The effect of bio-prepared zinc nanoparticles from the fungus *Verticillium lecanii* on combating third-instar larvae of the date moth *Ephestia cautella*

[1] Doaa Abdulmajeed Mohamed

eduhm230154@uosamarra.edu.iq

[1,2] Department of Biology, College of Education – University of Samarra

[2]Hisham naji hameed

Hisham.n370@uosamarra.edu.iq

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[2]Hisham naji hameed

Hisham.n370@uosamarra.edu.iq

Abstract:

The study was conducted in the Department of Biology - College of Education - University of Samarra, which aims to combat the third instar larvae of the date moth *Ephestia cautella* using bio-prepared zinc nanoparticles from the fungus *Verticillium lecanii* and comparing it with the biomass of the fungus *Verticillium lecanii* and finding out which is most effective in eliminating the third instar of the date moth *Ephestia. cautella* and biomass concentrations were used (1, 1.5, 2) g / L.

Only distilled water was used in the control plants, with a time period of (24, 48, 72) hours for each concentration, Three concentrations of zinc nanoparticles were used: (0.250, 0.125, 0.62) ml / liter , and only distilled water was used in the control laboratories, with a period of time of (24, 48, 72) hours for each concentration, and for each concentration, three replicates were used for each concentration, and 10 larvae of the date moth *Ephestia cautella* were placed in each replicate for each replicate of the

experiment, and the results of each were given: Biomass had high killing results at a concentration of 2 ml/L after 72 hours, and nano zinc at a concentration of 0.250 ml/L after 72 hours.

Introduction:

The date moth is an insect with complete metamorphosis and has four stages: the egg, the larva, the pupa, and the adult. The female date moth lays her eggs in groups or individually on the outer surface of the date [1].

Although the female insect lives for approximately 14 days, The eggs laid by one female are approximately 135 eggs, and approximately 90% of the eggs are laid in the first four days. The eggs are characterized by containing prominent lines on the surface of the egg, both longitudinally and transversely, arranged in 24 irregular rows, with the length of one egg ranging from (0.33 - 0.38 mm).

Their width ranges between (0.22 - 0.32 mm), and the eggs are white when first laid by the adult insect, then they turn orange before the hatching process, with clear longitudinal and transverse elevations on the outer surface [2],The hatching process may take from 3-4 days after the female lays her eggs, the date moth, *Ephestia cautella*, is a polyphagous insect that infects different types of stored foodstuffs, most notably dates, whether they are on palm trees or fallen on the ground or in stores, as well as feeding on many stored foodstuffs such as dried figs, raisins, tarshana, and grains. And their products, legumes, nuts, oilseeds, cocoa and other food families[3].

Nanocomposites are materials to which nanoparticles are added during the manufacture of these materials. As a result, the nanomaterials show an improvement in their properties. Nanotechnology is considered a broad field of scientific research and opens up a wide field in various fields.

It is considered the main advantage of modern insecticides, as it has a useful pesticide effect to eliminate insects. It is not harmful to the main environmental components, and the word nano (Nanos) is originally a Greek word that means dwarf and is used to describe materials with small sizes from (1−100 nanometers). Nanobjects are bio−manufactured using microorganisms such as fungi, bacteria, viruses, and nano−extracts.

One of the advantages of this method is that it is cheap and does not require Energy, fast and at the same time environmentally friendly [4].

Fungi that infect insects and products derived from fungi have been used in biological control of targeted insects, The *Verticillum lecanii* fungus is one of the most common fungi on target insect families and is used to eliminate insects that cause economic damage [5].

Aim of the Study:

1- Evaluation of the effectiveness of biomass prepared from the fungus *Verticillum lecanii* on third-instar larvae of the date moth *Cautella Ephestia.*

2- The effectiveness of a nano-prepared biological preparation from the *Verticillum lecanii* fungus on eliminating the larvae of the date moth *Ephestia cautella*.

key words: *Verticillium lecanii,, Ephestia cautella* , PDA, ZnoNPs, nano

Materials and Methods:

Medium Solid Potato Dextrose Agar (PDA):

Prepare the medium according to the manufacturer's instructions, HIMEDIA, by dissolving 39 grams of potato medium in a liter of distilled water, placing it in a 1000 ml glass baker, shaking well, closing the nozzle with a cotton plug, then sterilizing with an autoclave at a temperature of 121°C and a pressure of 15 pounds/inch for 15 minutes, then Leave the medium to cool and before it hardens, pour it into sterilized dishes.

