Molecular Science

AIMS Molecular Science, 11(4): 351–366.
DOI: 10.3934/molsci.2024021
Received: 20 June 2024
Revised: 26 September 2024
Accepted: 23 October 2024
Published: 30 October 2024

http://www.aimspress.com/journal/Molecular

Research article

Synthesis and application of zinc oxide nanoparticles in *Pieris brassicae* larvae as a possible pesticide effect

Isabella Martins Lourenço[1], Amedea Barozzi Seabra[1,*], Marcelo Lizama Vera[2,3], Nicolás Hoffmann[4,5], Olga Rubilar Araneda[5] and Leonardo Bardehle Parra[6,*]

[1] Center for Natural and Human Sciences (CCNH), Federal University of ABC (UFABC), Santo André, SP, Brazil
[2] Laboratory of Chemical Ecology, Department of Chemical Sciences, University of La Frontera, Temuco, Chile
[3] Programa de Doctorado en Ciencias Agroalimentarias y Medioambiente. Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera, Temuco, Chile.
[4] Department of Chemical Engineering, Universidad de La Frontera, Temuco, Chile
[5] Biotechnological Research Center Applied to the Environment (CIBAMA-BIOREN), Universidad de La Frontera, Temuco, Chile
[6] Departamento de Producción Agropecuaria, Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera, Temuco, Chile

* **Correspondence**: Email: amedea.seabra@ufabc.edu.br; leonardo.bardehle@ufrontera.cl.

Abstract: *Pieris brassicae* is commonly known as the cabbage moth and is a species known to be invasive, thereby causing serious damage to vegetables and subsequently leading to total crop loss. Formulations of nanopesticides can provide unique characteristics such as size and shape, in addition to having integrated properties in a single material, making them efficient in pest management and protection against diseases in a single material; it can be applied in small volumes, with a greater precision, lower input costs, and a potential reduction in environmental contamination. Nanotechnology is a type of alternative and highly effective technology in several sectors, mainly in agriculture and the enrichment and fortification of cultivars. Hydrothermal synthesis is a type of process used to obtain nanoparticles with a more uniform crystallinity and aging of nanocrystallites, where high temperatures and pressures help to reduce particle aggregation. Chemically synthesized metal nanoparticles, such as zinc oxide nanoparticles (ZnO NPs), can find wide applications and success against different types of pests, such as larvae. The present study focuses on the application of

different concentrations of ZnO NPs (12.5, 25, 50, 100, 200 and 400 mg/L) on the body surface of *P. brassicae* to verify their possible pesticide activity against these larvae. The results of this study suggest a non-intuitive pesticidal activity of ZnO NPs against cabbage moth larvae. The highest mortality percentage of larvae against the treatments occurred at the concentration of 200 mg/L of ZnO NPs, represented by a rate of 100% in the 72-h period of the experiment. Finally, the results of the present study with ZnO NPs and *P. brassicae* larvae suggest an initial trigger for future possibilities of exploration and more in-depth studies to clarify the interaction of ZnO NPs and the possible metabolic pathways triggered in these insect pests.

Keywords: *Pieris brassicae*; agricultural pests; zinc oxide nanoparticles; toxicity; nanotechnology

1. Introduction

Zinc oxide (ZnO) is a compound with semiconductor characteristics at room temperature. Depending on its conformation, ZnO can be found in either a hexagonal or a cubic coordination [1]. According to Klingshirn [1], in its most varied form, ZnO is used and incorporated annually in industrial sectors such as the pharmaceutical industry in well-dosed proportions as an additive for supplementation in animal and human food, and in the food industry as an excellent antimicrobial, which is biocompatible and cost-effective [2,3]. Nevertheless, ZnO nanoparticles (ZnO NPs) have currently been applied in agriculture and have been utilized as a biofortifier in cereal crops such as rice and corn, in addition to being excellent antibacterial materials [4,5]. Different studies in the literature have reported the positive effects of using ZnO NPs in different plants and vegetables on the nutritional content yield, the activation of the defense system, and an increased photosynthetic rate [6]. The effect of ZnO NPs depends on factors such as distribution, absorption, the type of charge of the NPs, and their interaction with parts of the plant such as trichomes, cuticles, the cell wall, the stomata, and root tissues, among others [7]. Zoufan et al. [8] demonstrated the activation of the antioxidant immune system in *Chenopodium murale* L. with the application of 250 mg/L of ZnO NPs after 6 days of treatment [8]. Pokhrel and Dubey [9] demonstrated that ZnO NPs dose-dependently inhibited cabbage seed germination [9]. Furthermore, Zhu et al. [10] demonstrated that the homogeneous accumulation of Zn through the foliar application of ZnO NPs relied on a positive surface charge potential and sizes of around 40 nm to penetrate the plant cytoplasm, which led to a better understanding of the metabolic mechanisms [10]. Another example [11] includes the use of insecticides to combat different types of pests.

In 2018, the global consumption of pesticides to control agricultural pests was around 2 million tons per year [12]. However, the increased use of pesticides to ensure crop performance has become excessive, which had led to an increased generation of waste that is harmful to the environment and human health [13]. The literature often mentions a consensus on the use of appropriate doses of pesticides and agrochemicals; however, on many occasions, doses higher than permitted were inappropriately used, thus leading to unsustainable agricultural practices [14]. The use of nanotechnology and related technologies that enable the targeted and controlled release of agrochemicals greatly contributes to the reduction of the doses applied to various materials, which ultimately improves the precision, productivity, and added economic value [13]. Synthetic pesticides are known for their hydrophobic characteristics and contain organic solvents, which implies a low solubility in water, thus leading to

an ineffectiveness and a prolonged accumulation over time. On the other hand, nanopesticides are part of a technology that allows for a better permeability, solubility, and biodegradability [13,14]. For centuries, the use of conventional insecticides to combat pests, insects, or even weeds has contributed to soil and water contamination, and in some cases, has caused damage to the food chain, animal, plant, and human health [15–17]. Therefore, the use of more effective and modern technologies is necessary. In this context, nanotechnology and nanomaterials are used to minimize contamination and the loss of nutrients, to increase the productivity level of plantations without polluting water and soil, and to protect them against biotic and abiotic factors [18,19]. Therefore, Ag, MgO, ZnO, and CuO nanoparticles are being extensively investigated as possible pest control materials that cause damage to the flora [11]. Therefore, due to their potentiated activities and high specificities, metallic and metallic oxide nanoparticles have demonstrated the potential to act as a nanopesticide because they can be used in low concentrations [19]. Studies initially reported the use of silica nanoparticles as carriers of pesticides such as validamycin and imidacloprid against *Rhyzopertha dominica* and *Sitophilus oryzae*, and demonstrated entomotoxicities greater than 90% [20]. In addition, Stadler et al. [21] demonstrated the entomotoxicities of nanostructured alumina against *Rhyzopertha dominica* and *Sitophilus oryzae* after 3 days of continuous treatment of wheat, thereby indicating an LD_{50} of 127 to 235 mg/kg [20]. Different concentrations of ZnO NPs alone or associated with other nanoparticles have been used as pesticidal and antifungal materials in agriculture [19]. Lili et al. [22] demonstrated the antifungal activity of ZnO NPs at concentrations of 0, 3, 6, and 12 mmol/L against post-harvest fungi *Penicillium expansum* and *Botrytis cinerea*, which prevented the growth and development of conidia and conidiophores, thus leading to the death of fungal hyphae [22]. However, in recent years, an emphasis has been placed on the use of ZnO NPs as a larvicide against urban insects such as *Culex tritaeniorhynchus* [23] and *Aedes aegypti* [24].

