *et al.* 2012; Azimpanah *et al.* 2022), offering them to protect crops from both insect pests and microbial infection.

In this context, our study aims to prepare and characterize ZnO NPs using *Polycladia Myrica* (S.G. Gmelin) algae extract employing two different methods, viz. hydrothermal and precipitation techniques. We also evaluated the entomotoxic efficacy of these biosynthesized ZnO NPs against the cowpea weevil and compared their effectiveness with chemically synthesized ZnO NPs. To our knowledge, no information exists on the entomotoxic potential of NPs prepared from *P. myrica* algae for pest management. Through this work, we hope to contribute to the development of sustainable and effective pest control strategies while mitigating the risks associated with conventional chemical insecticides.

Materials and methods

Materials

Urea and $\text{ZnNO}_3 \cdot 6\text{H}_2\text{O}$, NaOH were supplied by Merck. The brown algae were collected from the intertidal zone of Naftkesh coast, Bushehr province, in the northern Persian Gulf (approximately $28^\circ 59'\text{N}$, $50^\circ 50'\text{E}$). Samples were transported to the laboratory in sterile containers and washed with distilled water to remove epiphytes and sediments. Species name and its classification were supported by AlgaeBase (Guiry & Guiry 2021) and Dadolahi-Sohrab *et al.* (2012) as *P. myrica* (formerly known as *Cystoseira myrica*). The preparation of *P. myrica* algae extract was conducted as Mehrjo *et al.* (2021).

Preparation of ZnO-A and ZnO-D NPs by hydrothermal method

A mixture of $\text{ZnNO}_3 \cdot 6\text{H}_2\text{O}$ (25ml, 5.75 g), 12.5 ml of algae extract, and 5 ml of a 25% solution of urea (w/v) was transferred into a stainless-steel Teflon lined hydrothermal autoclave and heated at 120°C for 8 h in an oven. The mixture was cooled at room temperature and filtered. The resulting

precipitate was washed properly with deionized water and dried at 110°C for 4 h. The prepared powder was calcined at 600°C for 4 h (ZnO-A). The ZnO-D NPs were produced in the same manner as ZnO-A, without algae extract (Husairi *et al.* 2013).

Preparation of ZnO-B and ZnO-C NPs by precipitation method

ZnO B and ZnO C were synthesized based on a previously reported method (Mehrjo *et al.* 2021) with slight modifications. A mixture of 12.5 mL of algae extract, 25 mL of distilled water, and 5.75 g of $\text{ZnNO}_3 \cdot 6\text{H}_2\text{O}$ was stirred and heated at 100°C for 4 h. After that, 10 mL of 25% of NaOH solution (w/v) was added dropwise, and heating was continued for 2 h. The mixture was aged at room temperature for 24 h. The precipitated ZnO was filtered and washed with distilled water until the pH value reached 7. The white powder (ZnO) was calcined at 600°C for 4 h (ZnO-B). The ZnO-C NPs were prepared similarly, using 10 mL of 25% urea solution (w/v) instead of NaOH solution.

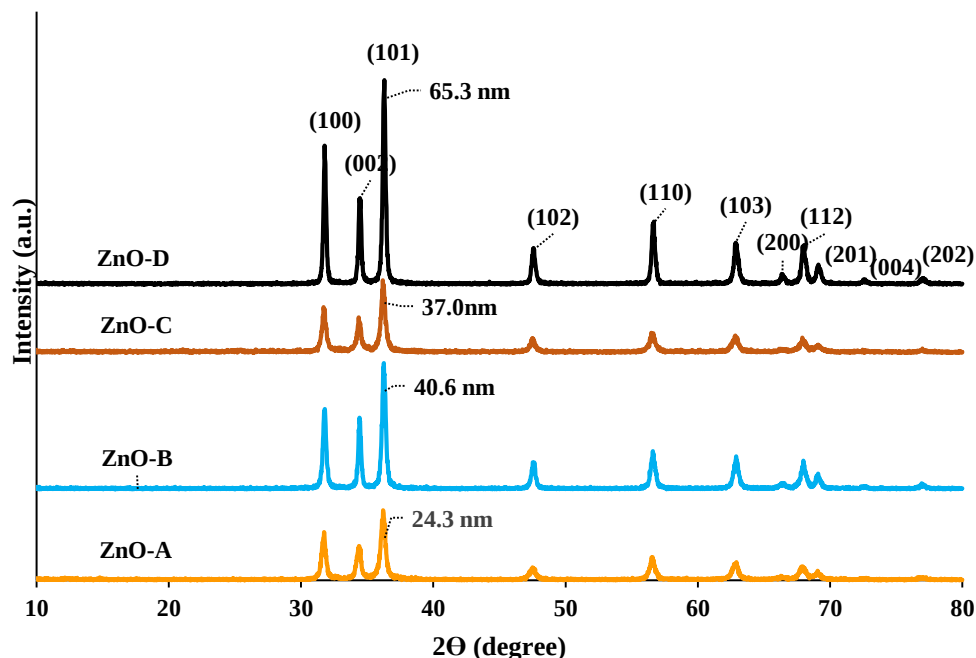


Figure 1. XRD patterns of ZnO-A, ZnO-B, ZnO-C, and ZnO-D nanoparticles. The intensity is shown in arbitrary units (a.u.). The numbers above the (101) peaks indicate the average crystallite size (in nm) calculated using the Debye–Scherrer equation.