Liquid potato dextrose medium (PDB) Potato Dextrose Broth:

Prepare the medium according to the manufacturer's instructions by dissolving 24 grams of powdered medium in 1 liter of distilled water. Distribute the medium into conical flasks with a capacity of 250 ml and plug their nozzles with cotton plugs. Then sterilize with an autoclave at a temperature of 121 °C and a pressure of 15 pounds/in2 for 15 minutes, Prepare this medium to obtain the biomass of the *Verticillium lecanii* fungus.

Activation of the fungus *V. lecanii*

Activating the fungal isolate by replanting it on new PDA media 7-9 days before starting to produce biomass in order to use it in preparing biomass.

Preparation of *V. lecanii* biomass

To obtain biomass, the fungal isolate was grown in a sterile Petri dish containing sterile Potato Dextrose Agar medium, and incubated at a temperature of 2 ± 26 °C and a relative humidity of 5 ± 85% for 7 days.

After that, four discs were taken from the colonies growing on the medium. The solid food PDA, with a diameter of 5 mm, was multiplied on the sterile liquid medium placed in a 1000 ml glass container with the addition of 125 mg of tetracycline to ensure that bacteria did not grow, while continuing to move the glass containers incubated at a temperature of 2 ± 26 °C for 21 days with daily manual shaking. Biomass after 21 days of incubation by using a glass funnel and filter paper.

After that, the biomass was washed with distilled water three times, followed by washing it with deionized water twice to get rid of all residual nutrient medium. Weighed 10 grams of fungal biomass using a sensitive balance and transferred to 1000 ml glass containers containing 250 ml deionized water were also incubated under the same conditions above with daily shaking using a shaker for 120 hours.

After the expiration of the period, the fungal biomass was filtered using filters to obtain the fungal biomass filtrate. The filtrate was collected and incubated at a temperature of 26 ± 2 °C and a relative humidity of 5 ± 75% until use [6].

The biomass is then dried to obtain a powder for experiments on the third instar larvae of the date moth.

For fungal biomass, concentrations of (1, 1.5, and 2) grams were used, with three replicates for each concentration. The control factor in the experiment was used only distilled water for 24, 48, and 72 hours. The concentrations gave high rates of death.

Bio-prepared nanocomposite from *Verticillium lecanii*

The nanocomposite zinc oxide (ZnoNPs) that was used in the study was obtained from the Ministry of Science and Technology in Baghdad Governorate / Iraq. The compound was in the form of a white-yellowish powder, with a particle size of less than 5 micrometers with a purity of 99% , The Ministry prepared the compound in A plastic box containing 7 grams. It was received in the form of a nanopowder with a particle size of less than 100 nanometers.

Preparation of nanocomposites (ZnoNPs):

Silver nanoparticles were manufactured by crushing the mushroom extract using an ultrasound device for five minutes, after which the previously prepared zinc oxide solution was placed on a hot plate with a magnetic stirrer for 30 minutes, after which the mushroom extract was added to the oxide solution.

Zinc in drops, then placed in an ultrasound machine for 30 minutes, then mixed with a magnetic mixer without heat for 30 minutes [7].

An amount of nano-zinc oxide was weighed 0.5 grams of powder and a drop of concentrated nitric acid was placed on it and mixed with the powder, noting the rise of vapors from the powder after mixing it with nitric acid.

Then the homogeneous material was placed in a glass beaker containing 1000 ml of distilled water with continuous stirring for 10 minutes. To ensure that the nanocomposite dissolves with water, and thus we have the main stock concentration.

After that, we conduct several dilutions to reach the concentrations required in the experiment, which are a concentration of 0.250, 0.125, 0.062) and for a period of time of (24, 48, 72, 96), with three replicates for each concentration and a coefficient was used. The control in this experiment was only distilled water.

