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Insecticidal evaluation of chitosan-based zinc oxide nanoparticles against *plutella xylostella* (Lepidoptera: Plutellidae)

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Abstract
The diamondback moth (*Plutella xylostella*), a major pest of cruciferous vegetables, has developed resistance to many conventional chemical and biological insecticides, necessitating the exploration of new pest control solutions. Bio-insecticides derived from plant-based compounds offer eco-friendly alternatives to synthetic pesticides. Recent advancements in synthesizing zinc oxide nanoparticles (ZnO NPs) using Chitosan (Cs) extracts have opened avenues for innovative approaches in agricultural pest management. This study aimed to evaluate the insecticidal potential of chitosan-coated zinc oxide nanoparticles (Cs-ZnO NPs) against *P. xylostella*. The nanoparticles were characterized using UV–vis spectroscopy, FTIR, SEM, TEM, EDX, and XRD techniques, confirming their structural and chemical properties. Laboratory bioassays revealed that Cs-ZnO NPs exhibited exceptional larvicidal and pupicidal activity, achieving 100% mortality at low concentrations (2–10 ppm). Toxicity assessments indicated LC_{50} values of 1.774 ppm for first-instar larvae and 7.784 ppm for pupae, significantly lower than those for chitosan, which ranged from 39.854 ppm to 111.338 ppm at dosages of 20–100 ppm. Additionally, treatment with Cs-ZnO NPs significantly reduced the longevity and fecundity of *P. xylostella* adults while severely disrupting their food utilization efficiency. These findings highlight the potential of Cs-ZnO NPs as a novel, effective, and sustainable insecticide for managing resistant agricultural pests, *P. xylostella*.

Keywords: *Plutella xylostella*, chitosan, ZnO, Nano-characterization, biopesticide

Introduction

The Diamondback moth (DBM), *Plutella xylostella* (Linnaeus) (Plutellidae: Lepidoptera), stands as the primary destructive pest affecting cruciferous crops, including cauliflower, cabbage, and mustard. This pest inflicts considerable economic damage, impacting 92% of farmers and leading to losses of up to 50%, with an estimated annual cost of US$ 168 million [1]. The harm inflicted by this insect on the host plant is connected to the feeding behaviours observed during the larval stage. The caterpillars often damage the parenchyma tissue, creating holes in the leaf surface and diminishing the area available for photosynthesis. The rapid reproduction rate (for instance, exceeding 20 generations annually in tropical areas) significantly escalates plant damage, rendering the complete harvesting of crops impractical unless containment strategies are implemented. Moreover, the limited presence of natural predators combined with the significant genetic diversity of this insect encourages the emergence of resistance to chemical pesticides [2]. Significant issues, such as the emergence of pesticide resistance and the return and epidemics of secondary pests, have been brought about by the lengthy history of careless pesticide use. Significantly, *P. xylostella* holds the distinction of being the first insect pest documented to exhibit resistance to DDT in 1953 in Java, Indonesia demonstrating resistance to almost all categories of insecticides throughout the years [3, 4]. Farmers rely significantly on conventional insecticides to manage *P. xylostella*; however, the pest's increasing resistance to nearly all classes of insecticides diminish their effectiveness. Furthermore, the overuse of insecticides leads to negative impacts on non-target organisms, including the decline of natural predators, which adds complexity to the management of *P. xylostella*. Considering the adverse impacts of insecticides, biological control offers a promising approach for the effective management of *P. xylostella* [1]. An immediate necessity exists for sustainable control solutions.

Nanotechnology is progressively finding applications in the production of pesticides and the management of pests. Nanotechnology has the potential to improve crop yield and facilitate the creation of innovative pest management solutions [5]. A study stated the application of nanocapsules or nanoparticles containing crop fortification agents, including pesticides, herbicides, repellents, and pheromones, has emerged as a method for pest control. Chitosan and other polymer-based nanomaterials have attracted significant interest across multiple domains within agriculture. Chitosan-derived nanoparticles exhibit potential biological activities owing to their biodegradable and nontoxic characteristics [6].

Chitin, present in certain seafood shells and fungal membranes, requires chemical transformation to yield CS polymers. The functionality, biodegradability, compatibility, safety, and potential antibacterial properties of these CS-NPs make them highly regarded bio-based nanomaterials suitable for a range of applications [7, 8]. Recently, metal-based nanomaterials have attracted significant interest because of their exceptionally accurate physicochemical properties and the wide range of potential applications across various fields, including technology, agriculture, healthcare, food safety, environmental remediation, and beyond [9, 10].