Pieris brassicae (Lepidoptera: Pieridae) is one of the most destructive and cosmopolitan pests of crucifers, and has been reported to feed on five main plant families (Tropaeolaceae, Resedaceae, Capparaceae, Papilinoaceae and Brassicaceae). Their young larvae feed on all parts of the host plant, including the seeds, leaves, and fruits of species such as cabbages and cauliflowers. This damage is considered important since a single larva can consume up to 74 to 80 cm^2 of the leaf area, which, in countries such as India, is equivalent to a 40% annual loss in different crops [25–28]. The serious damage caused to cabbage plantations by *P. brassicae* larvae is due to the fact that they directly attack the lower parenchyma of the leaf blades, thus causing irreversible damage to the plant [29]. Studies in the literature demonstrated the use of metallic nanoparticles against agricultural insects such as *Spodoptera frugiperda* (J. E. Smith, 1797), popularly known as fall armyworm, which is a serious pest that can cause damage to more than 350 species of plants, thus leading to a decrease in up to 70% of the yield in corn cultivars [30,31]. The larvae of *S. frugiperda* are difficult pests to combat as they are protected by the internal leaves of the plants, which, in turn, are protected by the excrement of these larvae, and consequently makes it difficult for the applied pesticide or insecticide to penetrate. In this way, problems of contamination and the excessive use of pesticides have arisen [32]. As a pest management and control strategy, alongside a less toxic and more environmentally friendly manner, nanotechnology has been used as a good technology to control the population growth of *Spodoptera litura* and *Plutella xylostella*, which are pests respectively known to infest tobacco, cotton, and cabbage crops [33,34]. In addition, depending on the nanoparticle concentration used to combat the agricultural pests, their phytotoxicities can be relatively low [35]. However, the most recent databases demonstrated a lack of data reporting the application of ZnO NPs to *Pieris brassicae* larvae. According

to studies found in the literature, it is known that metal oxide nanoparticles are widely used in agriculture and biological areas because they are materials that are easily absorbed and transported in the biological system [36]. However, to date, we have not observed conclusive studies on the mechanisms of cellular absorption, metabolism, and the accumulation of these materials in living organisms. In plants, the application of nanomaterials has shown promising results in combating stress caused by high salinity, droughts, and an increased antioxidant defense [37]. However, it is known that the use of metallic nanoparticles may be beneficial for some plant species and not for others, since a set of chemical and environmental factors can influence the interaction between plants and the environment, from the initial contact of the application until their complete metabolization by the organism [37]. For insects, the application of nanoparticles as a form of pesticides is also dependent on intrinsic characteristics such as their morphology, the surface charge, and the physicochemical properties [37]. Some authors reported the application of nanoparticles in insects with a great damaging potential, since this type of material penetrates the organism's exoskeleton and disrupts biological processes that involve bonds with phosphorus and sulfur within different types of proteins, thus leading to the denaturation of enzymes and damage to organelles [38,36]. In addition, metallic nanoparticles appear to decrease the permeability of the cytoplasmic membrane of different insects, thus leading to the loss of cellular function, proton motive force and cell death [36–38].

In this approach, nanoparticles appeared to generate intra- and extracellular oxidative stress by physical interactions with cellular organelles and enzymes that are involved in the reduction and oxidation catalysis process, in addition to a continuous exposure to nanoparticles over time through particle dissolution and disaggregation processes in the inserted medium. Some studies demonstrated that Ag NPs exerted harmful effects on the development of *Drosophila melanogaster* by accumulating reactive oxygen species (ROS) in their muscle tissues, thus resulting in cell apoptosis and damage to the genetic material; alternatively, for larvae of *Achaea janata* and *Spodoptera litura*, the same nanoparticles only led to an increase in the levels of antioxidant enzymes [39,40]. Therefore, there are few concrete studies in the literature that focused the metabolic pathways involved in each harmful mechanism generated in insects, especially in larvae of *P. brassicae*. Therefore, in a general way, the genotoxic effects of metal nanoparticles are explained by the production of ROS induced by metal ions among other inflammatory processes caused by OH• species, thus leading to enzymatic and molecular damage [36].

To verify the possible toxicity of ZnO NPs, adult larvae of *P. brassicae* were exposed to different concentrations of ZnO NPs synthesized by the hydrothermal method to test how these nanoparticles can influence their growth and mortality, thereby performing a brief comparison of the larvicidal effect of ZnO NPs and whether there is a dose-dependent toxicity. To our knowledge, this is the first report to evaluate the effects of ZnO NPs against the agricultural pest *P. brassicae*. The few studies published so far with ZnO NPs provide interesting findings against agricultural pests such as *Pieris rapae*, S*podoptera litura,* and *Spodoptera frugiperda*, in addition to other pests such as Coleoptera. Briefly, ZnO NPs that were added to the insect pest food demonstrated a toxic effect for most of them, depending on the concentration used. Among the most recent studies, this initial report presents the application of ZnO NPs in an unprecedented way on the body surface of the pest *P. brassicae*, thus indicating its practical and potential application as a pesticide. Based on the data obtained in the present study, we can leverage future enzymatic, molecular, and environmental experiments that complement the data that was already obtained.

2. Materials and methods

2.1. Materials

To prepare the ZnO nanoparticles (ZnO NPs), highly-pure deionized water (MS2000, Gehaka, São Paulo, resistivity of 18.2 MΩ·cm) was added to Zinc acetate dihydrate ($(Zn(CH^3COO)_2 \cdot 2H_2O)$ obtained from Sigma-Aldrich (St. Louis, MO, USA)), Sodium hydroxide (NaOH), and absolute ethyl alcohol (obtained from Labsynth (Diadema, São Paulo, Brazil)).

2.2. Synthesis of ZnO NPs

Samples of ZnO NPs were prepared by the hydrothermal method with oven heating [41]. The hydrothermal synthesis of the present study was prepared in a homogeneous aqueous medium and with the application of a temperature increase greater than 25 °C, which was used to facilitate the crystallization process of ZnO NPs [42,43]. An aqueous solution of zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$) was prepared in the proportion of 7.32% m/v in 30 mL of deionized water. Then, 30 mL of NaOH (1 mol/L) was slowly added to the zinc acetate solution under constant stirring. After the end of dripping, the final suspension, which was milky white in color, was transferred to the hydrothermal device at 170 °C for 10 h. The obtained material was washed three times with absolute ethyl alcohol and deionized water, followed by drying in an oven at 60 °C for 2 h.

Different techniques were previously reported by our group to characterize the synthesized ZnO NPs, including characterizations such as transmission electron microscopy and X-ray diffraction [41]. The surface morphology of ZnO NPs in the solid state was investigated by transmission electron microscopy (TEM, JEM-2100 Plus, 200 kV, JEOL, USA). The TEM analysis consisted of diluting and depositing the material on a carbon-coated grid and air drying at room temperature (25 °C).

