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Research Article

EVALUATION OF BIOLOGICALLY SYNTHESIZED ZINC OXIDE NANOPARTICLES FROM *PAECILOMYCES FARINOSUS* ON THE BIOLOGY OF SAWTOOTHED GRAIN BEETLE (*ORYZAEPHILUS SURINAMENSIS*)

[a]Tabark Ali Hasan, [b]Emad Ahmad Mahmood
[a] *Department of Biology, College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq.*
[b] *Department of Biology, AL-Farabi University College, Baghdad, Iraq.*

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ABSTRACT

The sawtoothed grain beetle, *Oryzaephilus surinamensis*, is a major pest of stored grains such as rice, wheat, barley, and legumes. This insect is highly adaptable and poses a persistent threat in diverse climatic conditions and storage environments. The present study aimed to determine the effect of the fungus *Paecilomyces farinosus* on the life stages (eggs, second and fourth instar larvae, and 24-hour-old pupae) of *O. surinamensis*. The fungus was tested at spore densities of 3×10^7, 3×10^5, and 3×10^3 spores/ml. The results showed that the highest mortality rates for eggs, second instar larvae, fourth instar larvae, and 24-hour-old pupae at a concentration of 3×10^7 spores/ml were 40.3%, 58.7%, 39.01%, and 55.6%, respectively. Furthermore, the study evaluated the impact of nano-zinc oxide (ZnONPs) on *O. surinamensis* at concentrations of 3000, 2000, and 1000 ppm. Various characteristics of the prepared ZnONPs, such as shape, size, and maximum absorption by ultraviolet spectroscopy, were analyzed. The results indicated 100% mortality of eggs treated with concentrations of 3000 ppm and 2000 ppm. The highest mortality rates for second instar larvae and 24-hour-old pupae were 80% and 20%, respectively, at a concentration of 2000 ppm. For fourth instar larvae, the highest mortality rate was 68.22% at a concentration of 3000 ppm. The study demonstrated that ZnONPs at all tested concentrations were more effective than the fungal suspension in causing mortality of eggs and second and fourth instar larvae. Moreover, the germination rate of rice grains treated with direct spraying was not affected by any of the treatments.

Corresponding Author: Tabark Ali Hasan
Email: tabark.bio89@ibnsina.edu.iq

INTRODUCTION

Grain crops are considered essential economic food sources for a significant portion of the global population due to their high content of crucial nutritional elements, such as proteins and carbohydrates. These crops play a vital role in food security and nutrition, providing the foundational dietary needs for billions of people. Rice, in particular, is a staple food for a large segment of the global population, especially in Asia, where it constitutes a major part of the daily diet (FAO, 2005). The significance of rice is further highlighted by its cultural,

economic, and social importance in many Asian countries. However, grain crops, including rice, are susceptible to infestations by various storehouse insects, which are economically significant pests. These pests compromise both the quantity and quality of stored grains, leading to substantial economic losses and presenting a global challenge to food security.

One of the most notable pests affecting stored grains is the saw-toothed grain beetle, *Oryzaephilus surinamensis*, belonging to the family Silvanidae and order Coleoptera. This insect is a primary pest of rice grains and other stored foodstuffs, including wheat, barley, and legumes (De Bach, 1964; Champ and Dyte, 1976). The widespread distribution and adaptability of *O. surinamensis* make it a persistent threat in various climatic conditions and storage environments. *O. surinamensis* is categorized as a secondary pest, meaning it primarily infests grains that have already been damaged by primary pests. This classification emphasizes the role of beetle in exacerbating the damage initiated by other pests, leading to a cumulative detrimental effect on stored grains. Effective management practices are necessary to prevent infestations, including thorough cleaning of storage facilities to remove potential breeding sites, securely sealing storage bags to prevent insect entry, and regulating temperature and humidity conditions to create an unfavorable environment for pest development (Kearn, 2006).

The saw-toothed grain beetle is characterized by its active and rapid movements, which enable it to spread quickly within storage facilities. It has a reddish-brown coloration (Elias, 2009), small eyes, biting mouthparts adapted for feeding on damaged grains, and functional wings despite its inability to fly. The beetle is small, measuring 2-3 mm in length, with a flattened body that allows it to penetrate narrow spaces within stored grains. Its name derives from the six prominent serrated teeth on each side of the thorax, a distinctive feature that aids in its identification (Rees, 2007). Moreover, *O. surinamensis* has three longitudinal ridges on the dorsal side of its thorax, further distinguishing it from other grain pests. The life cycle of *O. surinamensis* ranges from 17 to 50 days, depending on temperature conditions (Trematera and Reichmuth, 2000). Optimal conditions for its development include temperatures between 18 to 35°C and approximately 70% relative humidity. These conditions facilitate rapid population growth and increased infestation levels, highlighting the importance of maintaining controlled storage environments to mitigate the impact of this pest.

In addition to pesticides, there are alternative methods for controlling and preventing the spread of the saw-toothed grain beetle. Compared to chemical or physical approaches, biological management is a modern and safe strategy to limit the proliferation of these pests (Lord, 2001; Nazir et al., 2019; Khan et al., 2021).