ZnO NPs have been receiving significant attention as a promising alternative across multiple fields such as optical science, electronics, food packaging, and pharmaceuticals, due to their biocompatibility, low toxicity, and cost-effectiveness [10]. Previously a study showed remarkable

biocompatibility of ZnO at the nanoscale, making it particularly effective in a range of biomedical applications and drug delivery systems [11]. Consequently, numerous researchers have initiated the synthesis of ZnO NPs, which serve various purposes such as biological sensing, labelling, gene delivery, and therapeutic applications. This exhibits properties that are antifungal, antibacterial, and mosquitocidal [12]. Several studies have revealed that NPs, such as ZnO and CS, demonstrate significant antibacterial effects against both Gram-positive and Gram-negative bacteria [13, 14]. The application of chitosan coating on metal oxide nanoparticles, especially zinc oxide nanoparticles (ZnO NPs), has garnered significant interest due to its non-toxic, environmentally friendly nature and diverse applications [15, 16].

This study presents an eco-friendly approach for the synthesis of Chitosan-Coated Zinc Oxide Nanoparticles, characterised using various biophysical methods such as UV–vis spectroscopy, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), and Zeta potential analysis. This study aims to investigate the larvicidal and pupicidal effects, as well as the longevity and fecundity, of chitosan and Chitosan-Coated Zinc Oxide Nanoparticles (Cs-ZnO NPs) on *P. xylostella*. The purpose of this research is that the larvicidal and pupicidal effects, as well as the longevity and fecundity, of chitosan and Chitosan-Coated Zinc Oxide Nanoparticles (Cs-ZnO NPs) on *P. xylostella*. Additionally assessed the food utilisation behaviour of insect pests following treatment with Cs-ZnO nanoparticles. Furthermore, examining the polyphagous insect's perspective when exposed to Cs-ZnO NPs will contribute to the development of more effective pest control strategies.

Materials and methods

Collection and processing of crab shells

Mud crab shells (*Scylla Serrata*) were sourced from a local market in Coimbatore, Tamilnadu, India. Samples were maintained at low temperatures using ice throughout their transport to the laboratory. The exoskeletons of the crab were meticulously cut into smaller pieces using a meat tenderizer. Twenty grammes of crushed crab exoskeletons were accurately measured with a Mettle balance, subsequently labelled, and subjected to oven drying at 65 °C for a duration of four consecutive days to achieve a constant weight. The samples' dry weight was established, and the moisture content was assessed by calculating the differences between the wet and dry weights. The mean moisture content observed in the crab exoskeletons was 15.66%.

Isolation and Extraction of Chitosan from Crab Shell

The process of recovering chitosan included the washing of crushed crab exoskeletons using distilled water. Crushed crab exoskeletons were immersed in 1000 ml beakers containing boiling sodium hydroxide (2 and 4% w/v) for 1 hour to dissolve the proteins and sugars, thereby isolating the crude chitin. A 4% NaOH solution is utilized for the preparation of chitin, as indicated by the concentration employed at the Sonat Corporation [17]. Subsequently, the samples underwent boiling in sodium hydroxide. The beakers holding the shell samples were taken off the hot plate and permitted to cool for 30 minutes at room temperature [18]. The exoskeletons were subsequently pulverized into fragments measuring 0.5–5.0 mm with the aid of a meat tenderizer. The pure chitosan obtained was demineralized utilizing 1% HCl (v: v) and 2% NaOH, following the method outlined by Huang *et al*. (2004) [19]. A solution of 50% NaOH was introduced to demineralized chitosan and subjected to boiling at 100 °C for a duration of 2 hours on a hot plate, followed by a cooling period of 30 minutes at room temperature. The procedure was conducted again to deacetylate the chitosan powder, resulting in a creamy-white form of chitosan.

Preparation of CS-ZnO NPS

A solution of Chitoan at a concentration of 1.0 % (w/v) was prepared by dissolving it in a 1% (v/v) acetic acid. Following adequate dissolution, 45 ml of 0.1 M Zn $(CH_3COO)_2 \cdot 2H_2O$ was incorporated into 5 mL of chitosan solution. The pH of the combinations was adjusted by the addition of 0.1 M NaOH, followed by continuous mixing for 4 hours at 85°C to achieve a pH range of 9 - 10. Subsequently, the drying process was conducted at 120°C for a duration of 2.5 hours, followed by annealing for 4 hours at 450°C to produce CS-ZnO NPs [20, 21, 22].

Chitosan-Coated Zinc Oxide Nanoparticles (Cs-ZnO NPs) characterization

The analysis of the reaction mixture through UV-visible spectra confirmed the morphological and physicochemical attributes of the Cs-ZnO NPs. The following characterisation utilised methods including FESEM, TEM, and EDAX. XRD analysis was employed to assess the purity of the phase in the Cs-ZnO NPs. Furthermore, the dimensions of the particles and the related chemical groups with particular functions were analysed. FTIR spectroscopy was employed, as described by Ramimoghadam *et al*. (2013) [23].