Characterization of ZnO NPs

X-Ray diffraction (XRD) study

The crystal structure of ZnO was analyzed by X-ray diffraction (XRD) (X-ray diffractometer, Philips, PW1730, with Cu K α radiation, $\lambda = 0.15406$ nm) with 2θ in the range of 10–80 degrees.

FTIR study

Fourier transform infrared (FTIR) measurements were carried out using a Thermo, Nicolet 6700 equipped with a Smart Performer ATR accessory.

Morphological study

Field emission scanning electron microscopy (FE-SEM, HITACHI S-4500 microscope, Tokyo, Japan) was used to determine the morphologies and sizes of ZnO NPs.

Test insects

Adults of *C. maculatus* were taken from the culture, not exposed to insecticides, and maintained in the Entomology Laboratory, Persian Gulf University. They were reared on cowpeas (*Vigna unguiculata* L.) in a growth chamber at 27 ± 1 °C and $65 \pm 5\%$ RH in the darkness. Cowpeas were purchased from the local market and stored at -20 °C for 14 days to kill any live insects present. They were later conditioned at room temperature prior to use in bioassays. The adult insects were placed in ventilated plastic containers (15 cm height; 21 cm in diameter) containing ≈ 500 g cowpea seeds for oviposition. After 5 days, the parent insects were removed by sieving, and seeds containing eggs were kept in an incubator under the controlled conditions mentioned above until the F1 progeny emerged. Newly emerged adults were used for all bioassays.

Table 1. FWHM values and crystallite sizes of ZnO nanoparticles (ZnO-A to ZnO-D).

NP type	ZnO-A	ZnO-B	ZnO-C	ZnO-D
FWHM (degree)	0.3595	0.2152	0.2361	0.1338
D (nm)	24.3	40.6	37.0	65.3

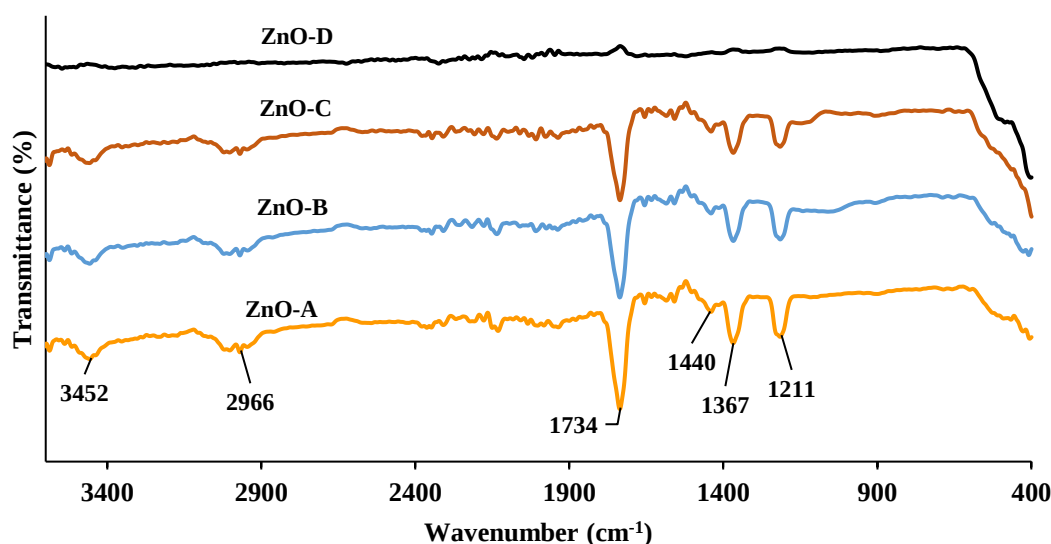


Figure 2. Fourier transform infrared spectroscopy (FTIR) spectrum of the synthesized ZnO nanoparticles.

Insecticidal efficacy

Cowpea seeds (40 g) were placed in 150 ml glass jars and treated separately with each ZnO NP type

at the individual dose of 100, 500, 1000, 1500 and 2000 mg/kg. Each jar was shaken manually for 5 min to ensure an even distribution of the ZnO NPs

on the seeds and then allowed to stand for 30 min. Each treated lot was divided into four replicates using plastic Petri dishes (80 × 15 mm), where five pairs of newly emerged *C. maculatus* (0-24 h old) were placed into each Petri dish containing 10 g of cowpea seeds (45-48 seeds). The dishes were sealed by perforated lids covered with muslin cloth for sufficient aeration. Untreated seeds were considered as the control group. The number of dead beetles was counted after 1, 3, and 5 days of exposure. Beetles were recognized dead when there was no response when their abdomen was gently probed with a hairbrush. All the adults were removed from the seeds on the seventh day, and the number of eggs laid was counted under a stereomicroscope (Helmut Hund GmbH, Germany). The oviposition inhibition percentages of *C. maculatus* were calculated following Badii *et al.* (2014):