2.3. Collection of Pieris brassicae

Pieris brassicae larvae in the third larval stage were collected from March to April 2023 in the Maquehue experimental field at the Universidad de La Frontera (S 39°13′48″ W 73°12′25″), 15.7 km from the Universidad de La Frontera campus located on Avenida Francisco Salazar, in the city of Temuco, Chile. *Pieris brassicae* larvae were collected with a tweezer and stored in a deep plastic container with a lid in the presence of small holes. The larvae contained in the plastic container were in contact with cabbage leaves harvested in the Maquehue experimental field, which served as food for the insects until the beginning of the experiment.

2.4. Tests with Pieris brassicae and ZnO NPs

The collected *P. brassicae* larvae were placed in 100x15 mm Petri dishes under the experimental conditions at room temperature (20–25 °C), where they remained for 72 h until the end of the experiment. Concentrations of 12.5, 25, 50, 100, 200, and 400 mg/L of the ZnO NPs were chosen, which were applied in a volume of 1 mL directly to the body surface of the larvae with the aid of a plastic sprayer. In addition to the negative control group, each concentration included n = 10 *P. brassicae* larvae. The negative control group was the only group that did not receive any type of

treatment. All insects were weighed before the exposure to ZnO NPs, followed by subsequent weighing at 24, 48, and 72 h after exposure. The third instar larvae were evaluated once a day for periods of 24, 48, and 72 h, where the mortality of each individual due to either the lack of movement and dehydration was recorded.

2.5. Statistical analysis

A two-way analysis of variance (ANOVA) with a significance level of 95% was used to determine the statistical differences between treatments in *P. brassicae*. In the weight differences (%), the ZnO NPs concentrations were analyzed using the Tukey test with a significance level of 95% using the Prism 9 software.

3. Results and discussion

3.1. Synthesis and characterization of ZnO NPs

In this work, ZnO NPs were synthesized by the hydrothermal technique, as previously reported by our group [41]. We have previously demonstrated that the crystal structure of the ZnO NPs can be confirmed through the phase reflections to the cubic structure of ZnO NPs (JSPDS card #36-1451), with a crystallite size of 39 nm [41]. These results agree with previously reported data, where either the grain size varied from 7 to 16 nm [42] or the sizes were below 60 nm [43]. Furthermore, ZnO NPs were found to have an average size of 118 ± 43 nm in the solid state, as represented by TEM, and reported in previous work by our group [41]. These results are in agreement with those observed by Rai and Yu [44], who reported ZnO NPs synthesized by the hydrothermal method with sizes of 100 to 150 nm in the solid state. Numerous studies demonstrated that the size and morphology of these nanoparticles were closely related to the use of the zinc precursor salt and the synthesis temperature [44]; additionally, ZnO NPs that were synthesized by the hydrothermal method varied in irregular hexagonal or spherical morphologies in the presence of clusters [42,45], thus corroborating the images obtained by TEM for ZnO NPs obtained in the present work (Figure 1).

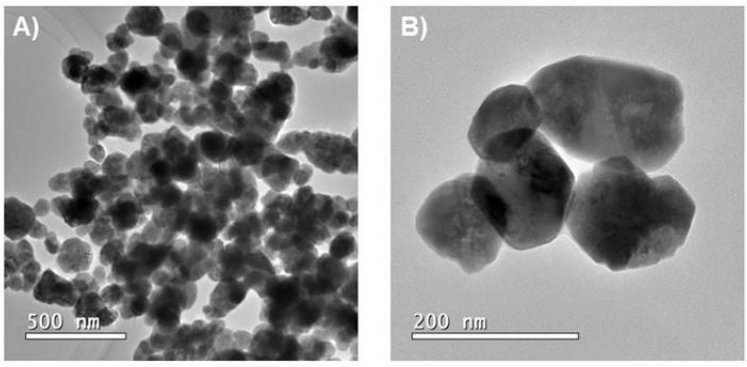

Figure 1. Representative TEM image illustrating the morphology corresponding to ZnO NPs in the solid state, synthesized in this work by the hydrothermal method. In (A) the image of ZnO NPs with an approximation on a scale of 500 nm and in (B) the image of ZnO NPs with an approximation on a scale of 200 nm.

3.2. Tests with Pieris brassicae and ZnO NPs

3.2.1. Weight differences by ZnO NPs application

Our findings on the weight differences (%) of *P. brassicae* by ZnO NPs application are presented in Table 1. Among the treatments, significant differences were determined among the 12.5, 25, 100, and 200 mg/L treatments and the negative control after 24 h. Significant values ranged from −10.24% to 15.15% of the weight differences for the negative control and the 25 mg/L treatments, respectively. Among the ZnO NPs application, significant differences were determined between the 25 mg/L and the 12.5 mg/L treatment, doubling the NPs concentration values that evidenced the 15.15% and −5.84% weight differences, respectively. After 48 h, the 25 mg/L treatment showed significant differences with the control, alongside the 12.5, 50, 100, 200, and 400 mg/L treatments. The highest lost weight difference (%) was −61.5% for the 25 mg/L treatment. All the remaining treatments showed weight losses. However, non-significant differences were observed, which were different from the 24 h treatments. Moreover, after 72 h, all results displayed a weight loss on the ZnO NPs, including the control treatment. The highest lost weight differences were determined on the 100 mg/L ZnO NPs application with a −16.5%. In this sense, significant differences can be observed between the 100 mg/L treatment and the 12.5, 50, and 200 mg/L treatments. Table 1 reveals weight differences of 0.29%, 0%, and 0% for the 12.5, 50, and 200 mg/L treatments, respectively. As was mentioned above, the 100 mg/L treatment showed the highest weight loss percentage, and the concentration results evidenced non-appreciable weight differences regarding the control measures.

Table 1. Difference in weights presented for Pieris brassicae after receiving different concentrations of ZnO NPs. Different letters imply significant differences. Uppercase letters indicate differences between treatment times. Lowercase letters indicate differences pair-wise comparison of least square means (mean ± SE, n = 10).