Rearing of *P. xylostella*

Larvae and adults of *P. xylostella* were gathered from cabbage crops located in Thondamuthur (10°59′24.9″N, 76°50′46.3″E, Coimbatore, Tamil Nadu, India). A total of 500 adult specimens were placed in a plastic cage measuring 50× 30× 30 cm^3 and were supplied with leaves from cabbage, *Brassica oleracea* L. var. botrytis (Brassicaceae), for the purpose of egg laying. Eggs were permitted to hatch on *B. oleracea* leaves, and larvae were kept at a temperature of 25±1 °C and 65±5% relative humidity (RH) under a 16L: 8D photoperiod within a growth chamber. Acute toxicity experiments were conducted using larval instars I–IV and pupae. Recently emerged male and female adults underwent testing for experiments focused on longevity and fecundity.

Toxicity against *P. xylostella*

Following the methodology outlined by Sengonca *et al*. (2006) [24], the F2 generation larvae were given cabbage leaf discs that had been subjected to chitosan at concentrations of 20, 40, 60, 80, and 100 ppm, or Cs-ZnO NPs at concentrations of 2, 4, 6, 8, and 10 ppm. After a duration of 24 hours, the subjects were moved to fresh cabbage leaves that had not undergone any treatment. The leaves underwent replacement daily, specifically every 24 hours. The mortality rate was recorded after a period of 96 hours. Each treatment involved five replicates, with each replicate consisting of 10 larvae.The formula of Hardstone *et al*. (2009) [25] was used to compute the percentage mortality.

Corrected mortality= (mortality in treatment-mortality in control)/ (100- mortality in control) × 100.
The larvae that remained alive were provided with untreated cabbage leaves as their food source until they entered the pupal stage. Pupal mortality was determined by subtracting the count of emerging adults from the overall number of pupae.

Impact on longevity and fecundity of *P. xylostella*
Newly emerged males and females of *P. xylostella* were placed in the wooden cages at a 1:1 sex ratio (n = 20). Ten hours post-emergence, the subjects were given an artificial diet comprising 20 mg of sucrose, 1 mL of honey mixed in 1 mL of sterile distilled water, along with 1 mL of an aqueous solution containing chitosan at concentrations of 20, 40, 60, 80, and 100 ppm, or Cs-ZnO NPs at concentrations of 2, 4, 6, 8, and 10 ppm. The control consisted of a diet that excluded chitosan and Cs-ZnO nanoparticles. The mean fecundity was determined by monitoring the daily egg-laying on five freshly selected leaves over a period of four consecutive days, divided by the number of females allowed to mate (n = 20). Daily assessments of mortality were conducted, and the average lifespan of each adult was determined.

Quantitative food utilization efficiency measures
A gravimetric method was used to assess weight gain, food intake, and faeces production by *P. xylostella*. Weight was measured with a monopan balance, precise to 0.1 mg. Newly moulted IV instar larvae were subjected to a starvation period of 3 hours. Following the measurement of the larvae's initial weight, each one was placed into its own distinct container. The larvae (n=20 per concentration, with five replicates for each) were permitted to consume measured amounts of both treated and untreated selected leaves over a duration of 24 hours. Subsequently, the larvae underwent another weighing process. The variation in the larvae's weight indicates the fresh weight acquired throughout the study period. Sample caterpillars were weighed, then subjected to oven drying for 48 hours at 60°C, after which they were reweighed to determine the percentage of dry weight of the experimental caterpillars. The leaves that remained at the end of each day were oven-dried and re-weighed to determine the percentage of dry weight of the diet that remained at the conclusion of each experiment, based on the total dry weight of the diet provided. Daily collections of faces were conducted, followed by weighing, oven drying, and subsequent reweighing to determine the dry weight of the excreta. The experiment was conducted over a span of four days, with observations meticulously recorded at 24-hour intervals. Consumption, growth rates, and post-digestive food utilisation efficiencies (all based on dry weight) were determined using the methodology established by Waldbauer, (1968) and Slansky and Scriber, (1985 [26, 27]
Consumption index (CI) = E/TA
Relative growth rate (RGR) = P/TA
Approximate digestibility (AD) = 100 (E-F)
Efficiency of conversion of ingested food (ECI) = 100 P/E
Efficiency of conversion of digested food (ECD) = 100 P/ (E-F)
Where,
A = mean dry weight of animal during experiment
E = dry weight of food eaten
F = dry weight of f food eaten
P = dry weight gained by the insect
T= duration of experimental period