$$\% \text{ Oviposition inhibition} = \frac{L_c - L_t}{L_c} \times 100$$

Where L_c = No. of eggs laid on control, and L_t = mean number of eggs laid on treated seeds. The eggs were allowed to develop into adults. After an additional 40 days, the number of F1 progenies produced and the weight of damaged seeds were recorded. The percentages of weight loss of cowpea seeds were calculated according to Harris & Lindblad (1978) formula:

$$\% \text{ WL} = \frac{IW - FW}{IW} \times 100$$

Where WL= Weight loss, IW= Initial weight, and FW= Final weight. All bioassays were conducted under controlled laboratory conditions at 27 ± 1 °C, $65 \pm 5\%$ RH, and continuous darkness.

Statistical analysis

Adult mortality percentage was corrected with that in control according to Abbott's formula (Abbott 1925). The corrected mortality data and data on oviposition inhibition percentage, number of F1 progeny production, and seed weight loss percentage were examined for normality using

Kolmogorov-Smirnov test ($P < 0.05$). If the data were normal, no transformation was performed (Zar 1999). The corrected mortality data were then subjected to a 3-way ANOVA using the general linear model (GLM) procedure with NP type, dose rate and exposure time as the main effects. The oviposition inhibition percentage, number of F1 progeny production, and seed weight loss percentage data were subjected to 2-way ANOVA with NP type and dose rate as the main effects. The means were separated by the Tukey–Kramer (HSD) test ($P = 0.05$) using SAS software (version 9.1) (SAS Institute, 2003).

Results

Characterization of ZnO NPs

X-Ray diffraction (XRD) study

The X-ray diffraction (XRD) patterns of fabricated ZnO NPs are shown in Figure 1. The ZnO NPs planes with miller indices values of 100, 002, 101, 102, 110, 103, 200, 112, 201, 004, and 202 are in accordance with peaks at 2θ angles of 31.7° , 34.4° , 36.3° , 47.5° , 56.6° , 62.3° , 66.5° , 67.9° , 69.1° , 72.6° , and 77° , respectively. The X-ray diffraction peaks belong to the ZnO hexagonal wurtzite structure and match with standard data of Joint Committee on Powder Diffraction Standards (JCPDS) card No. 36-1451. No characteristic peaks of impurities were seen. The average crystallite size was calculated using the Debye-Scherrer's equation:

$$D(\text{\AA}) = \frac{K\lambda}{(\beta \cos \theta)}$$

Where D is the average crystallite size ($\text{\AA}$), K is the shape factor (0.9), λ is the X-ray wavelength (Cu $K\alpha$, 1.5406 $\text{\AA}$), θ is the Bragg angle, and β is the full width at half maximum (FWHM) (Mehrjo *et al.* 2021). The strongest peak, corresponding to the (101) reflection plane at $2\theta = 36.3^\circ$, was used for the calculations. The corresponding FWHM values and the calculated crystallite sizes for ZnO-A to ZnO-D are summarized in Table 1. The average crystallite size was determined to be 24.3, 40.6, 37.0, and 65.3 nm for ZnO-A, ZnO-B, ZnO-C, and ZnO-D NPs,

respectively.

FTIR study

The ATR-FTIR spectra of the synthesized ZnO NPs are presented in Figure 2. For all samples, the bands present between 600 and 400 cm^{-1} may be assigned to the Zn–O stretching modes (Azimpanah *et al.* 2022). The FTIR spectrum of fabricated ZnO NPs using algae extract (ZnO-A, ZnO-B, and ZnO-C NPs) were similar and revealed the presence of various phytochemicals (Lodeiro *et al.* 2006). The observed peaks at about 3452 cm^{-1} were attributed to vibrations of O-H groups (phenols/alcohols). The vibrations of aliphatic C-H bonds were observed at about 2800–3000 cm^{-1} . The bands at 1211 cm^{-1} may be referred to C-O-C the vibration (glycosidic linkage). The bands at 1367 cm^{-1} may be attributed to vibration of CH₃ groups and also, the possible presence of C-N stretching vibration (Gheda *et al.* 2021). The stretching vibrations of C=O bonds in

different functional groups were observed at 1734 cm^{-1} .

Morphological study

Figure 3 presents the typical FE-SEM images of the fabricated ZnO NPs at 200 nm. Different sizes and morphological characteristics of ZnO NPs depended upon the synthesis method and precursor materials. The FE-SEM images demonstrate that both nanoparticles of ZnO-A and ZnO-C NPs are in the form of a porous plate (Figure 3a, 3c). However, significantly thinner plates are seen for ZnO-A NPs. FE-SEM results indicated that the mean thickness is 34.5 nm and 43.6 nm for ZnO-A and ZnO-C NPs, respectively. Figure 3b revealed that ZnO-B NPs have irregular sheet-like shapes and a mean thickness of 44.5 nm. Figure 3d indicates that ZnO-D NPs are composed of irregularly shaped and some hexagonal-like particles with diameters between 56–71 nm.