Statistical Analysis:

The results analyzed statistically by applying the statistical program (MINITAB VER.17) according to the Anova analysis test (Anova). The mathematical averages were compared according to the Duncuns Multiple Range test and at a possibility of $0.05 \geq p$ [8].

Results :

The results of the table (1) and the effect of the interaction of the different concentrations (0.062, 0.125, 0.250 mg/L) of the zinc oxide nano composite, as well as the time period for the death of the third instar of the date moth, showed that there were significant differences in the killing rates due to the interaction between the concentrations and the duration of exposure, as the highest percentage of killing was for the third instar larvae. The third was 96.7% at a concentration of 2500 after 96 hours of treatment, while the lowest killing percentage was 23.3% at a concentration of 0.062 after 24 hours of treatment, while averages of the killing percentage as a result of the concentrations showed that the highest killing percentage was at a concentration of 0.250 reaching 72.5%, while The lowest average kill rate at a concentration of 0.062 was 59.2%.

As for the average kill rate based on the duration of killing, the highest kill rate after 96 hours was 70.0%, and the lowest kill rate after 24 hours was 24.2%.

From the results it was shown that the percentage Larval killing increased with increasing concentration and duration of treatment. The results of the study were consistent with the findings of [9].

As they indicated that the zinc oxide nanocomposite had an effective effect in combating the red flour beetle T. castaneum compared to the pesticide malathion, as the results showed that there was a significant effect of the nanocomposite.

Zinc oxide affects the percentage of killing, productivity and weight loss of whole grains. These percentages increase with increasing concentration and duration of exposure, as these particles cause deformities and dehydration of the insect and provide protection for the grain by reducing the rate of the first generation of the insect T. castaneum and then reducing the percentage of weight loss in the grain. It was also similar The results of this study are based on the findings of [10].

Who indicated the effect of nano composites, including zinc oxide, in protecting grains from infection with the Khabra insect for a period of up to 40 days, where the percentage of weight loss was 0.67, 0.73, and 3.44%, while the percentage of weight loss was 3.44%.

The loss in the control treatment is 11.74%. Zinc oxide nanoparticles have been used to develop pesticides due to their antimicrobial, physical and some other properties [11]; [12].

Table (1) shows the effect of nano-zinc oxide (ZNPs) on the mortality rates of third-instar larvae of the date moth.

Concentration Average	Time				Concentration ml /l
	96 Hours	72 Hours	48 Hours	24 Hours	
7.25 A	9.67 a	8.67 b	6.33 d	4.33 f	0.250
6.25 B	9.33 ab	7.33 c	5.33 e	3.00 g	0.125
5.92 B	9.00 b	7.33 c	5.00 e	2.33 h	0.062
0.0 C	0 i	0 i	0 i	0 i	Control
	7.00 a	5.83 b	4.17 c	2.42 d	Time average

Small letters that are similar horizontally mean that there are no significant differences between them.

The results of the table (2) and the effect of the interaction of different concentrations (2, 1.5, 1) g/Lon biomass as well as the time period for the death of the third instar of the date moth showed that there were significant differences in the killing rates due to the interaction between the concentrations and the duration of exposure, as the highest percentage of killing for the third instar larvae was 93.3 % at concentration 2 after 72 hours of treatment, while the lowest killing rate was 23.3% at concentration 1 after 24 hours of treatment, while averages of the killing rate as a result of the concentrations showed that the highest killing rate was at concentration 2, reaching 72%, The lowest average kill rate at concentration 1 was 23.3%.

As for the average kill rate based on the duration of killing, the highest kill rate after 72 hours was 50.0%, and the lowest kill rate after 24 hours was 28.3%.

From the results it was shown that the percentage of Killing third instar larvae of date moth increased with increasing concentration and duration of treatment. The results of this study agreed with [13].

Through the use of suspensions of three types of chrysogenum fungi. *penicillium*, *V.lecanii, Aspergillus.niger*) in its effect on the larval stages of *C.quinquefasciatus* mosquitoes, as the insect-pathogenic fungus *V.lecanii* outperformed the rest of the fungi in influencing mosquito larvae with percentages of death reaching 20, 16.66, 13.33, and 10% after 48 hours of treatment and rose to 63.33), 60, 56.66 and 53.33 after 96 hours of treatment.