Results and discussion
Characterization of essential oil loaded Chitosan nanoparticles
UV–Visible Spectroscopy
The study performed using the UV-vis spectrophotometer for Cs-ZnO NPs indicated a band at around 316 nm. The symmetrical structures of this band demonstrated a uniform dispersion, confirming the presence and stability of the particles (Fig. 1a). A prior study revealed that ZnO nanoparticles display a distinct absorption band in the 280–400 nm region [28]. It has been previously noted that ZnO/CS composites demonstrate a significant absorption band within the range of 350–380 nm [29]. The spectra validate the arrangement of the ZnO nanoparticles on the chitosan surface, leading to the formation of the ZnO/CS composite. Furthermore, the observation of blue shift in the UV spectra indicates the formation of the CS/ZnO nanocomposite, likely attributed to the presence of zinc oxide [30, 31]. Oh *et al.* (2019) [32] demonstrated the UV-Visible spectrum of chitosan nanoparticles, highlighting a prominent peak at 320 nm with sharp intensity.

FTIR studies
The FTIR transmittance spectra of Cs-ZnO NPs, illustrated in Fig. 1b, display distinctive bands within the range of 600-4000 cm^{-1} at particular peaks: 3322.75 cm^{-1}, 2917.77 cm^{-1}, 2135.78 cm^{-1}, 1644.02 cm^{-1}, 1390.42 cm^{-1}, 1149.37 cm^{-1}, 1034.42 cm^{-1}, 893.844 cm^{-1}, 813.813 cm^{-1}, and 753.066 cm^{-1}.For instance, the broad band noted at 3322.75 cm^{-1} and the minor absorption peak near 1390.42 cm^{-1} are associated with O–H stretching, which is characteristic of alcohol and phenol compounds. The peaks observed at 2917.77 cm^{-1} and 753.066 cm^{-1} correspond to the stretching of C-H bonds in alkanes. The absorption peak observed at approximately 2135.78 cm^{-1} is attributed to the C-H modes characteristic of aromatic compounds. The observed peaks at 1644.02 cm^{-1}, 893.844 cm^{-1}, and 813.813 cm^{-1} are indicative of C=C stretching vibrations present in alkene compounds. The C-O stretch in primary and secondary alcohol groups is observed at 1149.37 cm^{-1} and 1034.42 cm^{-1}. In earlier studies by Jayachandran *et al.* (2021) [33], the broad band identified at 3273 cm^{-1} was linked to the O–H stretching typical of phenolic compounds. The alkene group was detected at 1624 cm^{-1} as reported by Hu *et al.* (2021) [34]. A recent study conducted by Mohammed *et al.* (2023) [35] examined deep bands observed in the range of 1250 to 1100 cm^{-1}. The observed bands are associated with C–O stretching vibrations, serving as indicators of organic compounds, and possibly playing a role in the environmentally friendly synthesis of Cs-ZnO nanoparticles.

X-ray diffraction studies
The phase and crystalline structure were analysed through X-ray diffraction of Cs-ZnO NPs, leading to the recognition of a distinct and specific diffraction pattern (Fig. 1c). The Cs-ZnO NPs displayed distinct peaks at 2θ values of 31.97, 34.81, 36.87, 47.98, 56.91, 62.91, 66.93, 68.51, 69.62, 72.74, and 77.87, which align with the (hkl) diffraction planes (100), (002), (101), (102), (110), (103), (200), (112), (201), (004), and (202) respectively. The exact peaks

observed aligned perfectly with the pattern recorded in the JCPDS card No: 36-1451, thereby validating the presence of a hexagonal phase structure in the synthesised ZnO nanoparticles [36]. The broad diffraction peak at 2θ ~ 20◦ was observed in the diffraction pattern of chitosan-ZnO nanocomposites, indicating the presence of semi-crystalline chitosan and confirming that the hexagonal ZnO nanoparticles were effectively integrated into the chitosan matrix [37]. The identification of phases in the XRD pattern demonstrates the effective synthesis of CS-ZnO NPs. The size of the crystallites in the nanoparticles was determined utilising the Scherrer formula (Tamanna *et al*., 2024) [38].