Time (h) / Weight difference (%) / Concentration (mg/L)	24	48	72
Control	$-10.24 \pm 2.35^{\mathbf{Ac}}$	$0.29 \pm 8.26^{\mathbf{Aab}}$	$-11.88 \pm 12.57^{\mathbf{Aabc}}$
400	$-18.50 \pm 31.17^{\mathbf{Aacd}}$	$-1.40 \pm 8.39^{\mathbf{Abd}}$	$-12.83 \pm 11.76^{\mathbf{Aabc}}$
200	$2.91 \pm 6.50^{\mathbf{Aad}}$	$-0.59 \pm 1.29^{\mathbf{Abd}}$	$0.00 \pm 0.00^{\mathbf{Aab}}$
100	$2.06 \pm 6.5^{\mathbf{Aabd}}$	$-5.72 \pm 4.80^{\mathbf{ABabd}}$	$-16.5 \pm 10.35^{\mathbf{Bc}}$
50	$0.63 \pm 26.84^{\mathbf{Abcd}}$	$-1.62 \pm 0.33^{\mathbf{Abd}}$	$0.0 \pm 0.00^{\mathbf{Aa}}$
25	$15.15 \pm 9.81^{\mathbf{Ad}}$	$-61.52 \pm 9.15^{\mathbf{Bc}}$	$-6.59 \pm 11.30^{\mathbf{Cabc}}$
12.5	$-5.84 \pm 24.11^{\mathbf{Aabc}}$	$2.04 \pm 5.40^{\mathbf{Ab}}$	$0.29 \pm 0.82^{\mathbf{Aa}}$

Once the experiment was conducted, significant differences were determined by the time application treatment. In this context, the 25 mg/L treatment showed significant differences at 24, 48, and 72 h. Detailed results evidenced a 15.15% increase in the weight differences after 24 h, thus showing the highest weight gain from all the experiments. Nonetheless, after 48 h, the highest lost weight was registered by the 25 mg/L application, thus representing −61.52%. In the end, a weight loss of −6.59% was registered after 72 h of the 25 mg/L treatment of ZnO NPs. Finally, the 100 mg/L

treatment of ZnO NPs evidenced significant differences between 24 and 72 h. The recorded values were 2.06% and −16.50%, respectively.

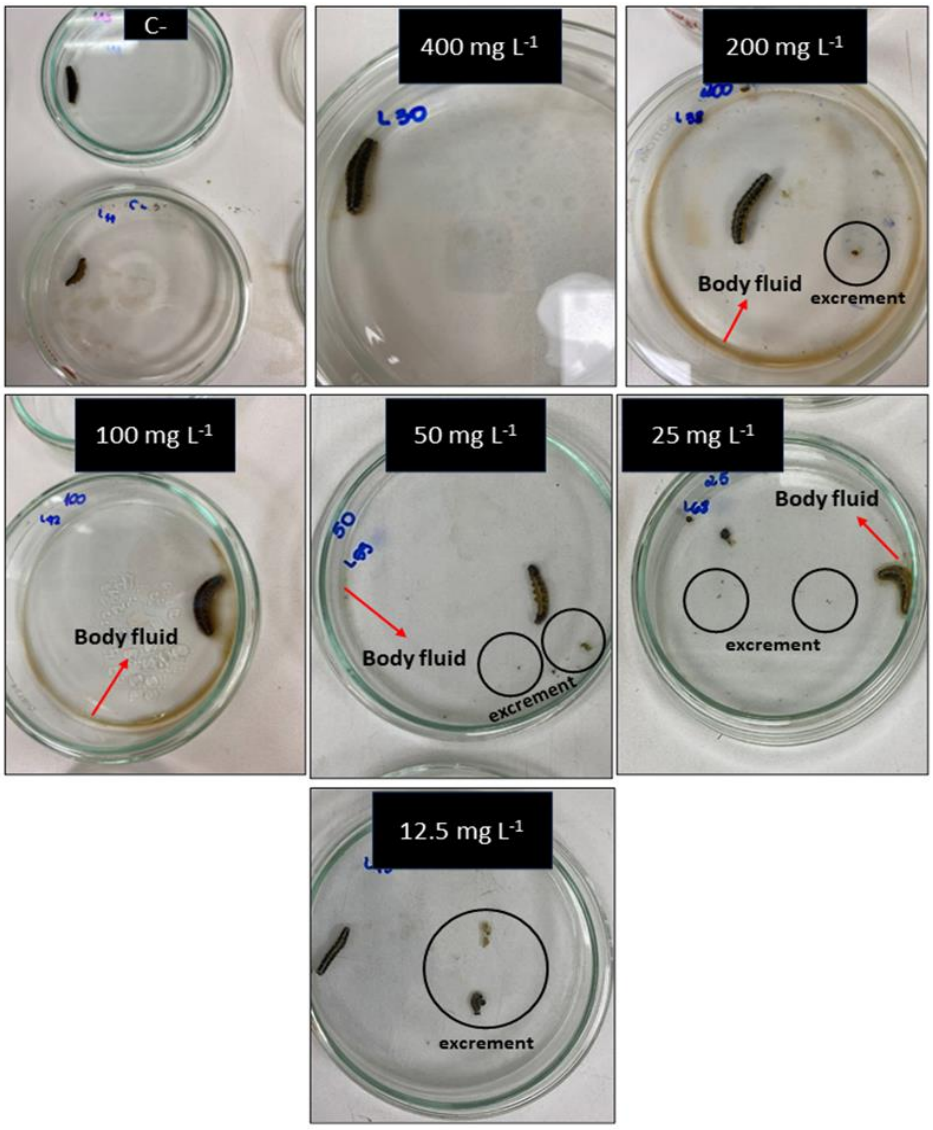

Figure 2. *Pieris brassicae* larvae in 48 h of experiment in the presence of excrement (black circle) at concentrations of 400, 200, 100, 50, 25, and 12.5 mg/L of ZnO NPs compared to negative control larvae that received no treatment. It is also noted in most concentrations the loss of body fluid (red arrow), possibly indicating dehydration of the insect.

Nowadays, little is known about the mechanism of ZnO NPs against pests such as *P. brassicae* larvae. It is known that the corporal application of metallic nanoparticles in insects leads to toxicity due to their penetration in the exoskeleton and, therefore, to the accumulation of nanomaterials in the intracellular space, inducing processes of oxidative stress and, consequently, to the imbalance of the homeostatic activity of enzymes and cellular organelles [46–48]. The significant weight reduction mainly observed at the lowest concentrations of 12.5, 25, 100, and 200 mg/L of ZnO NPs may possibly be related to the lower amount of nanoparticles present in suspension, thus indicating that they are less

prone to agglomeration [49,50], allowing for a greater contact and absorption on the body surface of the larva of *P. brassicae*. Therefore, the initial 24 h of the experiment possibly resulted in an initial trigger of physiological stress that led to the loss of body mass. Figure 2 illustrates *P. brassicae* larvae in the Petri dish after 24 h of the bodily application of ZnO NPs at concentrations of 200, 100, 50, 25, and 12.5 mg/L. Black circles indicate the presence of fecal droppings, which indicates a rapid stress response against the treatment received, compared to the negative control, which does not have fecal droppings or liquid from the larvae. The significant weight loss of the subjects primarily treated with the 25 mg/L concentration compared to the 12.5, 50, 100, 200, and 400 mg/L concentrations after 24 h of the experiment may be closely correlated with the amount of excrement released and the loss of body fluid caused by stress induced by the treatments with ZnO NPs, which led to the gradual dehydration of the insect. The present results are mostly based on behavioral studies [46], thus requiring a more in-depth study on the physiological and metabolic characteristics of *P. brassicae* against ZnO NPs even after 72 h of the experiment.

3.2.2 Mortality percentage

The mortality percentage was determined from the weight difference experiment. Therefore, 24, 48, and 72 h measures were considered (Figure 3). Initially, after 24 h, the control experiment showed no mortality percentage. However, the 200 to 400 mg/L ZnO NPs applications showed increases in the insect mortality percentage, with 20% and 33% values, respectively. Indeed, a strict and positive slope can be observed in Figure 3a, thus suggesting a dose-dependent behavior. Therefore, the mortality percentage increased beyond the 12.5, 25, 50, and 200 ZnO NPs mg/L threshold. The control measures showed a 10% mortality.