Biological control methods often employ entomopathogenic fungi (Javed et al., 2019; Shehzad et al., 2021, 2022; Jabbar et al., 2022), which invade the cuticle layer of insects and decompose it. This decomposition facilitates the entry of the fungus into the internal parts of the insect, leading to infection and eventual death. One such fungus used to control the saw-toothed grain beetle is *Paecilomyces farinosus*. Studies have demonstrated that this fungus has essential properties for being an effective entomopathogen, resulting in high mortality rates among infected insects (Harney, 1989).

Nanotechnology has also emerged as a promising tool in controlling insect pests. This approach involves the synthesis of nano-sized particles, which exhibit unique properties distinct from the bulk metals from which they are derived. Nano-structured materials are recognized for their potent effectiveness, even at low concentrations, making them environmentally friendly options (Nowack, 2009; Shahbaz et al., 2023). The efficacy of nano-materials is largely attributed to their high surface area-to-volume ratio, a characteristic of their small size (Gahlawat et al., 2016). Among these materials, nano-zinc oxide has been utilized effectively in pest control.

Among these materials, nano-zinc oxide (ZnONPs) has been effectively used. ZnONPs are inorganic chemical substances with the formula ZnO, typically appearing as a white powder. They are recognized for their safety, effectiveness, non-toxicity, high strength, light weight, small size, excellent chemical reactivity, large surface area, and high stability (Kadhim et al., 2019).

The integration of nanotechnology in pest management leverages the unique physical and chemical properties of nanoparticles. For instance, nano-zinc oxide can be synthesized in various forms, such as powders or coatings, to create barriers against pests or to deliver lethal doses to insects upon contact. These nanoparticles can disrupt essential biological processes in insects, leading to their death. Furthermore, the use of nanotechnology in pest control is promising due to its

potential to reduce the reliance on traditional chemical pesticides, thereby minimizing environmental impact and resistance development in pests.

The combination of biological control methods and nanotechnology offers a synergistic approach to pest management. By integrating these advanced techniques, it is possible to achieve sustainable and effective control of the saw-toothed grain beetle and other storage pests. This integrated pest management strategy not only addresses the immediate problem of insect infestation but also contributes to long-term sustainability in agricultural practices.

Therefore, the present study aimed to assess the effectiveness of both the fungus *Paecilomyces farinosus* and zinc oxide nanoparticles in controlling the saw-toothed grain beetle, *Oryzaephilus surinamensis*. The specific objectives were:

1. To assess the impact of varying concentrations of *P. farinosus* suspensions on different life stages of the saw-toothed grain beetle and
2. To evaluate the effects of different concentrations of biologically manufactured zinc oxide nanoparticles (ZnO NPs) on various stages of the saw-toothed grain beetle.

MATERIALS AND METHODS

Collection and rearing of the saw-toothed grain beetle

Insects were collected from various food items infested by them. These insects were reared in the laboratory using amber rice grains, ensuring that the grains were free from infestation by other insects. They were placed in plastic containers measuring 25 cm by 15 cm, with 25 males and 25 females of the sawtoothed grain beetle, and supplemented with 12 g of dry yeast. The containers were sealed tightly with organza cloth.

The insects were then transferred to an incubator. All experiments were conducted in the incubator at a temperature of 27±2°C and a relative humidity of 70±2%, as described by Al-Jabr (2006). The insect colony was reared for four generations prior to the experiments to eliminate any potential pesticide residues.

Development of fungus colonies

The fungal isolate was obtained and grown in Petri dishes containing Potato Dextrose Agar (PDA), which was sterilized using an autoclave at a temperature of 121°C and a pressure of 1 bar for 15 min. After cooling the medium, 125 mg of the antibiotic tetracycline was added to prevent bacterial growth. The dishes were then transferred to an incubator for 8 days before starting the experiments (Al-Dosky, 2007).

Studying the effect of concentrations of the fungus *P. farinosus* on some aspects of the life performance of the sawtoothed grain beetle

Preparation of fungal suspension

Five milliliters of distilled and sterilized water was added to a dish containing an 8-day-old fungal culture. The spores were separated and collected using an L-shaped instrument. The contents of the dish were then filtered through Whatman No.1 mm filter paper placed on a glass funnel. An additional 5 ml of distilled and sterilized water was added to ensure complete passage of the fungal spores, resulting in a fungal suspension. This suspension was prepared in concentrations of 3×10^3, 3×10^5, and 3×10^7 spores/ml. The tubes containing the fungal concentrations were stored at 4°C in dark conditions (Lacey, 1997).

Experimental setup

The experiments were conducted in an incubator at a temperature of 27±2°C. Each experiment included three replications, with each treatment and each replication consisting of 20 samples. The control treatment used 4 ml of distilled water instead of the fungal suspension.