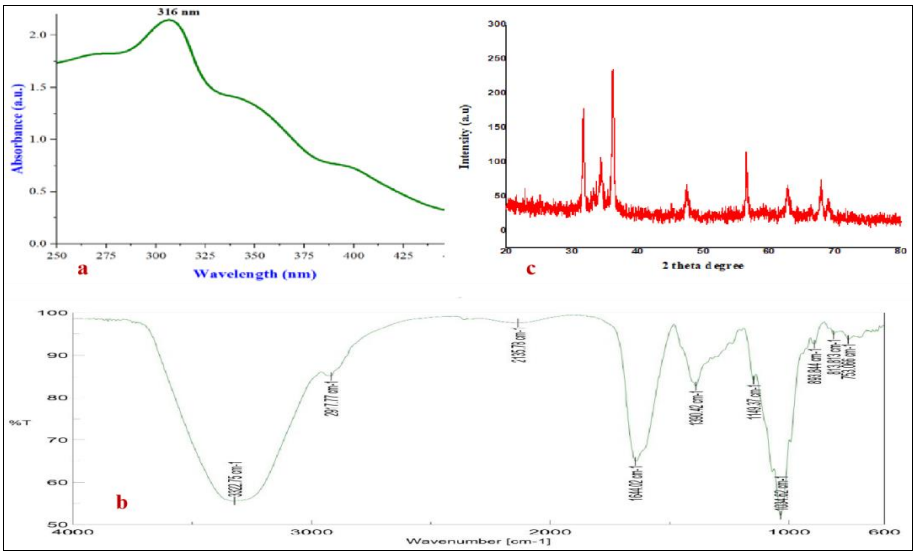

Fig 1: a) UV-visible spectrum, b) FTIR and c) XRD analysis of Chitosan synthesized ZnO nanoparticles

Studies on morphology and elements

SEM and TEM Analysis

The morphological characteristics of Cs-ZnO nanoparticles are being examined using Field Emission Scanning Electron Microscopy (FESEM) to determine the shape of the ZnO nanoparticles. The SEM analysis demonstrates the spherical morphology of the synthesised nanoparticles, indicating the presence of agglomerated particles as illustrated in Figure 2a. Distinct clustering with uneven particle forms, occasionally resembling spherical and oval shapes, along with a multitude of pores, is noted. Several studies have explored how surface morphology influences the synergistic activity of ZnO [33]. The SEM analysis verifies the spherical morphology of the ZnO nanoparticles, characterised by smooth edges, aligning with earlier studies (Gandhi *et al*., 2017; Elumalai *et al*., 2015) [39, 40].

The surface structure and particle size of the Cs-ZnONPs were investigated using Transmission Electron Microscopy (TEM), as illustrated in Figure 2b (a-c). The TEM image (Figure 2b(a)) shows agglomerated particles of synthesised Cs-ZnO NPs, which display both spherical and hexagonal shapes, with an average particle size of 19.78 nm. The team utilised high-resolution transmission electron microscopy (HR-TEM) to confirm the spherical shape of the nanoparticles. Faisal *et al*. (2021) [36] highlighted the application of aqueous fruit extracts from Myristica fragrans in the environmentally friendly synthesis of zinc oxide (ZnO) nanoparticles led to morphologies that varied from spherical to hexagonal. The synthesis of Chitosan-Coated Zinc Oxide Nanoparticles was validated through a comparison of particle sizes obtained via transmission electron microscopy and X-ray diffraction, which substantiated the effective fabrication of the nanoparticles [15].

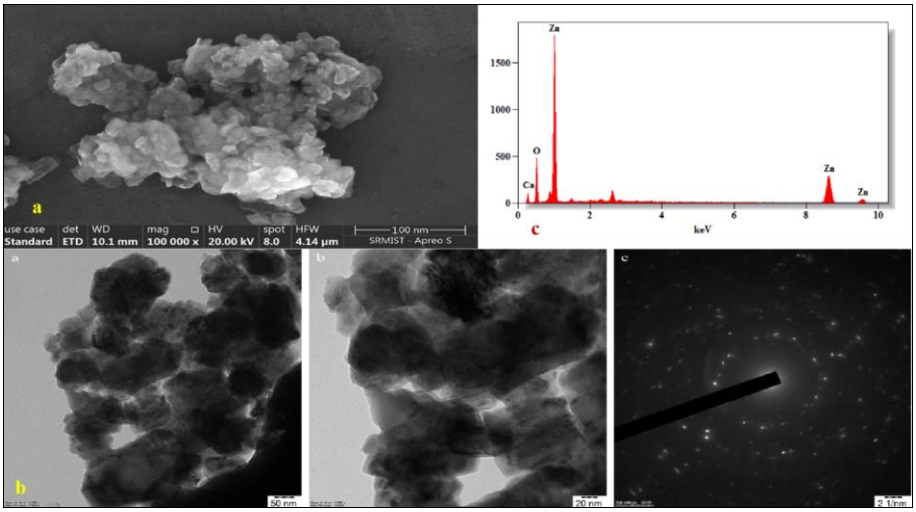

Fig 2: a) SEM, b) TEM and c) Energy dispersive X-ray analysis of Chitosan synthesized ZnO nanoparticles

Energy Dispersive X-ray Analysis (EDX) Spectrum of Cs-ZnO NPs

The elemental composition of Cs-ZnO NPs was obtained using Energy Dispersive X-ray Spectroscopy (EDS). The EDS analysis revealed that Cs-ZnO consisted solely of Zn, O, and Ca atoms. The expected stoichiometric mass percentages of Zn (68%), O (20%), and Ca (12%) in the compound Cs-ZnO NPs are presented in Figure 2c. The analyses conducted using energy-dispersive X-ray Spectroscopy (EDS) in our study reveal consistent results for the synthesised nanoparticles. In a previous investigation conducted by Fakhari *et al*. (2019) [41], the elemental composition was documented, revealing that zinc constituted 80.3% and oxygen accounted for 19.7% of the total composition.