Figure 3b revealed a different mortality percentage behavior 48 h after the ZnO NPs concentration application. Interestingly, the percentage dose response shifts as the concentration increases, with a noticeable change in the slope's trend. This shift reveals a biphasic dose-response throughout the experiment, thus indicating a potential hormetic effect described on *Artemia salina* aquatic organisms by applying magnetite nanoparticles [51]. Initially, the 12.5 and 25 mg/L treatments showed negative slope tendencies, since the mortalities decreased from 20% to 60%. As the ZnO NPs concentration increases, a positive slope can be observed from 25 to 50 mg/L, thus representing an increase from 20% to 50%. Thus, between the 12.5 and 50 mg/L treatments, a curve interpretation describes a negative parabola curve mortality response. The same response interpretation can be appreciated between 50 to mg/L, where the values range from 50% to 70%, respectively. At the same time, the minimum value is represented at the 100 mg/L treatment with 0% mortality. In contrast, once the ZnO NPs concentration increases from 100 to 200 mg/L, a 70% mortality index can be reported as the maximum percentage after 48 h. A drop in the slope is represented between 200 to 400 mg/L, thereafter showing 0% mortality after 48 h. The negative control measure was 10% after 48 h, which is the same value observed in the 24 h period of exposure to ZnO NPs. In summary, from the observed non-intuitive mortality report, it is suggested that this mortality pattern can be explained by a hormonal behavior of the larvae as the application of ZnO NPs to *P. brassicae* increases. Finally, the same trend is reported after 72 h (Figure 3c). Congruently, from 48 to 72 h, the mortality behavior persists. However, the highest percentage mortality activity of the entire experiment was 100% with the application of 200 mg/L of ZnO NPs after 72 h. Additionally, the lowest percentage of mortality was recorded at a dose of 100 mg/L with 20%. The negative control increased the percentage from 10% to 50% between 48

and 72 h. The fact that the negative control did not receive any concentration of ZnO NPs could possibly highlight the larvae mortality over time for other metabolic reasons that would need to be studied in detail. Since the previously mentioned hormetic response occurs after 48 hours, non-linear fit models are unsuitable to determine the lethal concentration for 50% of the population (LC50). Therefore, this value cannot be reported in this work.

In the literature, a study conducted by Abd El-Wahab and Anwar [52] reported that the amount of 0.01 g led to a 100% mortality of *Spodoptera litura* larvae, while copper nanoparticles caused disability and dark gray coloration in larvae, thus leading to a 33.3% mortality. In addition, an enzymatic analysis of an increase in hemocytes that contained a large number of apoptotic cells was observed in *S. litura* insects within a period of 24 h exposure to nanoparticles. A decrease in the enzymes superoxide dismutase and ascorbate peroxidase was observed when faced with 1000 mg/kg of ZnO NPs as compared to the control group [53]. In another study, Eskin and Narullahoğku (2022) [54] added concentrations of 100, 500, 1000, 3000, and 5000 ppm of ZnO NPs to the diet of *Galleria mellonella* larvae, thus indicating that the larvae fed with all five concentrations of ZnO NPs reached the last larval stage; however, the time of pupal maturation in all groups was considered higher, but without a statistical significance [52]. Finally, the same tendency is reported after 72 h (Figure 3c). Logically, from 48 to 72 h, the mortality behavior persists. Nonetheless, the highest mortality percentage activity from the whole experiment was 100% by applying 200 mg/L of ZnO NPs after 72 h. The lowest mortality was recorded at 100 mg/L with a 20% index. The control measure increased from 10% to 50% between 48 and 72 h, thus evidencing a natural mortality decay.

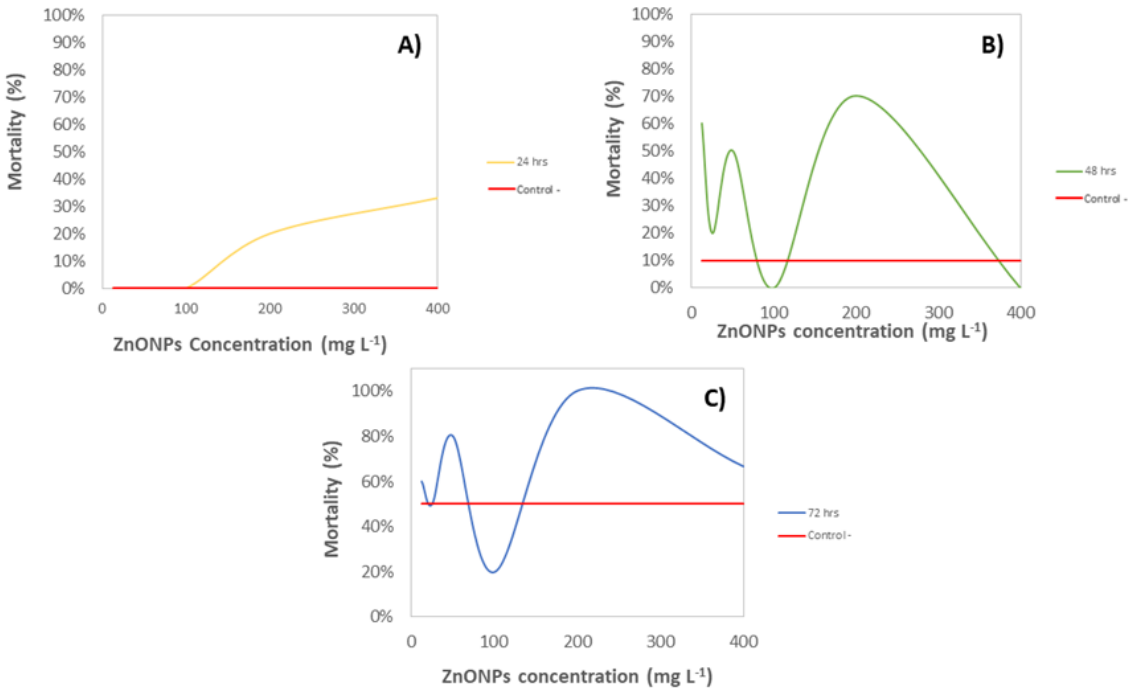

Figure 3. Mortality trend graphs of *Pieris brassicae* that received concentrations of 12.5, 25, 50, 100, 200 and 400 mg/L of ZnO NPs in the periods of 24 h, 48 h and 72 h of the experiment.