Effect of different concentrations of fungal suspension on the development of 24-hour-old eggs

The eggs were sprayed with 4 ml of fungal suspensions at concentrations of 3×10^3, 3×10^5, and 3×10^7 spores/ml, from a distance of 15-25 cm to ensure complete coverage. The treated eggs were then individually placed into separate Petri dishes, and their development was monitored. The parameters recorded included egg mortality rate, incubation period, larval mortality rate, duration of the larval stage (days), pupal mortality rate, duration of the pupal stage (days), and adult emergence rate.

Effect of different concentrations of fungal suspension on the second and fourth larval instars and their development

Second and fourth instar larvae were treated with 4 ml of fungal suspensions at the aforementioned concentrations, following the method described above. The recorded parameters included larval mortality rate, average duration of each larval instar (days), average duration of the entire larval stage, pupal mortality rate, and adult emergence rate.

Effect of different concentrations of fungal suspension on the pupal stage and its development

Pupae were treated with 4 ml of fungal suspensions by spraying, following the aforementioned procedure. The parameters recorded included pupal mortality rate, average duration of the pupal stage (days), and adult emergence rate.

Studying the effect of different concentrations of zinc oxide nanoparticles prepared by biological methods on certain life performance aspects of the saw-toothed grain beetle

Preparation of aqueous extract of the biomass of the *P. farinosus* fungus

The fungal isolate was cultivated in a sterile Petri dish containing PDA and incubated at 26 ± 2°C and 85 ± 5% relative humidity for one week. Subsequently, four discs were extracted from the growing PDA colonies and transferred to a liquid medium of sterilized Potato Sucrose (PS) in a 1000 ml glass container. To prevent bacterial growth, 125 mg of tetracycline was added. The container was continuously agitated at 26 ± 2°C for three weeks.

After incubation, the fungal biomass was collected using filter paper and a glass funnel, then washed three times with distilled water and twice with deionized water to remove any remaining nutrient medium. Twenty grams of the fungal biomass were weighed and transferred to a 1000 ml glass container containing 500 ml of deionized water. This mixture was incubated for three days with daily shaking. Afterward, the biomass was filtered using a 0.4 mm filter, and the filtrate was incubated at 26 ± 2°C and 75 ± 5% relative humidity until use (Al-Shammari, 2015).

Preparation of zinc oxide nanoparticles (ZnONPs) by precipitation method

Zinc oxide nanoparticles were synthesized using the precipitation method. Twenty grams of zinc acetate were added to 200 ml of the aqueous extract of the fungal biomass at 37°C. The mixture was incubated in a shaker incubator for 24 hours in a dark environment. The mixture was then centrifuged, and the sediment was washed three times with deionized water. The precipitate was dried using a microwave, and the formation of nanoparticles was confirmed by a color change. Solutions of zinc oxide nanoparticles were prepared at concentrations of 1000, 2000, and 3000 ppm from the resulting powder.

Characterization of zinc oxide nanoparticles

The optical properties of the zinc oxide nanoparticles were studied using ultraviolet-visible (UV-vis) spectroscopy. The particle size and crystalline structure were analyzed using a scanning electron microscope (SEM) (Chorachoo et al., 2013). The height and size of the three-dimensional nanoparticles were determined using an atomic force microscope (AFM) (Phang et al., 2021).

Effect of different concentrations of zinc oxide nanoparticles on 24-hour-old eggs and their development

The eggs were sprayed with 4 ml of various concentrations of zinc oxide nanoparticles from a distance of 15-25 cm to ensure complete coverage. Each treated egg was placed in an individual Petri dish, and their development was monitored. The parameters recorded included egg mortality rate, egg incubation rate, larval mortality rate, duration of the larval stage (days), pupal mortality rate, duration of the pupal stage (days), and percentage of adult emergence.

Effect of different concentrations of zinc oxide nanoparticles on the second and fourth larval instars and their development

Second and fourth instar larvae were treated by spraying with 4 ml of various concentrations of zinc oxide nanoparticles, following the procedure described above. The parameters recorded included larval mortality rate, average duration of the larval stage (days), pupal stage rate, pupal mortality rate, and percentage of adult emergence.

Effect of different concentrations of zinc oxide nanoparticles on the pupal stage and its development

The pupae were treated by spraying with 4 ml of various concentrations of zinc oxide nanoparticles, following the procedure described above. The parameters recorded included pupal mortality rate, average duration of the pupal stage (days), and percentage of adult emergence.

The influence of various concentrations of fungal suspension on the germination rate of rice amber grains

One hundred grams of rice grains were subjected to direct spraying with different concentrations of fungal suspension for each treatment. For the control treatment, seeds were treated solely with distilled water. Treated grains were thoroughly dried and subsequently packed into jute bags. After a week of storage, the germination rate was assessed by selecting 40 seeds per replicate across three replicates per treatment. Seeds were placed on filter paper moistened

with distilled water inside Petri dishes and maintained under controlled laboratory conditions. The germination process was monitored daily at a temperature of 26 ± 2°C, and germination rates were recorded for each treatment.

The impact of various concentrations of zinc oxide nanoparticle solution on the germination rate of amber seeds

In this study, the seeds were treated with varying concentrations of zinc oxide nanoparticle solution, following the methodology described above.