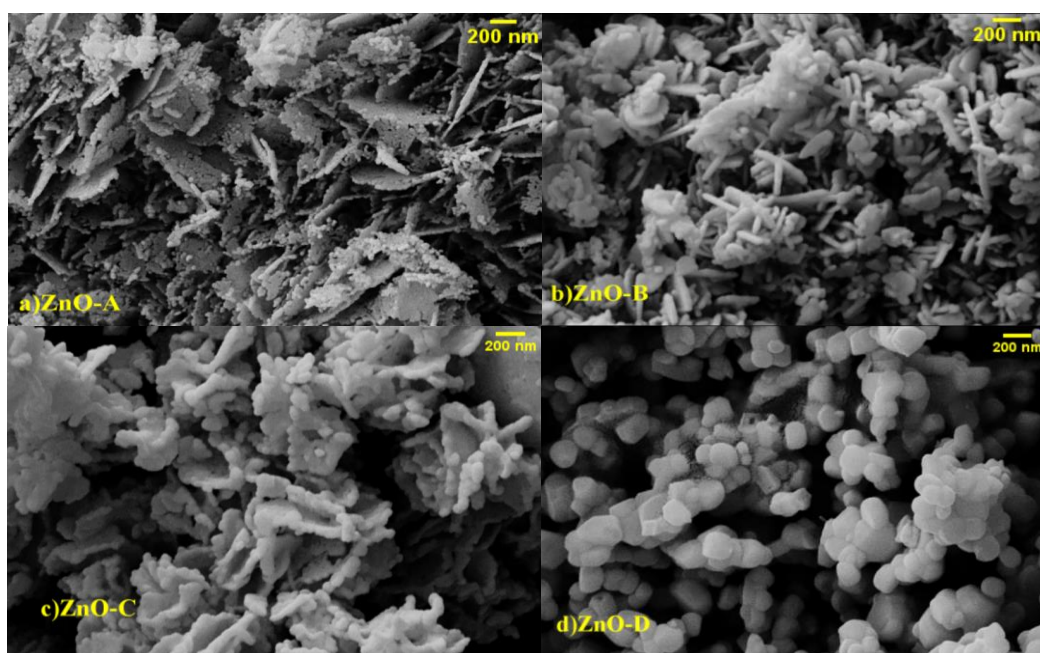


Figure 3. FE-SEM images of ZnO nanoparticles (a) ZnO-A, (b) ZnO-B, (c) ZnO-C, and (d) ZnO-D.

Adult mortality

The mortality of *C. maculatus* adults was significantly affected by NP type ($F = 110.28$; $df = 3, 180$; $P < 0.0001$), dose rate ($F = 82.81$; $df = 4, 180$; $P < 0.0001$), exposure time ($F = 2036.49$; $df = 2, 180$; $P < 0.0001$), and their associated interactions

($F = 3.94$; $df = 24, 180$; $P < 0.0001$). NP type $\times$ exposure time ($F = 37.40$; $df = 6, 180$; $P < 0.0001$), NP type $\times$ dose rate ($F = 9.83$; $df = 12, 180$; $P < 0.0001$), and dose rate $\times$ exposure time ($F = 22.23$; $df = 8, 180$; $P < 0.0001$) interaction effects on adult mortality were also significant.

Mortality was very low and did not exceed 10% at 2000 mg kg⁻¹ of ZnO-B NPs, after one day of treatment (Table 2). After 3 days of exposure, the mortality significantly increased to 50% when *C. maculatus* adults were exposed to ZnO-B NPs at 2000 mg kg⁻¹. ZnO-A NPs also caused nearly 40% insect mortality at high doses of 1000, 1500, and 2000 mg kg⁻¹ at this time (Table 2). A significant increase in insect mortality was observed for all the treatments after 5 days of exposure. More than 80% of *C. maculatus* adults died when ZnO-A NPs were applied at 500 to 2000 mg kg⁻¹. ZnO-C NPs also killed more than 80% of adults at 1000, 1500, and 2000 mg kg⁻¹. Nearly 90% of adult mortality was observed when ZnO-B NPs were applied at 2000 mg kg⁻¹, whereas at this dose rate, ZnO-D NPs caused 59.8% mortality (Table 2).

Table 2. Mean mortality percentage $\pm$ SE of *Callosobruchus maculatus* adults after 1, 3 and 5 days exposure to cowpeas treated with four different ZnO NPs at 5 dose rates.