Larvicidal and Pupicidal Toxicity

The insecticidal potential of chitosan extract derived from crab shell was assessed at different concentrations (twenty, forty, sixty, eighty, and hundred ppm) targeting the larval young instars and pupae stages of the diamondback, *P. xylostella*. At a concentration of 100 ppm, the first instar larvae exhibited a mortality rate of 79.96%, which progressively decreased to 69.86%, 64.08%, and 55.52% in the second to fourth instar stages, with a final rate of 44.1% observed in pupae. The mortality data were analysed to determine the LC_{50} and LC_{90} values, yielding LC_{50} values of 39.854 ppm, 53.342 ppm, 73.319 ppm, 89.003 ppm, and 111.338 ppm, alongside LC_{90} values of 128.603 ppm, 155.169 ppm, 168.726 ppm, 191.498 ppm, and 216.517 ppm for the first, second, third, fourth instars, and pupal population, as presented in Table 1. It is important to highlight that the control treatment showed no instances of mortality.

The chitosan synthesised ZnO NPs (Cs-ZnO NPs) demonstrated significant larvicidal and pupicidal effects against the diamondback moth, *P. xylostella*, achieving 100% mortality at a concentration of 50 ppm. The LC_{50} values for Cs-ZnO nanoparticles concerning the instar larval stages (I, II, III, IV) and pupae of diamondback, *P. xylostella*, were established as 1.774 ppm, 2.917 ppm, 3.980 ppm, 6.246 ppm, and 7.784 ppm, respectively. The calculated LC_{90} values were determined to be 7.182 ppm, 9.602 ppm, 13.440 ppm, 16.234 ppm, and 17.475 ppm, as presented in Table 2.

The results align with the observations made by Namasivayam *et al*. (2018) [42], who noted a comparable dose–mortality relationship for *N. Rileyi* chitosan nanoparticles when tested on various larval instars of *S. litura*. The observed LC_{50}values of nanoparticles exhibited a rising trend corresponding to the advancement in larval age. This trend in larval mortality may be associated with the tolerance of larval instars during their growth phase. The median lethal concentration (LC_{50}) of *M. anisopliae*–chitosan nanoparticles targeting second instar *P. xylostella* larvae in laboratory conditions was found to be 14.44 ppm. This contrasts with the LC_{50} of *B. brongniartii* Fe^0NPs, which was recorded at 58 ppm against second instar *S. litura* larvae, as noted by Xu *et al*. (2020) [43]. In earlier work, Paulraj *et al*. (2017) [44] indicated that the nano-formulation of chitosan tripolyphosphate PONNEEM demonstrated the highest larvicidal activity of 90.2% at a concentration of 0.3%, along with growth regulating effects on *H. armigera* larvae at significantly low concentrations.

Table 1: Larvicidal and pupicidal effect of chitosan extract from crab shell against the diamondback moth, *Plutella xylostella*

Larval and pupal stage	Larval and pupal mortality (%) (Mean±S.D) Concentration (ppm)					LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.*=4)
	20	40	60	80	100		Lower	Upper		
Larva I	37.5±1.9	49.8±2.4	64.0.±1.7	71.0±3.2	79.9±0.9	39.854 (128.603)	28.235 (111.265)	48.255 (158.734)	*x*= 0.014 *y*= -0.576	0.561n.s
Larva II	32.4±2.6	42.0±2.2	57.1±2.6	64.0±2.6	69.8±1.6	53.342 (155.169)	42.939 (130.643)	62.328 (201.6787)	*x*= 0.013 *y*= -0.671	1.097 n.s
Larva III	23.8±2.4	32.7±1.0	42.4±2.1	53.7±2.4	64.0±1.9	73.319 (168.726)	64.815 (142.609)	84.262 (216.7401)	*x*=0.013 *y*= -0.985	0.011n.s
Larva IV	19.6±1.8	26.7±2.6	35.7±1.3	45.6±1.7	55.5±2.3	89.003 (191.498)	78.423 (158.455)	106.086 (256.279)	*x*= 0.013 *y*= -1.113	0.010 n.s
Pupae	12.4±1.5	20.6±1.7	26.1±1.0	35.4±2.1	44.1±2.7	111.338 (216.517)	96.288 (175.609)	140.102 (301.884)	*x*= 0.012 *y*= -1.357	0.220 n.s