Statistical analysis

Data analysis was performed using the Statistical Analysis System (SAS) to evaluate the influence of different parameters on the studied traits based on a Completely Randomized Design (CRD). Significant differences between means were determined using the Least Significant Difference (LSD) test at a probability level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Effect of *P. farinosus* suspension concentrations on *O. surinamensis* eggs and development

The results presented in Table 1 demonstrate the impact of applying three different concentrations of *P. farinosus* fungal suspension (3×10^7, 3×10^5, and 3×10^3 spores/ml) on the eggs of the grain saw-toothed beetle. The observed egg mortality rates were 40.3%, 19.5%, and 3%, respectively, for each of the aforementioned concentrations, in contrast to the control treatment, which recorded a mortality rate of 0%. No significant differences in the duration of egg incubation were noted.

The highest larval mortality rate of 6.8% was recorded at the concentration of 3×10^7 spores/ml, compared to 1% in the control treatment. Statistical analysis revealed that the larval stage duration was longest at 21.2 days with the 3×10^7 concentration, compared to 17.1 days in the control treatment. No pupal mortality was observed across all treatments and concentrations, including the control.

The duration of the pupal cycle increased to 6.9 days with the 3×10^3 concentration, compared to 4.8 days in the control treatment. Additionally, the rates of adult emergence decreased to 52.9% and 74.2% at concentrations of 3×10^7 and 3×10^5 spores/ml, respectively, compared to 99% in the control treatment. Statistical analysis confirmed significant differences between the treatments.

Treatment with the fungal suspension resulted in egg destruction, attributed to the presence of polar and non-polar fats in the outer layer of the eggshell. These fats are decomposed by fungal spores, facilitated by the formation of appressoria, which helps the fungus adhere to the integument, thereby affecting the life and vitality of the embryo (Gindin et al., 2009; Ment et al., 2010).

Table 1. The effect of different concentrations of *P. farinosus* fungus suspension on the eggs and development of *O. surinamensis*.

Treatment Fungus spores /ml	% Egg Mortality	Rate of Egg Incubation (day)	% larva Mortality	Period of Larval instar (day)	% Pupal mortality	Pupal instar (day)	% Adult emergence
3×10^7	40.3	5.9	6.8	21.2	0	6.9	52.9
3×10^5	19.5	5.1	6.3	20.4	0	6	74.2
3×10^3	3	4.8	5	19	0	5	92
Control	0	4.5	1	17.1	0	4.8	99
L.S.D	8.37	1.51 N.S	1.27	3.08	0 N.S	1.02	9.24

Effects of various concentrations of *P. farinosus* suspension on the second larval instar of *O. surinamensis* and its development

The results presented in Table 2 demonstrated that all concentrations of the *P. farinosus* fungus suspension significantly increased the mortality rates of second-instar larvae. Specifically, concentrations of 3×10^7, 3×10^5, and 3×10^3 spores/ml resulted in mortality rates of 58.7%, 59%, and 50.5%, respectively, compared to the control treatment, which had a mortality rate of 5%. Statistical analysis revealed no significant differences among the various fungus treatments.

The maximum duration of the second larval stage was observed to be 28.01 days at a concentration of 3×10^7 spores/ml. In comparison, the durations for the concentrations of 3×10^5 and 3×10^3 spores/ml were 25

and 23.20 days, respectively, compared to 18 days for the control treatment.

For the pupae, all concentrations resulted in a mortality rate. The highest pupal mortality rate was 9.3% at 3×10^7 spores/ml, which decreased to 4.7% at 3×10^3 spores/ml, with no mortality observed in the control treatment. Furthermore, the adult emergence rate was 32% at 3×10^7 spores/ml, increasing to 40.8% at 3×10^3 spores/ml, compared to 95% in the control treatment.

The results indicated that second-instar larvae are particularly susceptible to the fungus suspension, likely due to their thin chitinous walls, which render them more vulnerable to fungal enzyme activity. This vulnerability facilitates the fungus's entry and subsequent consumption of the larvae's internal contents, leading to increased mortality. Moreover, previous research has demonstrated that varying concentrations of a suspension containing the fungus *Lecanicillium lecanii* resulted in a 60% mortality rate in second-instar larvae of the saw-toothed grain beetle at a concentration of 1×10^3 spores/ml (Mahmood and Tawfeeq, 2017).

Table 2. The effect of different concentrations of the fungus suspension *P. farinosus* on the second larval instar of *O. surinamensis* and its development.

Treatment Fungus spores /ml	% Larval Mortality	Average of larval period starting from the second larval instar /day	Average of pupal instar period /day	% Pupal mortality	% Adult emergence
3×10^7	58.7	28.01	4.6	9.3	32
3×10^5	59	25	4.2	5.1	35.9
3×10^3	50.5	23.20	4.3	4.7	40.8
Control	5	18	4.1	0	95
L.S.D	8.12	3.90	2.10	1.28	9.75

Effect of *P. farinosus* suspension concentrations on fourth instar larvae of *O. surinamensis* and their development

The results in Table 3 showed that the highest mortality rate of fourth-instar larvae was 39.01% at a concentration of 3×10^7 spores/ml, compared to 35.6% and 30.3% at concentrations of 3×10^5 and 3×10^3 spores/ml, respectively. The control treatment recorded 0% mortality. Regarding the duration of the fourth instar, no significant differences were observed between the different concentrations used.