NP type	Dose rate (mg kg ⁻¹)	Exposure time (days)			$F_{2,9}, P$
		1	3	5	
ZnO-A	100	0.0 $\pm$ 0.0 dB	7.5 $\pm$ 1.0 efgB	59.8 $\pm$ 6.7 cdeA	68.56, <.0001
	500	2.5 $\pm$ 0.3 cC	32.5 $\pm$ 2.3 cB	83.9 $\pm$ 5.3 abcA	147.36, <.0001
	1000	2.5 $\pm$ 0.2 cC	40.0 $\pm$ 4.0 bB	89.3 $\pm$ 6.2 abA	103.40, <.0001
	1500	5.0 $\pm$ 0.6 bC	38.4 $\pm$ 5.1 bcB	97.3 $\pm$ 2.7 aA	193.65, <.0001
	2000	2.5 $\pm$ 0.3 cC	42.5 $\pm$ 3.2 bB	97.3 $\pm$ 2.7 aA	384.65, <.0001
ZnO-B	100	0.0 $\pm$ 0.0 dC	12.5 $\pm$ 1.4 eB	41.1 $\pm$ 3.1 efA	114.18, <.0001
	500	5.0 $\pm$ 0.3 bC	20.0 $\pm$ 2.0 dB	54.5 $\pm$ 2.7 deA	168.33, <.0001
	1000	5.0 $\pm$ 0.3 bB	20.0 $\pm$ 2.0 dB	59.8 $\pm$ 6.7 cdeA	48.43, <.0001
	1500	5.0 $\pm$ 0.6 bC	25.0 $\pm$ 2.0 dB	54.4 $\pm$ 2.7 edA	158.57, <.0001
	2000	10.0 $\pm$ 1.1 aC	50.0 $\pm$ 7.0 aB	89.3 $\pm$ 6.9 abA	52.65, <.0001
ZnO-C	100	0.0 $\pm$ 0.0 dB	0.0 $\pm$ 0.0 hB	22.3 $\pm$ 2.7 fA	69.49, <.0001
	500	0.0 $\pm$ 0.0 dB	2.5 $\pm$ 0.2 hB	67.8 $\pm$ 6.2 bcdA	115.96, <.0001
	1000	0.0 $\pm$ 0.0 dB	10.0 $\pm$ 1.1 efB	81.2 $\pm$ 6.7 abcA	126.04, <.0001
	1500	2.5 $\pm$ 0.3 cC	20.0 $\pm$ 2.0 dB	83.9 $\pm$ 5.3 abcA	167.31, <.0001
	2000	0.0 $\pm$ 0.0 dC	20.0 $\pm$ 2.0 dB	94.6 $\pm$ 5.3 aA	227.15, <.0001
ZnO-D	100	2.5 $\pm$ 0.3 cB	5.0 $\pm$ 0.3 fghB	41.1 $\pm$ 3.1 efA	143.03, <.0001
	500	0.0 $\pm$ 0.0 dB	2.5 $\pm$ 0.3 ghB	38.4 $\pm$ 5.1 efA	52.47, <.0001
	1000	2.5 $\pm$ 0.3 cB	5.0 $\pm$ 0.3 fghB	54.5 $\pm$ 6.7 deA	54.46, <.0001
	1500	0.0 $\pm$ 0.0 dB	5.0 $\pm$ 0.3 fghB	54.5 $\pm$ 6.7 deA	59.69, <.0001
	2000	0.0 $\pm$ 0.0 dB	5.0 $\pm$ 0.3 fghB	59.8 $\pm$ 2.7 cdeA	452.80, <.0001
$F_{19,60}, P$		52.15, <.0001	34.96, <.0001	18.96, <.0001	

Means followed by different upper case letters in each row and lower case letter within each column are significantly different (GLM, Tukey's HSD $P < 0.05$).

Concerning progeny emergence, the main effects of NP type ($F = 160.32$; $df = 4, 75$; $P < 0.0001$), dose rate ($F = 22.31$; $df = 4, 75$; $P < 0.0001$), and their associated interactions ($F = 4.70$; $df = 16, 75$; $P < 0.0001$) were significant. The mean number of progeny in the control seeds was 175.5 ± 3.2 . ZnO-C NPs at 100 and 500 mg kg⁻¹, and ZnO-

Oviposition, progeny emergence, and seed weight loss

The ZnO NP type ($F = 202.59$; $df = 3, 60$; $P < 0.0001$), dose rate ($F = 26.05$; $df = 4, 60$; $P < 0.0001$), and their associated interactions ($F = 5.96$; $df = 12, 60$; $P < 0.0001$) had significant effects on oviposition inhibition percentage. Among the treatments, ZnO-A NPs recorded the highest oviposition inhibition, ranging from 35.1-44.9%, and did not significantly differ among doses except for 100 mg kg⁻¹. However, for ZnO-B and ZnO-C NPs the highest oviposition inhibition was observed at 2000 mg kg⁻¹. The oviposition inhibition was deficient when adults were exposed to ZnO-D NPs and did not exceed 5.5% (Table 3).

D NPs at 100 to 1500 mg kg⁻¹ had no significant effects on the number of F1 progenies compared to the untreated control (Table 3). Among the treatments, ZnO-A at 500 to 2000 mg kg⁻¹ doses and ZnO-B at 2000 mg kg⁻¹ showed significantly lower F1 progenies than other treatments. The percent reduction in number of progenies produced on ZnO

NPs-treated seeds in comparison to untreated cowpeas was between 1.7 to 45.6% (Table 3).