Table 2: Larvicidal and pupicidal effect of Cs-ZnO Against the diamondback moth, *Plutella xylostella*

Larval and pupal stage	Larval and pupal mortality (%) (Mean±S.D) Concentration (ppm)					LC_{50} (LC_{90})	95% Confidence Limit LC_{50} (LC_{90})		Regression equation	χ^2 (*d.f.*=4)
	2	4	6	8	10		Lower	Upper		
Larva I	55.7±2.7	69.1±2.0	79.8±3.4	91.8±1.6	100±0.0	1.774 (7.182)	0.651 (6.475)	2.546 (8.179)	*x*= 0.237 *y*= -0.420	4.792 n.s
Larva II	48.2±2.1	55.4±1.4	66.8±2.6	82.3±1.1	94.8±0.8	2.917 (9.602)	1.868 (8.593)	3.670 (11.1347)	*x*= 0.192 *y*= -0.559	4.643 n.s
Larva III	42.2±1.6	48.9±2.5	56.3±2.4	71.8±2.6	80.7±1.6	3.980 (13.440)	2.724 (11.502)	4.867 (16.948)	*x*=0.135 *y*= -0.539	1.404 n.s
Larva IV	31.5±1.0	37.5±2.0	46.0±2.1	58.5±2.4	70.3±1.4	6.246 (16.234)	5.344 (13.667)	7.200 (21.058)	*x*= 0.128 *y*= -0.801	0.756 n.s
Pupae	23.5±3.6	30.6±1.9	37.6±0.8	51.9±2.1	62.4±2.0	7.784 (17.475)	6.896 (14.698)	9.015 (22.650)	*x*= 0.132 *y*= -1.029	0.537 n.s

Impact of Cs-ZnO NPs on *P. xylostella* longevity and fecundity

The impact of Cs-ZnO NPs on decreasing adult lifespan and reproductive capacity in the diamond-back moth *P. xylostella* is illustrated in Table 3. After administering chitosan extracts derived from crab shell at a concentration of 100 ppm, the lifespan of males was reduced to 8.0 days, in contrast to the control group, which sustained an average of 17.80 days. In a similar manner, the application of chitosan resulted in a decrease in female longevity to 11.1 days, whereas the control group sustained an average of 24.20 days. The application of chitosan led to a notable decrease in fecundity; the control group yielded 875 eggs, while the treatment groups generated 330 eggs at concentrations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm, respectively (Table 3).

The chitosan synthesised ZnO NPs (Cs-ZnO NPs) demonstrated adult longevity and fecundity in the diamond-back moth *P. xylostella*, as shown in Table 3. After administering chitosan extracts derived from crab shell at a concentration of 10 ppm, the lifespan of males was reduced to 4.6 days, in contrast to the control group, which sustained longevity of 17.80 days. In a similar manner, the administration of chitosan resulted in a decrease in female longevity to 5.0 days, whereas the control group exhibited longevity of 24.20 days. The application of chitosan led to a notable decrease in fecundity; the control group yielded 875 eggs, while the treatment groups generated 145.6 eggs at concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, respectively (Table 3). This investigation represents the initial evaluation of the impact of chitosan synthesised ZnO NPs on the lifespan and reproductive capacity of moth parasites. Additional evidence reinforces the idea that chitosan-synthesized ZnO NPs are harmful to agricultural pests.

Table 3: Impact of Chitosan extract from crab shell and Cs-ZnO NPs on longevity and fecundity of the crop pest *plutella xylostella*.

Treament	(ppm)	Adult longevity (days)		Fecundity (Nos.)
		Male	Female	
Chitosan	Control	17.8±1.4	24.2±0.6	875.1±0.9
	20	16.4±2.9	19.2±2.0	840.2±1.0
	40	14.8±1.0	17.1±2.9	771.3±1.4
	60	12.2±2.8	16.4±1.1	560.2±1.6
	80	11.2±1.7	14.5±2.1	458.1±1.4
	100	8.0±1.0	11.1±2.0	330.9±0.2
Cs-ZnO NPs	2	13.9±2.5	14.1±1.0	670.9±1.8
	4	8.1±1.8	12.0±1.2	472.8±1.7
	6	7.2±1.0	8.8 ±1.7	344.1±0.6
	8	6.1±1.1	7.2±1.0	228.7±0.8
	10	4.6±1.2	5.0±0.7	145.6±1.6

In a similar vein, Ammar and Abd-ElAzeem, (2021) [45] demonstrated a decrease in longevity for both male and female *Eariasinsulana* (Frauenfeld) following the application of gelatin copper bio-NPs. The morphological and genetic traits of *H. armigera* exhibited similarities to those of the species examined by Queiroz-Santos *et al*. (2018) [46]. The highest mortality was observed with Cs-ZnO NPs at elevated concentrations, in contrast to the use of chitosan alone. This could be attributed to the combined influence of the nanoparticles.