The results also indicated that the longest pupal stage duration was 7 days at a concentration of 3×10^7 spores/ml, compared to 5.5 days in the control treatment. Table 3 further showed that the highest pupal mortality rate was 11.04% at a concentration of 3×10^7 spores/ml, which decreased to 7.60% at a concentration of 3×10^3 spores/ml, while the control treatment had a mortality rate of 1%.

The adult emergence rate was highest at 62.10% with a concentration of 3×10^3 spores/ml, and decreased to 49.95% at a concentration of 3×10^7 spores/ml, compared to 99% in the control treatment.

From these results, it is evident that the second larval stage is more susceptible than the fourth larval stage. This may be due to the thinner cuticle layer and incomplete hardening in the second larval stage.

Table 3. The effect of different concentrations of *P. farinosus* fungus suspension on the fourth instar larvae of *O. surinamensis* and their development.

Treatment Fungus spores /ml	% Larval Mortality	Average of fourth stage period (days)	Average of pupal stage (days)	% Pupal mortality	% Adult emergence
3×10^7	39.01	8.8	7	11.04	49.95
3×10^5	35.6	8	6.3	9.13	55.27
3×10^3	30.3	8.61	6	7.60	62.10
Control	0	8.3	5.5	1	99
L.S.D	3.31	N.S	1.7	1.43	6.42

Effect of different concentrations of *P. farinosus* Suspension on the parthenogenesis and development of 24-hour-old *O. surinamensis*

The results presented in Table 4 showed a clear effect on the mortality rate of pupae. The highest mortality rate was recorded at 55.6% when treated with a *P. farinosus* mushroom suspension at a concentration of 3×10^7 spores/ml, while the mortality rate decreased to 48.14% at a concentration of 3×10^3 spores/ml. The control treatment recorded a mortality rate of 0%. There were no significant differences among treatments in the average duration of the pupal cycle.

The highest rate of adult emergence was recorded at 51.86% at the concentration of 3×10^3 spores/ml, decreasing to 44.40% at a concentration of 3×10^7 spores/ml, compared to the control treatment, which had an adult emergence rate of 100%. The mortality of pupae is attributed to the fact that insect-pathogenic fungi attack the bodies of insects at all stages of their development. They penetrate the insects through chitin- and protein-degrading enzymes they secrete, leading to a localized infection that spreads to other tissues, causing a systemic infection. This process includes the multiplication of the fungus by producing yeast cell-like structures and depleting the nutrients in the body cavity and fat bodies, leading to starvation and, consequently, the death of larvae, pupae, or adult insects (Ali and Ren, 2010). Mahmood and Mahmood (2013) also indicated that the *P. farinosus* fungus affects the mortality rate of 24-hour-old pupae of the Khapra beetle, *Trogoderma granarium*, increasing it to 65%.

Characterization of zinc oxide nanoparticles (ZnONPs)

Color changes were observed when the biomass extract of the *P. farinosus* fungus was added to zinc acetate, transitioning from yellow to a white precipitate (Figure 1). After centrifugation and drying, the precipitate appeared as a bright white powder.

These changes indicated the formation of zinc oxide particles. Ultraviolet spectroscopy measurements revealed that the zinc oxide nanoparticles produced by the fungus exhibited optimal absorption for the surface plasmon signal at a wavelength of 281 nm (Figure 2). Scanning electron microscopy (SEM) analysis revealed that the zinc oxide nanoparticles were spherical, with an average size of 32 nm (Figure 3). Atomic force microscopy (AFM) provided a three-dimensional image of the nanoparticles, detailing their distribution and roughness (Figure 4).

Figure 1. Color changes observed during zinc oxide nanoparticle preparation using the fungus *P. farinosus*. (1). Cell-free extract with zinc acetate after incubation. (2). Sedimentation of nanoparticles after centrifugation.