The effects of NP type ($F = 135.69$; $df = 4, 75$; $P < 0.0001$), dose rate ($F = 19.59$; $df = 4, 75$; $P < 0.0001$), and their associated interactions ($F = 4.02$; $df = 16, 75$; $P < 0.0001$) on seed weight loss were significant. Mean weight loss in seeds treated with ZnO-C NPs at 100 and 500 mg kg⁻¹ and ZnO-D NPs

at all applied doses was higher than 45% and showed no significant difference compared to the untreated control. However, in the other treatments, mean weight loss was significantly lower than the control. Mean weight loss at the maximum applied dose (2000 mg kg⁻¹) was 23.8, 28.1, and 33.5% for ZnO-A, ZnO-B and ZnO-C NPs, respectively (Table 3).

Table 3. Oviposition inhibition percentage of *Callosobruchus maculatus*, number of F₁ progeny produced, and weight loss percentage on cowpeas treated with ZnO NPs at different dose rates.

NP type	Dose rate (mg kg ⁻¹)	Oviposition inhibition (%)	Number of F ₁ progeny (% reduction)	Weight loss (%)
ZnO-A	100	16.5 ± 0.5 ^{c-f}	148.1 ± 3.3 ^{b-e} (15.6)	44.0 ± 1.5 ^{bcd}
	500	35.1 ± 1.4 ^{ab}	112.8 ± 1.3 ^g (35.7)	36.5 ± 0.5 ^{def}
	1000	43.3 ± 5.4 ^a	96.6 ± 2.5 ^g (44.9)	29.6 ± 0.6 ^{fgh}
	1500	44.9 ± 3.2 ^a	96.5 ± 2.8 ^g (45.0)	29.3 ± 1.2 ^{fgh}
	2000	44.9 ± 1.6 ^a	95.4 ± 3.5 ^g (45.6)	23.8 ± 0.9 ^h
ZnO-B	100	25.9 ± 1.1 ^{bcd}	129.8 ± 3.2 ^f (21.0)	34.4 ± 1.3 ^{efg}
	500	26.5 ± 3.4 ^{bc}	129.4 ± 1.1 ^f (26.7)	33.1 ± 1.5 ^{efg}
	1000	27.3 ± 1.8 ^{bc}	126.8 ± 3.9 ^f (27.7)	31.7 ± 1.3 ^{efg}
	1500	28.2 ± 3.1 ^{bc}	125.7 ± 0.9 ^f (28.4)	30.2 ± 2.3 ^{fgh}
	2000	39.9 ± 4.2 ^a	95.5 ± 5.0 ^g (45.5)	28.1 ± 1.3 ^{gh}
ZnO-C	100	9.4 ± 0.5 ^{efg}	157.0 ± 2.0 ^{a-d} (10.5)	46.0 ± 1.0 ^{ab}
	500	14.7 ± 1.0 ^{def}	150.4 ± 2.3 ^{a-e} (14.3)	45.9 ± 1.0 ^{ab}
	1000	20.1 ± 2.4 ^{cde}	143.5 ± 0.4 ^{cde} (18.2)	38.1 ± 2.8 ^{cde}
	1500	21.1 ± 0.5 ^{cd}	137.7 ± 4.7 ^{def} (21.5)	36.0 ± 0.1 ^{ef}
	2000	24.2 ± 0.1 ^{bcd}	133.7 ± 1.1 ^{def} (23.8)	33.5 ± 2.0 ^{efg}
ZnO-D	100	2.3 ± 0.1 ^g	172.5 ± 14.7 ^{ab} (1.7)	48.3 ± 1.1 ^{ab}
	500	2.6 ± 0.3 ^g	169.2 ± 11.1 ^{abc} (3.5)	47.2 ± 1.4 ^{ab}
	1000	5.3 ± 0.6 ^{fg}	157.8 ± 1.6 ^{a-d} (10.1)	46.0 ± 1.0 ^{ab}
	1500	5.5 ± 0.7 ^{fg}	150.7 ± 1.4 ^{a-e} (14.1)	44.9 ± 0.1 ^{abc}
	2000	5.1 ± 1.0 ^{fg}	148.4 ± 4.7 ^{b-e} (15.5)	44.8 ± 0.1 ^{abc}
Control	-	-	175.5 ± 3.2 ^a	52.2 ± 2.6 ^a
<i>F</i>		41.23	25.87	31.67
<i>df</i>		19, 60	20, 63	20, 63
<i>P</i>		<.0001	<.0001	<.0001

Means followed by different lower case letters within each column are significantly different (GLM, Tukey's HSD $P < 0.05$).