Grisakova et al. [55] reported that different insects have different types of sensitivities to different natural or synthetic components. In his study, they demonstrated that the application of a Neem leaf extract at different concentrations was able to lead to a significant mortality of *P. brassicae* in a period of 4 days [54]. A possible explanation for the mortality of *Pieris* larvae and the lack of continuity in their growth may be connected to the hormone secretion disorder that prevents metamorphosis, which subsequently led to damage in epithelial cells, body weight, and muscle tissues [54]. In the present study, as expected, the mortality of *Pieris* did not present a significant percentage of mortality in 24 h; however, it demonstrated a linear trend of mortality that followed in the 48 h of the experiment. At 48 h of the experiment, it visibly showed mortality at concentrations from 25 to 50 mg/L, thus representing an increase of 20% to 50%, respectively. This indicated a dose-dependent response to the application of ZnO NPs, which could be observed for the concentrations from 50 to 200 mg/L, where the mortality values varied from 50% to 70%, respectively. These results corroborated the loss of mass presented in item 3.2.1, where the most affected larvae were those that received a concentration of 25 mg/L of ZnO NPs, as compared with concentrations of 400, 200, 100, 50, and 12.5 mg/L. The highest concentrations did not show any significance to the detriment of the concentration of 25 mg/L, possibly indicating that the ZnO NPs may take a longer time to be absorbed by the body surface of the larvae of *P. brassicae* because they are more susceptible to agglomeration; however, as they are in a greater proportion, they can generate a greater stress in the larvae, thereby inducing a greater loss of body fluid and fecal excrement (Figure 2). In addition, at concentrations of 25, 50, 100, 200 and 400 mg/L, Figure 2 illustrates larvae with dark colors and the presence of body liquid in their surroundings, possibly resulting from the loss of liquid caused by the application of the ZnO NPs treatments. Apparently, as a potent material, ZnO NPs can be attributed the ability to absorb and abrade the body layer that protects the insects; in some cases, this can be attributed to the cuticle wax of some insects, thus leading to water loss, dehydration, and death by desiccation, as observed in Figure 2 of the present work [55].

Contrary to our results, Eskin and Narullahoğku [54] reported that only high concentrations of ZnO NPs (1000, 3000, and 5000 ppm) significantly decreased the pupal weights of greater wax moth, *Galleria mellonella* (Lepidoptera: Pyralidae), which is one of the best model organisms in ecotoxicology tests. To date, the literature found in the databases demonstrated the toxicity of ZnO NPs applied to *P. brassicae* feed, thus indicating a dose-dependent type of toxicity at concentrations ranging from 100 to 175 ppm [31,52,56]. Similar to our results, Shabir et al. [56] reported a 100% mortality of the 3rd instar larvae of the noctuid *Spodoptera litura* (Lepidoptera), which is an important cotton and tobacco pest, using ZnO NPs coated with a ginger plant extract (*Zingiber officinale*), but at a concentration of 500 ppm [57]. The results presented in the present study demonstrate that ZnO NPs at different concentrations have a toxic effect, thereby creating a dose-dependent response against *P. brassicae* larvae, indicating the potential pesticidal effect that zinc oxide nanoparticles may have.

Our results suggest that exposure to toxicological ZnO NPs in *Pieris brassicae* exhibits a hormetic behavior, which is characterized by a two-phase response to stressors: low doses stimulate beneficial effects, while high doses inhibit numerous processes and result in adverse outcomes [58]. Hormesis has been extensively documented in studies of algae and plants, where nanomaterials can either increase or decrease the accumulation of transport genes involved in heavy metal uptake and translocation, which subsequently affects the nutrient status [59]. Elements released from these nanomaterials may accumulate in the insect tissues; however, the physiological implications of this accumulation remain unclear. This present study opens new avenues for further studies aimed to better elucidate the following: (i) the underlying mechanisms of ZnO NPs as pesticide agent; (ii) the long

term effects of these nanoparticles; (iii) their impact on non-targeted organisms and/or the environment; (iv) the effects of ZnO NPs on a broader range of pests, besides *Pieris brassicae larvae*, to strengthen the use of ZnO NPs as a versatile nanopesticide; (v) a comparison of the effectiveness of ZnO NPs with other pesticides, traditionally used for these purposes; and (vi) to test broader concentrations of ZnO NPs. This present work reports, for the first time, the effects of ZnO NPs on *Pieris brassicae larvae*, thus highlighting the potential pesticide effects of ZnO NPs and *enhancing* the understanding of the responses to ZnO NPs application. Future investigations are welcome.

4. Conclusions

Chemical ZnO NPs were synthesized by the hydrothermal method, which enabled the formation of nanoparticles by employing a high temperature and pressure after the reduction of zinc acetate dihydrate with sodium hydroxide. Through initial studies on the application of ZnO NPs on the body surface of *Pieris brassicae* larvae, it can be pointed out that different concentrations of these nanoparticles could possibly generate a stress effect on the larvae, thus causing a possible metabolic imbalance hitherto unknown, but capable of inducing a toxic effect against these pests, leading to their death. This result can be corroborated with the mortality percentages observed when the 100 and 200 mg/L treatments of ZnO NPs were applied, with a 70% increase in the mortality rate after 48 h. Although no linear trend in the mortality rate was observed after 24 h, the initial data reported in the present study demonstrated good prospects for the use of these nanoparticles as a potential pesticide.

Author contributions

Conceptualization, A.B.S., O.R.A., and L.B.P.; methodology, I.M.L., M.L.V. and N.H..; validation, I.M.L., M.L.V., and N.H; formal analysis, I.M.L., M.L.V., and N.H.; resources, A.B.S., O.R.A., and L.B.P.; writing—original draft preparation, I.M.L.; writing—review and editing, I.M.L., A.B.S., M.L.V., N.H., O.R.A., and L.B.P.; supervision, O.R.A. and L.B.P..; project administration, A.B.S., O.R.A., and L.B.P..; funding acquisition, A.B.S., O.R.A., and L.B.P. All authors have read and agreed to the published version of the manuscript.

Acknowledgments

The authors are grateful to the São Paulo Research Foundation (FAPESP, grant number 2022/00321-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant number 313117/2019–5). INCT NanoAgro #405924/2022-4. This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001, CAPES-INCT NanoAgro #88887.953443/2024-00 ANID/FONDAP/15130015 and ANID/FONDAP/1523A0001.

Conflicts of interest

The authors have no conflict of interest that are relevant to the content of this article.