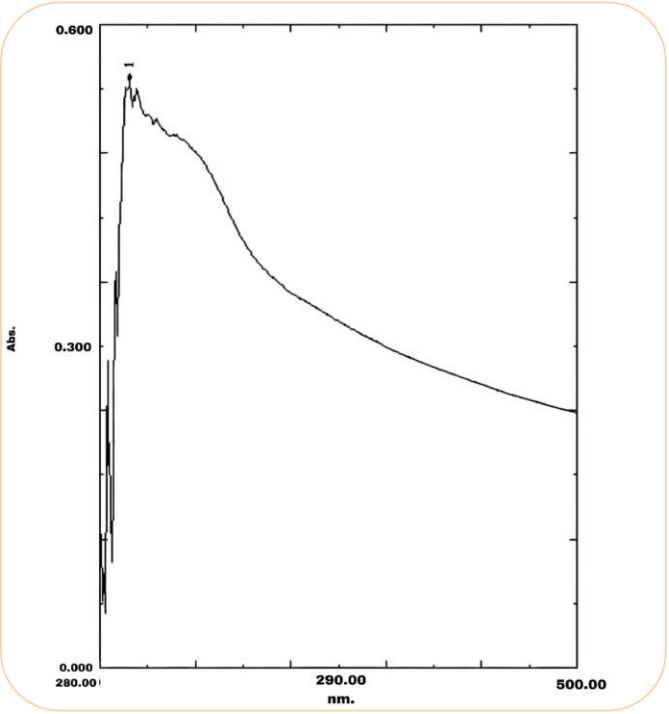

Figure 2. Absorption spectrum of zinc oxide nanoparticles using UV-VIS spectroscopy.

Table 4. Effect of different concentrations of *P. farinosus* suspension on the pupal stage and development of 24-hour-old *O. surinamensis*.

Treatment Fungus spores/ml	% Mortality of the pupal stage	Average of pupal stage (days)	% Adult emergence
3×10^7	55.6	5.81	44.40
3×10^5	49.02	4.62	50.98
3×10^3	48.14	4.98	51.86
Control	0	5.02	100
L.S.D	3.03	1.73 N.S	7.43

The effects of ZnONPs at different concentrations on 24-hour-old eggs of *O. Surinamensis* and their development

The results in Table 5 showed that different concentrations of zinc oxide nanoparticles had a significant impact on saw-toothed grain beetle eggs. A mortality rate of 100% was recorded at concentrations of 3000 and 2000 ppm, while a rate of 92% was observed at 1000 ppm, which differed significantly from the control treatment that showed no egg mortality. The incubation period for eggs was 7.32 days at 1000 ppm, whereas the control treatment had an incubation period of 4.20 days.

Larval mortality reached 100% at 1000 ppm, compared to only 2% in the control treatment. Table 5 indicated that treatments with zinc oxide nanoparticles did not affect the average duration of the larval stage, pupal mortality rates, average pupal stage duration, or rates of adult emergence, in comparison to the control treatment which recorded 16.03 days, 0%, 5.3 days, and 98%, respectively.

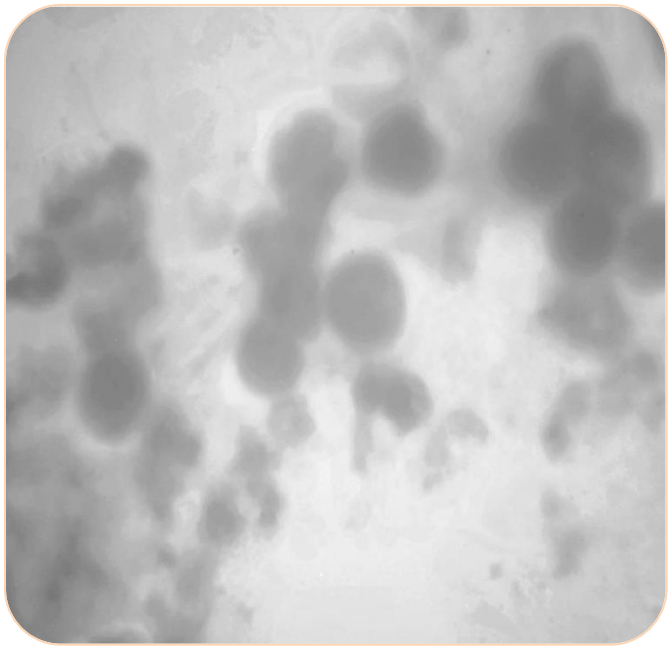

Figure 3. SEM image of zinc oxide nanoparticles.