The high rate of death rates in the first larval ages with the last instar and adults is attributed to the incompleteness of the defense cells in the first larval ages, in addition to the lack of thickness of the cuticle layer, or it may be explained by changes in the biological and chemical composition of the insect's body wall, such as the presence of toxic compounds, which may To prevent the germination of fungal spores [14].

The results of other studies that are consistent with this study showed what was mentioned by [15].

that they were more sensitive to infection by *E. cautella*. The results showed that individuals of the first larval stages of the date moth were exposed to biological factors (bacteria and fungi) from later ages. The ability of the fungus to adhere to the body The insect, its structure, the germination tube and adhesion organ, and the amount of enzymes secreted by the fungus, such as chitinase, lipase, and protease enzymes, played a major role in destroying the insect's body.

The current study showed that increasing the concentration and duration of treatment of biologically prepared biomass from mushrooms has a significant impact on eliminating third-instar larvae of the date moth.

Table (2) shows the effect of biosynthetic mass from the fungus *V. lecanii*. On third instar larvae of the date moth *E. cautella*

Concentration Average	Time			Concentration ml /l
	72 Hours	48 Hours	24 Hours	
3.33 C	4.00 e	3.67 e	2.33 f	1
5.56 B	6.67 bc	6.33 c	3.67 e	1.5
7.22 A	9.33 a	7.00 b	5.33 d	2
0.0 D	0 g	0 g	0 g	Control
	5.00 a	4.25 b	2.83 c	Time average

Small letters that are similar horizontally mean that there are no significant differences between them.

Conclusion:

1-The fungus *V. lecanii* showed high efficiency in the biosynthesis of zinc oxide nanoparticles.

2- Biologically prepared nanoparticles have a promising future in controlling insect pests.

3. Treatment with zinc nanoparticles led to the killing of third-instar larvae of the date moth three days after treatment, with a direct relationship between concentrations and killing rates.

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تأثير جزيئات الزنك النانوية المحضرة حيويا من الفطر *Verticillium lecanii* في مكافحة يرقات العمر الثالث لفراشة التمر *Ephestia cautella*

1,2

كلية التربية[1,2] , قسم علوم الحياة – جامعة سامراء

دعاء عبدالمجيد محمد [1]

eduhm230154@uosamarra.edu.iq

هشام ناجي حميد [2]

Hisham.n370@uosamarra.edu.iq

المستخلص:

أجريت الدراسة في قسم علوم الحياة – كلية التربية – جامعة سامراء، وتهدف إلى مكافحة يرقات العمر الثالث لعثة التمر *Ephestia cautella* باستخدام جزيئات الزنك النانوية المحضرة حيوياً من فطر Verticillium lecanii ومقارنتها مع الكتلة الحيوية للفطر *Verticillium lecanii* ومعرفة أيهما أكثر فعالية في القضاء على العمر الثالث لعثة التمر *Ephestia*. واستخدمت تراكيز من النانو والكتلة الحيوية (1، 1.5، 2) غرام/لتر.

تم استخدام الماء المقطر فقط في محطات المراقبة وبمدة زمنية (24، 48، 72) ساعة لكل تركيز، وتم استخدام ثلاث تراكيز من جزيئات الزنك النانوية: (0.250، 0.125، 0.62) مل/لتر، والماء المقطر فقط. تم استخدام مختبرات المقارنة بفترة زمنية (24، 48، 72) ساعة لكل تركيز، ولكل تركيز تم استخدام ثلاث مكررات لكل تركيز، وتم وضع 10 يرقات من فراشة التمر *Ephestia cautella* في مكررات للمقارنة.

تم إعطاء نتائج كل مكرر لكل مكرر للتجربة: أظهرت الكتلة الحيوية نتائج قتل عالية عند تركيز 2 مل/لتر بعد 72 ساعة، ونانو زنك بتركيز 0.250 مل/لتر بعد 72 ساعة.

الكلمات المفتاحيه: *Verticillium lecanii*, *Ephestia cautella* , PDA, ZnoNPs, nano