References

1. Klingshirn CF, Meyer BK, Waag A, et al. (2010) *Zinc oxide: From fundamental properties towards novel applications*, Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-10577-7
2. Espitia PJP, Otoni CG, Soares NFF (2016) Zinc oxide nanoparticles for food packaging applications. In: *Antimicrobial food packaging*, Cambridge, MA, USA: Academic Press, 425–431. https://doi.org/10.1016/B978-0-12-800723-5.00034-6
3. Alves Z, Ferreira NM, Figueiredo G, et al. (2022) Electrically conductive and antimicrobial agro-food waste biochar functionalized with zinc oxide particles. *Int J Mol Sci* 23: 8022. https://doi.org/10.3390/ijms23148022
4. Taheri M, Qarache HA, Qarache AA, et al. (2015) The effects of zinc-oxide nanoparticles on growth parameters of corn (SC704). *STEM Fellowship J* 1:17–20. https://doi.org/10.17975/sfj-2015-011
5. Abdallah Y, Liu M, Ogunyemi SO, et al. (2020) Bioinspired green synthesis of chitosan and zinc oxide nanoparticles with strong antibacterial activity against rice pathogen *Xanthomonas oryzae* pv. oryzae. *Molecules* 25: 4795. https://doi.org/10.3390/molecules25204795
6. Thounaojam TC, Meetei TT, Devi YB, et al. (2021) Zinc oxide nanoparticles (ZnO-NPs): A promising nanoparticle in renovating plant science. *Acta Physiol Plant* 43: 136. https://doi.org/10.1007/s11738-021-03307-0
7. Wang X, Yang X, Chen S, et al. (2016) Zinc oxide nanoparticles affect biomass accumulation and photosynthesis in *Arabidopsis*. *Front Plant Sci* 6: 1243. https://doi.org/10.3389/fpls.2015.01243
8. Zoufan P, Baroonian M, Zargar B (2020) ZnO nanoparticles-induced oxidative stress in *Chenopodium murale* L, Zn uptake, and accumulation under hydroponic culture. *Environ Sci Pollut Res* 27: 11066–11078. https://doi.org/10.1007/s11356-020-07735-2
9. Pokhrel LR, Dubey B (2013) Evaluation of developmental responses of two crop plants exposed to silver and zinc oxide nanoparticles. *Sci Total Environ* 452: 321–332. https://doi.org/10.1016/j.scitotenv.2013.02.059
10. Zhu J, Wang J, Zhan X, et al. (2021) Role of charge and size in the translocation and distribution of zinc oxide particles in wheat cells. *ACS Sustain Chem Eng* 9: 11556–11564. https://doi.org/10.1021/acssuschemeng.1c04080
11. Hameed RS, Hassan ZN, Shafeeq MAA (2022) Bioactivity of insecticides nanoparticles for pest control: Review. *Nat J Pharm Sci* 2: 17–23. https://doi.org/10.22271/27889262.2022.v2.i1a.31
12. Zhang L, Yan C, Guo Q, et al. (2018) The impact of agricultural chemical inputs on environment: Global evidence from informetrics analysis and visualization. *Int J Low-Carbon Technol* 13: 338–352. https://doi.org/10.1093/ijlct/cty039
13. Fincheira P, Hoffmann N, Tortella G, et al. (2023) Eco-efficient systems based on nanocarriers for the controlled release of fertilizers and pesticides: Toward smart agriculture. *Nanomaterials* 13: 1978. https://doi.org/10.3390/nano13131978
14. Kumar A, Choudhary A, Kaur H, et al. (2021) Smart nanomaterial and nanocomposite with advanced agrochemical activities. *Nanoscale Res Lett* 16: 156. https://doi.org/10.1186/s11671-021-03612-0

15. Del Prado-Lu JL (2015) Insecticide residues in soil, water, and eggplant fruits and farmers' health effects due to exposure to pesticides. *Environ Health Prev Med* 20: 53−62. https://doi.org/10.1007/s12199-014-0425-3
16. Aktar W, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol* 2:1−12. https://doi.org/10.2478/v10102-009-0001-7
17. Sahoo D, Mandal A, Mitra T, et al. (2018) Nanosensing of pesticides by zinc oxide quantum dot: An optical and electrochemical approach for the detection of pesticides in water. *J Agric Food Chem* 66: 414−423. https://doi.org/10.1021/acs.jafc.7b04188
18. Chen H, Seiber JN, Hotze M (2014) ACS select on nanotechnology in food and agriculture: A perspective on implications and applications. *J Agric Food Chem* 62: 1209–1212. https://doi.org/10.1021/jf5002588
19. Kaur A, Bhatt DP, Raja L (2022) Applications of nanotechnology in agriculture, with a focus on insect pest management. *NanoWorld J* 8: S76-S82. https://doi.org/10.17756/nwj.2022-s1-015
20. Sahayaraj K (2014) Nanotechnology and plant biopesticides: An overview. In: *Advances in plant biopesticides*, New Delhi: Springer, 279–293. https://doi.org/10.1007/978-81-322-2006-0_14
21. Stadler T, Buteler M, Weaver DK (2010) Novel use of nanostructured alumina as an insecticide. *Pest Manag Sci* 66: 577–579. https://doi.org/10.1002/ps.1915
22. He L, Liu Y, Mustapha A, et al. (2011) Antifungal activity of zinc oxide nanoparticles against *Botrytis cinerea* and *Penicillium expansum*. *Microbiol Res* 166: 207–215. https://doi.org/10.1016/j.micres.2010.03.003
23. Vijayakumar S, Vinoj G, Malaikozhundan B, et al. (2015) *Plectranthus amboinicus* leaf extract mediated synthesis of zinc oxide nanoparticles and its control of methicillin resistant *Staphylococcus aureus* biofilm and blood sucking mosquito larvae. *Spectrochim Acta A* 137: 886–891. https://doi.org/10.1016/j.saa.2014.08.064
24. Roopan SM, Mathew RS, Mahesh SS, et al. (2019) Environmental friendly synthesis of zinc oxide nanoparticles and estimation of its larvicidal activity against *Aedes aegypti*. *Int J Environ Sci Technol* 16: 8053–8060. https://doi.org/10.1007/s13762-018-2175-z
25. Hasan F, Ansari MS (2011) Effects of different brassicaceous host plants on the fitness of *Pieris brassicae* (L.). *Crop Prot* 30: 854–862. https://doi.org/10.1016/j.cropro.2011.02.024
26. Chahil GS, Kular JS (2013) Biology of *Pieris brassicae* (Linn.) on different Brassica species in the plains of Punjab. *J Plant Prot Res* 53: 53–59.
27. Ali S, Ullah MI, Arshad M, et al. (2017) Effect of botanicals and synthetic insecticides on *Pieris brassicae* (L., 1758) (Lepidoptera: Pieridae). *Turk Entomol Derg* 41: 275–284. https://doi.org/10.16970/entoted.308941
28. Mazurkiewicz A, Tumialis D, Pezowicz E, et al. (2017) Sensitivity of *Pieris brassicae*, *P. napi* and *P. rapae* (Lepidoptera: Pieridae) larvae to native strains of *Steinernema feltiae* (Filipjev, 1934). *J Plant Dis Prot* 124: 521–524. https://doi.org/10.1007/s41348-017-0118-4
29. Karnavar GK (1983) Studies on the population control of *Pieris brassicae* L. by *Apanteles glomeratus* L. *Int J Trop Insect Sci* 4: 397–399. https://doi.org/10.1017/S1742758400002460
30. Montezano DG, Specht A, Sosa-Gomez DR, et al. (2016) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *Afr Entomol* 26: 286–300. https://doi.org/10.4001/003.026.0286