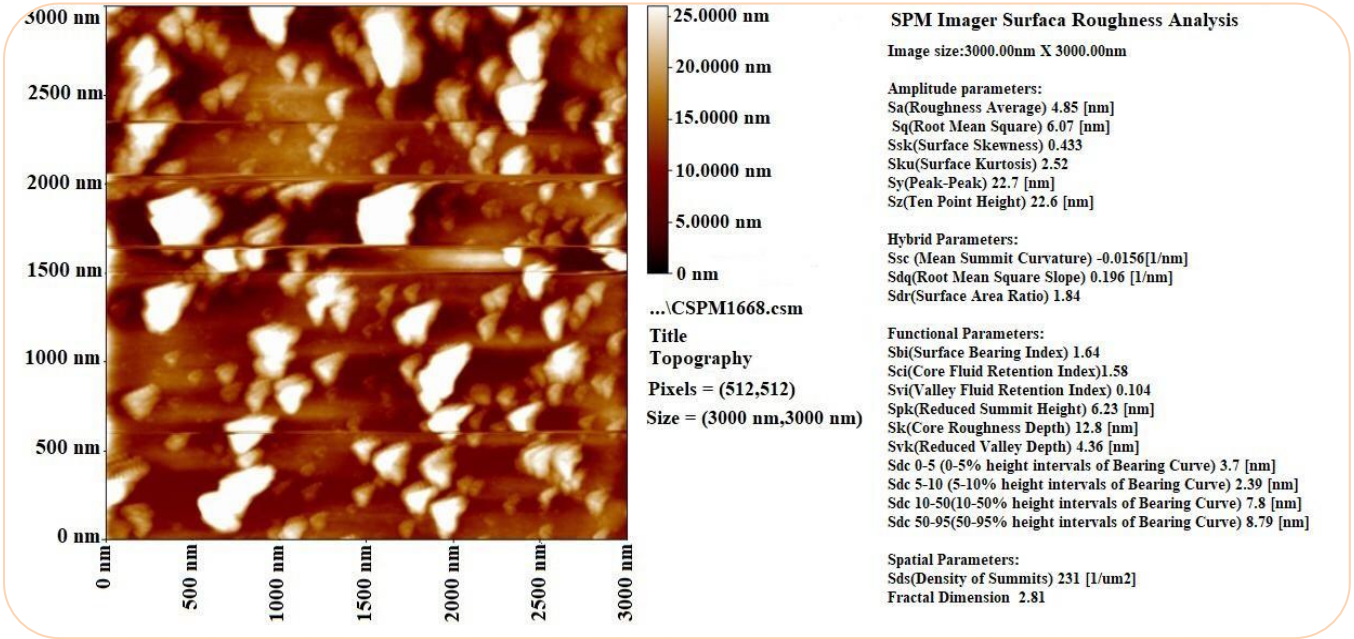

Figure 4. Three-dimensional image of zinc oxide nanoparticles showing distribution and surface roughness.

Table 5. The effect of different concentrations of ZnONPs on 24-hour-old eggs of *O. Surinamensis* and their development.

Treatment Concentration ppm	% Egg mortality	Average of egg incubation (days)	% Larval mortality	Average of the larval stage period (days)	% Pupal mortality	Average of pupal stage (days)	% Adult emergence
3000	100	-	-	-	-	-	-
2000	100	-	-	-	-	-	-
1000	92	7.32	100	-	-	-	-
Control	0	4.20	2	16.03	0	5.3	98
L.S.D	9.30	3.50	11.34	2.11	-	2.40	11.70

The Effect of various concentrations of ZnONPs on the second larval instar and development of *O. surinamensis*

The results in Table 6 demonstrated a clear impact i.e. different concentrations of zinc oxide nanoparticles significantly affected the second larval stage of the saw-toothed grain beetle. The mortality rate of second instar larvae reached 80% at a concentration of 2000 ppm, decreasing to 71.30% and 78.8% at 1000 ppm and 3000 ppm, respectively, while the control treatment recorded a mortality rate of 7%. The duration of the larval stage was 36, 32, and 33 days at concentrations of 3000 ppm, 2000 ppm, and 1000 ppm, respectively, compared to 16 days in the control treatment. Additionally, the highest percentage of pupal mortality, 12%, occurred at 3000 ppm, decreasing to 6.1% at 1000 ppm, with no pupal mortality in the control treatment. The longest pupal duration, 7.8 days, was observed at 3000 ppm, compared to 4 days in the control treatment.

Regarding adult emergence, the highest rate, 22.6%, was recorded at 1000 ppm, decreasing to 13% and 9.3% at 2000 ppm and 3000 ppm, respectively, while the control treatment had a rate of 93%. Statistical analysis indicated significant differences among treatments. Another study using nano-zinc oxide at a concentration of 300 mg/kg on the second and third larval instars of the Khapra beetle, *T. granarium*, resulted in a mortality rate of 22.3%. This effect is attributed to the incomplete cuticle layer and changes in the chemical and biological composition of the insect's body wall during larval growth periods (Mohammadali and AL-Rubaie, 2022).

Table 6. The effect of different concentrations of ZnONPs on the second larval instar and development of *O. surinamensis*.

Treatment Concentration ppm	% Larval mortality	Average of the larval period starting from 2nd instar	% Pupal mortality	Average of pupal stage period (days)	% Adult emergence
3000	78.7	36	12	7.8	9.3
2000	80	32	7	6.9	13
1000	71.30	33	6.1	7	22.6
Control	7	16	0	4	93
L.S.D	10.30	3.72	1.4	1.03	8.74

The effect of ZnONPs at various concentrations on the fourth larval instar of *O. surinamensis* and its development

The findings, displayed in Table 7, demonstrated that fourth-instar larvae had a lower mortality rate than second-instar larvae. The highest death rate was recorded for fourth larval stage saw-toothed grain beetles treated with zinc oxide nanoparticles i.e. 68.22% at a concentration of 3000 ppm, decreasing to 58% and 47% at concentrations of 2000 ppm and 1000 ppm, respectively. The mortality percentage in the control treatment was 0%. Results also showed the longest average duration of the fourth larval stage at a concentration of 2000 ppm, recorded at 12.50 days, decreasing to 11.30 days and 10.01 days at concentrations of 3000 ppm and 1000 ppm,

respectively, whereas the control treatment recorded 8.90 days.