Food Utilization measures

Table 4 shows the food consumption measurements for *P. xylostella* VI instar larvae after they were treated with Cs-ZnO nanoparticles and S. chitosan extract made from crab shell. The consumption index of larvae of *P. xylostella* remained unaffected by chitosan, even at the elevated concentration of 100 ppm tested, suggesting that it does not repel the insect. The initial leaf disc choice bioassay assessed the efficacy of the synthesised Cs-ZnO nanoparticles in reducing insect feeding activity. The consumption index of Cs-ZnO nanoparticles has shown a significant decrease, demonstrating a relationship that is dependent on the dosage. At a concentration of 10 ppm, the Cs-ZnO nanoparticles demonstrate significant reduction. The findings regarding weight gain, CI, AD, ECI, and ECD of *P. xylostella* align closely with the report by Murugan *et al*. (2018) [47]. For example, the application of azadirachtin and various triterpenes derived from neem led to a decrease in RGR, RCR, ECI, and ECD in moths [48]. Devi *et al*. (2014) [49] indicated that *H. armigera* subjected to synthesised AgNPs derived from *E. hirta* exhibited a notable decrease in the Consumption index (CI), efficiency of ingested ECI, and digested ECD, resulting in a reduction of the regulative growth rate (RGR) from 4.86 to 2.00 mg mg day. Shukla and Patel, (2011) [50] examined how ECI and ECD differed in *S. litura* when fed on various host plants, including tobacco, Chinese cabbage, cowpea, sweet potato, and banana (cv Grand Naine leaves).

Table 4: Impact of Chitosan extract from crab shell and Cs-ZnO NPs on food utilization of the crop pest *Plutella xylostella*

Treatment (ppm)		CI	RGR	ECI (%)	ECD (%)	AD (%)
Control		26.61±2.01^{a}	12.28±2.24^{a}	31.29±1.65^{a}	36.48±1.96^{a}	69.32±1.24^{a}
Chitosan	20 ppm	25.12±2.33^{b}	11.51±1.22b	29.55±1.57^{b}	34.20±1.20^{b}	67.24±1.28^{b}
	40 ppm	23.61±2.24^{c}	10.14±1.64^{c}	26.81±1.24^{c}	30.11±1.46^{c}	64.18±2.46^{c}
	60 ppm	19.56±1.28^{d}	8.61±1.55^{d}	23.63±1.54^{d}	26.44±1.81^{d}	59.44±2.44^{d}
	80 ppm	18.40±2.46^{e}	8.10±0.76^{e}	20.54±1.81^{e}	24.93±1.66^{e}	55.43±0.51^{e}
	100 ppm	16.61±1.94^{f}	7.91±1.81^{f}	18.41±2.20^{f}	19.21±1.25^{f}	50.27±2.01^{f}
Cs- ZnONPS	2 ppm	21.16±2.22^{a}	8.31±0.68^{a}	27.45±1.10^{a}	32.64±1.18^{a}	56.09±1.36^{a}
	4 ppm	19.28±2.38^{b}	7.53±1.10^{b}	23.01±1.47^{b}	27.23±1.14^{b}	52.21±1.89^{b}
	6 ppm	15.20±1.46^{c}	7.10±1.45^{c}	18.98±2.38^{c}	21.96±0.02^{c}	45.94±1.49^{c}

	8 ppm	11.22±2.44[d]	6.81±0.23[d]	14.66±1.75[d]	18.23±0.94[d]	40.33±0.24[d]
	10 ppm	9.30±1.06[e]	5.09±1.62[e]	10.61±2.08[e]	15.04±0.07[e]	36.66±0.81[e]

Within each tested product, values followed by the same letter(s) are not significantly different (ANOVA, Tukey's HSD, α=0.05.

Conclusion

The chitosan extracted from freshwater crab shells and chitosan-coated zinc oxide nanoparticles exhibited significant effects on the growth, development, and insecticidal activity against crop pest, *P. xylostella* larvae. The insecticidal properties of chitosan-coated ZnO nanoparticles present a promising strategy for pest control, offering a potential alternative that minimizes the toxicological impact on the environment. This research contributes valuable insights that could aid in the development of novel approaches to combat biological insecticide resistance.

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Declaration of competing interest

The author (s) declare (s) that there is no conflict of interest regarding the publication of this paper.

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