Discussion

The preparation of ZnO NPs was confirmed by XRD analysis. These diffraction peaks of the ZnO phase belong to the hexagonal structure and were reported in previous studies (Mehrjo *et al.* 2021; Azimpanah *et al.* 2022). The crystalline structure of ZnO NPs can be distinguished from the sharp peaks. Both phase and molecular structures specified by XRD and FTIR patterns indicated the formation of ZnO crystalline nanoparticles. The order of ZnO NPs from the smallest to largest particle size was ZnO-A, ZnO-C, ZnO-B, and ZnO-D. ZnO-A and ZnO-C NPs, synthesized by two different methods, displayed the shape of a porous plate. Larger ZnO-

B NPs exhibited an irregular sheet-like shape. Biosynthesized ZnO NPs from *P. myrica* algae extract, ZnO-A, ZnO-B, and ZnO-C NPs, were smaller than chemically synthesized ZnO-D NPs. Numerous studies have reported the biosynthesis of ZnO NPs using algae extracts as a simple, low-cost, and eco-friendly method for the production of nanoparticles (Azizi *et al.* 2014; Bhattacharya *et al.* 2020; Elrefaey *et al.* 2022). The *P. myrica* algae extract, as a stabilizing agent, could prevent the agglomeration of the ZnO nanocrystallites (Mehrjo *et al.* 2021; Alprol *et al.* 2023).

The study presented here contributes valuable insights into the potential of zinc oxide

nanoparticles (ZnO NPs) as an innovative biopesticide solution. Insecticidal effects of ZnO NPs on stored-product insect species have been documented in previous studies (Hamza 2012; Das *et al.* 2019). The present study demonstrated the efficiency of synthesized ZnO NPs against *C. maculatus* adults. Despite the promising results, it is important to note that even at the highest dose of 2000 mg/kg, complete mortality was not achieved after 5 days of exposure resulted in 59.8-97.3% mortality. The efficacy of nanoparticles against stored product insects depends greatly on particle morphology (Vani & Brindhaa 2013; Lazarevic *et al.* 2018). Our results also indicated the dependence of ZnO NPs toxicity on shape and particle size, related to different synthesis conditions. Among the ZnO NPs, porous ZnO-A NPs with the smallest particle size (24.3 nm), followed by ZnO-C and ZnO-B, were the most toxic nanoparticles in controlling *C. maculatus* while ZnO-D NPs were the least effective. The smaller size and larger surface area of particles and their porosity probably increase insecticidal properties due to the higher adsorption capacity of cuticular fatty acids and break the wax layer of insect cuticle (Ulrichs *et al.* 2006; Katoch *et al.* 2024). Nevertheless, the increase in the adsorption with an increase in the NPs doses leads to death in a shorter time than those at lower doses. Moreover, the efficacy of the ZnO NPs generally increased with the increasing dose rate. In the study conducted by Das *et al.* (2019), the mortality of *Sitophilus oryzae* (L.) adults consistently increased as the dose of ZnO NPs and exposure time increased, which agrees with our findings. Insects' mortality due to ZnO NPs could be related to attaching nanoparticles to the cuticle followed by adsorption of epicuticular lipids in the exoskeleton of the insects, which exacerbates the water loss, causing death due to insect dehydration (Ibrahim *et al.* 2022). This toxicity mechanism was reported in many earlier studies on nanozeolites (Lu *et al.* 2017), nano-alumina (Lazarevic *et al.* 2018; Ismail *et al.* 2021), and inert dust (Prasanth *et al.* 2015).

Our findings indicate that the biosynthesized ZnO-A, ZnO-B, and ZnO-C nanoparticles exhibited

superior efficacy compared to the chemically synthesized ZnO-D nanoparticles in reducing beetle progeny and minimizing seed weight loss. The synthesized ZnO-A NPs at 1000 to 2000 mg kg⁻¹ caused more than 40% oviposition deterrence in beetles exposed to ZnO NPs-treated seeds. Reduced oviposition following exposure of adults to ZnO NPs may be due to fewer adult survivors or suffering of insects because of desiccation or blockage of spiracles (Arumugam *et al.* 2016). On the other hand, ZnO NPs may interfere with movement and mating behavior in adult insects on treated seeds. According to Reyma (2007), inspecting the treated seed coats by females of *Callosobruchus chinensis* (L.) before egg laying causes the accumulation of diatomaceous earth (DE) particles on different body parts of the insects, especially ovipositor, antennae, and mouthparts. Such accumulations of DE may inhibit sensory receptors on these organs, causing reduced activity or survival rate. A significant reduction of 35.7 to 45.6% in progeny number was also found for insects on cowpeas treated with ZnO-A NPs at 500 to 2000 mg kg⁻¹. Such reduction in beetle progeny production and subsequently less weight loss in seeds can be attributed to several mechanisms, including the parental mortality following exposure to the NPs or the loose attachment of eggs on the seed surface treated with ZnO NPs, leading to a reduction in the number of larvae being able to bore into the cowpeas (Badii *et al.* 2014). Progeny suppression of stored-product insects treated with inert dust or nanomaterials has been reported in previous studies (Kljajic *et al.* 2010; Kabir & Wulgo 2014; Badii *et al.* 2014; Lu *et al.* 2017; Lazarevic *et al.* 2018). However, potential health risks of nanomaterials on the human health should be considered. NPs applied to seeds can uptake by human body through ingestion, skin contact, and inhalation, which can cause cytotoxicity effects (Lewinski *et al.* 2008). Toxic effects have already been documented on biological systems at the pulmonary, cardiac, reproductive, renal, cutaneous and cellular levels, while nanoparticles can be distributed throughout the body, including the interior of cells (Hoet *et al.* 2004; Ostiguy *et al.*

2006). Immediate application of all useful measures, based on the precautionary principle, is necessary to limit exposure and protect the health of potentially exposed individuals.