For concentrations of 3000 ppm, 2000 ppm, and 1000 ppm, the pupal mortality percentages were 19%, 14.5%, and 10%, respectively, compared to 0% in the control treatment. The longest average duration of the pupal instar (8 days) was recorded at a concentration of 3000 ppm, compared to 4.90 days in the control treatment. Moreover, the emergence rate of adults was 12.78% when treated with 3000 ppm, while it was 100% in the control treatment. Statistical analysis indicated significant differences among the treatments.

Furthermore, research revealed that using nano-zinc oxide at a quantity of 300 mg/kg during the fourth and fifth larval stages of the Khapra beetle, *T. granarium*, resulted in a mortality rate of 16.8%. The high effectiveness of nano composites like silica oxide and zinc oxide is attributed to the small size of their molecules. The different active components in each nano composite contribute to varying levels of efficiency, determined by the nanomaterial's ability to adhere to the insect's body wall or the exterior of treated grains (Mohammadali and AL-Rubaie, 2022; Khalil et al., 2019).

Table 7. The impact of different concentrations of ZnONPs on the fourth larval instar of *O. surinamensis*, and its development.

Treatment Concentration ppm	% Larval mortality	Average of larval stage period starting from 4th instar (days)	% Pupal mortality	Average of pupal stage (days)	% Emergence of adult
3000	68.22	11.30	19	8	12.78
2000	58	12.50	14.5	7.20	29.20
1000	47	10.01	10	7	43
Control	0	8.90	0	4.90	100
L.S.D	9.25	2.74	4.8	1.10	9.80

The impact of various concentrations of ZnONPs on the pupal stage and development of *O. surinamensis* at 24 hours

Table 8 showed the percentage of pupal mortality of the saw-toothed grain beetle at 24 hours of age using various concentrations of zinc oxide nanoparticles. The highest mortality rate, 20%, was observed at a concentration of 2000 ppm, while it decreased to 17% and 15.8% at concentrations of 1000 ppm and 3000 ppm, respectively, compared to the 0% mortality in the control treatment.

The longest average duration of the pupal stage, 7.53 days, was recorded at 1000 ppm, decreasing to 5.50 days at 2000 ppm. The emergence rate of adults was 84.2% at 3000 ppm, and 83% and 80% at 1000 ppm and 2000 ppm, respectively, compared to 100% in the control treatment. Statistical analysis indicated no significant difference among the concentrations compared to the control treatment.

The impact of *P. farinosus* fungus suspension and ZnONPs solution on the germination rate of amber grain seeds treated via direct spraying

The results in Table 9 showed that the germination rates of amber rice grains were measured after three months of treatment. The treatments involving the suspension of the fungus *P. farinosus* and the nano-zinc oxide solution did not exhibit any significant differences among them. Specifically, the germination rates of rice grains treated with the *P. farinosus* suspension via direct spraying were 98.05%, 98%, and 95% for concentrations of 3×10^3, 3×10^5, and 3×10^7 respectively. Meanwhile, germination rates using zinc oxide nanoparticle concentrations of 3000, 2000, and 1000 recorded 95.32%, 96.00%, and 96.30% respectively, compared to the 100% control treatment.

Table 8. The effects of different concentrations of ZnONPs on the pupal stage of *O. surinamensis* at 24 hours of age and during its development.

Treatment Concentration ppm	% Pupal mortality	Average of the pupal stage (days)	% Adult emergence
3000	15.8	6.72	84.2
2000	20	5.50	80
1000	17	7.53	83
Control	0	3.94	100
L.S.D	3.8	2.04	9.36

Table 9. Effects of ZnONP solution and *P. farinosus* fungus suspension on seed germination rate in amber cultivar via direct spray.

Treatment	% Germination of amber seeds after 3 months
Control	100
Fungus at a concentration of 3×10^3	98.05
Fungus at a concentration of 3×10^5	98
fungus at a concentration of 3×10^7	95
Zinc oxide nanoparticles at a concentration of 1000	96.30
Zinc oxide nanoparticles at a concentration of 2000	96
Zinc oxide nanoparticles at a concentration of 3000	95.32
L.S.D. value	5.72 N.S

N.S. = not significant.

The results demonstrated that solutions containing zinc oxide nanoparticles consistently outperformed spore suspensions of the fungus *P. farinosus* across all concentrations. They notably increased rates of egg mortality and mortality among second and fourth instar larvae. Specifically, the second larval instar showed a significantly higher susceptibility compared to the fourth larval instar. No significant effect was observed on the germination rates of rice grains treated with either the fungal suspension or varying concentrations of zinc oxide nanoparticles.

AUTHORS' CONTRIBUTIONS

EAM and TAH designed, formulated, laid out the study and conducted the experiments; TAH collected, arranged, analyzed the data and provided technical assistance; EAM supervised the work; EAM and TAH wrote the manuscript; EAM proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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