31. Pittarate S, Rajula J, Rahman A, et al. (2021) Insecticidal effect of zinc oxide nanoparticles against *Spodoptera frugiperda* under laboratory conditions. *Insects* 12: 1017. https://doi.org/10.3390/insects12111017
32. Pittarate S, Perumal V, Kannan S, et al. (2023) Insecticidal efficacy of nanoparticles against *Spodoptera frugiperda* (J.E. Smith) larvae and their impact in the soil. *Heliyon* 9: e16133. https://doi.org/10.1016/j.heliyon.2023.e16133
33. Debnath N, Das S, Seth D, et al. (2011) Entomotoxic effect of silica nanoparticles against *Sitophilus oryzae* (L.). *J Pest Sci* 84: 99–105. https://doi.org/10.1007/s10340-010-0332-3
34. Murugan K, Wei J, Alsalhi MS, et al. (2017) Magnetic nanoparticles are highly toxic to chloroquine-resistant *Plasmodium falciparum*, dengue virus (DEN-2), and their mosquito vectors. *Parasitol Res* 116: 495–502. https://doi.org/10.1007/s00436-016-5310-0
35. Raju IM, Rao TS, Lakshmi KVD, et al. (2019) Poly 3-Thenoic acid sensitized, copper doped anatase/brookite TiO_2 nanohybrids for enhanced photocatalytic degradation of an organophosphorus pesticide. *J Environ Chem Eng* 7: 103211. https://doi.org/10.1016/j.jece.2019.103211
36. Tunçsoy BS (2018) Toxicity of nanoparticles on insects: A review. *Adana Sci Technol Univ* 1: 49–61.
37. Khan M, Khan MAS, Borah KK, et al. (2021) The potential exposure and hazards of metal-based nanoparticles on plants and environment, with special emphasis on ZnO NPs, TiO_2 NPs, and AgNPs: A review. *Environ Adv* 6: 100128. https://doi.org/10.1016/j.envadv.2021.100128
38. Rai M, Kon K, Ingle A, et al. (2014) Broad-spectrum bioactivities of silver nanoparticles: The emerging trends and future prospects. *Appl Microbiol Biotechnol* 98: 1951–1961. https://doi.org/10.1007/s00253-013-5473-x
39. Yasur J, Pathipati UR (2015) Lepidopteran insect susceptibility to silver nanoparticles and measurement of changes in their growth, development and physiology. *Chemosphere* 124: 92–102. https://doi.org/10.1016/j.chemosphere.2014.11.029
40. Mao BH, Chen ZY, Wang YJ, et al. (2018) Silver nanoparticles have lethal and sublethal adverse effects on development and longevity by inducing ROS-mediated stress responses. *Sci Rep* 8: 2445. https://doi.org/10.1038/s41598-018-20728-z
41. Lourenço IM, Freire BM, Pieretti JC, et al. (2023) Implications of ZnO nanoparticles and S-Nitrosoglutathione on nitric oxide, Reactive oxidative species, photosynthetic pigments, and ionomic profile in rice. *Antioxidants* 12: 1871. https://doi.org/10.3390/antiox12101871
42. Madathil ANP, Vanaja KA, Jayaraj MK (2007) Synthesis of ZnO nanoparticles by hydrothermal method, *Nanophotonic Mater IV* 6639: 47–55. https://doi.org/10.1117/12.730364
43. Ismail AA, El-Midany A, Abdel-Aal EA, et al. (2005) Application of statistical design to optimize the preparation of ZnO nanoparticles via hydrothermal technique. *Mater Lett* 59: 1924–1928. https://doi.org/10.1016/j.matlet.2005.02.027
44. Rai P, Yu YT (2012) Citrate-assisted hydrothermal synthesis of single crystalline ZnO nanoparticles for gas sensor application. *Sensor Actuat B-Chem* 173: 58–65. https://doi.org/10.1016/j.snb.2012.05.068
45. Vlazan P, Ursu DH, Irina-Moisescu C, et al. (2015) Structural and electrical properties of TiO_2/ZnO core–shell nanoparticles synthesized by hydrothermal method. *Mater Charact* 101: 153–158. https://doi.org/10.1016/j.matchar.2015.01.017

46. Benelli G (2018) Mode of action of nanoparticles against insects. *Environ Sci Pollut Res* 25: 12329–12341. https://doi.org/10.1007/s11356-018-1850-4
47. Jiang X, Miclăuş T, Wang L, et al. (2015) Fast intracellular dissolution and persistent cellular uptake of silver nanoparticles in CHO-K1 cells: Implication for cytotoxicity. *Nanotoxicology* 9: 181–189. https://doi.org/10.3109/17435390.2014.907457
48. Rai M, Kon K, Ingle A, et al. (2014) Broad-spectrum bioactivities of silver nanoparticles: The emerging trends and future prospects. *Appl Microbiol Biotechnol* 98: 1951–1961. https://doi.org/10.1007/s00253-013-5473-x
49. Peng YH, Tso CP, Tsai YC, et al. (2015) The effect of electrolytes on the aggregation kinetics of three different ZnO nanoparticles in water. *Sci Total Environ* 530–531: 183–190. https://doi.org/10.1016/j.scitotenv.2015.05.059
50. Fouda A, Hassan SED, Salem SS, et al. (2018) In-vitro cytotoxicity, antibacterial, and UV protection properties of the biosynthesized Zinc oxide nanoparticles for medical textile applications. *Microb Pathogenesis* 125: 252–261. https://doi.org/10.1016/j.micpath.2018.09.030
51. Hoffmann N, Tortella G, Hermosilla E, et al. (2022) Comparative toxicity assessment of eco-friendly synthesized superparamagnetic iron oxide nanoparticles (SPIONs) in plants and aquatic model organisms. *Minerals* 12: 451. https://doi.org/10.3390/min12040451
52. Abd El-Wahab RA, Anwar EM (2014) The effect of direct and indirect use of nanoparticles on cotton leaf worm, *Spodoptera littoralis*. *Int J Biol Sci* 1: 17–24.
53. Fouda A, Hassan SE, Salem SS, et al. (2018) In-vitro cytotoxicity, antibacterial, and UV protection properties of the biosynthesized zinc oxide nanoparticles for medical textile applications. *Microb Pathogenesis* 125: 252–261. https://doi.org/10.1016/j.micpath.2018.09.030
54. Eskin A, Nurullahoğlu ZU (2022) Effect of zinc oxide nanoparticles (ZnO NPs) on the biology of *Galleria mellonella* L. (Lepidoptera: Pyralidae). *J Basic Appl Zool* 83: 54. https://doi.org/10.1186/s41936-022-00318-2
55. Grisakova M, Metspalu L, Jõgar K, et al. (2006) Effects of biopesticide Neem EC on the large white butterfly, *Pieris brassicae* L. (Lepidoptera, Pieridae). *Agron Res* 4: 181–186.
56. Thakur P, Thakur S, Kumari P, et al. (2022) Nano-insecticide: Synthesis, characterization, and evaluation of insecticidal activity of ZnO NPs against *Spodoptera litura* and *Macrosiphum euphorbiae*. *Appl Nanosci* 12: 3835–3850. https://doi.org/10.1007/s13204-022-02530-6
57. Asghar MS, Sarwar ZM, Almadiy AA, et al. (2022) Toxicological effects of silver and zinc oxide nanoparticles on the biological and life table parameters of *Helicoverpa armigera* (Noctuidae: Lepidoptera). *Agriculture* 12: 1744. https://doi.org/10.3390/agriculture12101744
58. Alian RS, Dziewiecka M, Kedziorski A (2021) Do nanoparticles cause hormesis? Early physiological compensatory response in house crickets to a dietary admixture of GO, Ag, and GOAg composite. *Sci Total Environ* 788: 147801. https://doi.org/10.1016/j.scitotenv.2021.147801
59. Agathokleous E, Araminiene V, Belz RG, et al. (2019) A quantitative assessment of hormetic responses of plants to ozone. *Environ Res* 176: 108527. https://doi.org/10.1016/j.envres.2019.108527

AIMS Press