In conclusion, our findings indicated higher efficacy of biosynthesized ZnO-A, ZnO-B, and ZnO-C NPs than chemically prepared ZnO-D NPs to protect stored cowpeas against *C. maculatus*. The application ZnO NPs on treated seeds presents a significant impact on the behavior and reproductive success of *C. maculatus* adults. Additional experiments are required to improve the most effective ZnO-A NPs by increasing porosity and decreasing particle size. Overall, the integration of ZnO NPs into pest control strategies could offer a promising avenue for enhancing the protection of

stored grains while minimizing reliance on traditional chemical pesticides. Further research is warranted to fully understand the mechanisms at play and to optimize the application of these nanomaterials for sustainable pest management along with test their effects on percent germination and physical properties of the cowpea seeds. In addition, potential risks of synthesized ZnO NPs on human health should be explored.

Acknowledgements

The authors gratefully acknowledge the financial and technical supports provided by the Department of Plant Protection and Chemistry Department, Persian Gulf University. We also thank Fereshteh Iranpour for her kind help in lab work.

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بررسی کارایی حشره‌کشی نانوذرات اکسید روی سنتز شده با عصاره جلبک قهوه‌ای *Polycladia myrica* علیه سوسک چهار نقطه‌ای حبوبات (Col.: *Callosobruchus maculatus* (Col.: Chrysomelidae: Bruchinae)

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دریافت: ۱۴۰۴/۰۵/۰۸ بازنگری: ۱۴۰۴/۱۰/۰۷ پذیرش: ۱۴۰۴/۱۱/۲۰

چکیده

خطرات زیست‌محیطی و سلامت انسان مرتبط با حشره‌کش‌های شیمیایی، بررسی‌های گسترده‌ای را برای یافتن روش‌های جایگزین برای محافظت از محصولات انباری برانگیخته است. اخیراً، نانوذرات به عنوان جایگزین‌های امیدوارکننده‌ای برای محصولات شیمیایی مصنوعی در نظر گرفته شده‌اند. در این مطالعه، نانوذرات روی (ZnO NPs) با استفاده از عصاره جلبک *Polycladia myrica* سنتز شدند و با استفاده از پراش اشعه ایکس (XRD)، طیف‌سنجی مادون قرمز (FTIR) و میکروسکوپ الکترونی روبشی نشر میدانی (FE-SEM) شناسایی شدند. سه نوع مختلف از نانوذرات روی، ZnO-A، ZnO-B و ZnO-C، با استفاده از عصاره جلبک و با دو روش مختلف سنتز شدند. فعالیت حشره‌کشی آنها علیه سوسک چهار نقطه‌ای حبوبات، *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) روی لوبیا چشم بلبلی انبارشده ارزیابی و با نانوذرات روی سنتز شده به صورت شیمیایی ZnO-D مقایسه شد. نانوذرات ZnO-A، ZnO-B و ZnO-C بیوسنتز شده، فعالیت بالاتری را علیه *C. maculatus* در مقایسه با نانوذرات ZnO-D نشان دادند. نانوذرات متخلخل ZnO-A با کوچکترین اندازه ذرات (۲۴/۳ نانومتر) به عنوان سمی‌ترین نانوذرات تعیین شدند که پس از پنج روز قرار گرفتن در معرض آنها، بالاترین میزان مرگ و میر حشرات بالغ *C. maculatus* را ایجاد کردند، در حالی که نانوذرات ZnO-D کمترین تأثیر را در کنترل *C. maculatus* داشتند. کاهش قابل توجه تخم‌ریزی (۳۵/۱ تا ۴۴/۹ درصد) و کاهش نتاج (۳۵/۷ تا ۴۵/۶ درصد) نیز برای حشراتی که در معرض لوبیا چشم بلبلی تیمار شده با نانوذرات ZnO-A با غلظت ۵۰۰ تا ۲۰۰۰ میلی‌گرم در کیلوگرم قرار داشتند، مشاهده شد. میانگین کاهش وزن دانه‌های لوبیا چشم بلبلی تیمار شده با نانوذرات ZnO-A، ZnO-B و ZnO-C در غلظت ۲۰۰۰ میلی‌گرم در کیلوگرم به ترتیب ۲۳/۸، ۲۸/۱ و ۳۳/۵ درصد بود که اختلاف معنی‌داری نسبت به شاهد (۵۲/۲ درصد) داشتند. نتایج این بررسی نشان می‌دهد که نانوذرات روی بیوسنتز شده می‌توانند به عنوان یک جایگزین مناسب برای کنترل *C. maculatus* در لوبیا چشم بلبلی انبار شده در نظر گرفته شوند.

کلمات کلیدی: سوسک چهار نقطه‌ای حبوبات، *Polycladia myrica*، کنترل آفت، نانوتکنولوژی

