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Effect of two nanoparticles and bacteria spores on some biological aspects of waxworm Galleria mellonella (Lepidoptera;Pyralidae)

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Abstract. The aim of this study is to control the major Waxworm Galleria mellonella, pests that, cause damage to honeybee colonies due to feeding their larvae on wax combs using safe alternatives such as nanomaterials and biological control using spores of *Bacillus thuringiensis* bacteria and the use of concentrations, 10^6 10^7، 10^8 and 10^9 cells/ml of D.W on the egg stage, and the efficiency of zinc oxide nanoparticles and titanium dioxide in controlling the insect was tested using concentrations (5000,1000,500,100) ppm. The practical study was conducted in the insect lab /Department of Biology/College of Education for Pure Sciences at Mosul University. The nanomaterials and bacterial spores used showed a significant effect in the incubation period, as the longest period was 17.667 days in zinc oxide at a concentration of 100 ppm, and the percentage of hatching was as low as 26,000% in bacterial spores at a concentration 10^6 cells/ml and duration The larval and pupal stages and the mortality rate in them, where the longest period in the larval stage was 39.333 days in TiO2 treatment at concentration 5000ppm, and the highest percentage of mortality was 82.1% in bacterial spores at concentration 10^6 cells/ml in addition to the number of emerging insects and sexual ratio, as significant differences were shown between the treatments.

Keywords: Nanoparticles, ZiO, TiO2, Galleria mellonella, Bacillus thuringieneses

Introductio

Lives in most parts of the world except the polar regions. Honeybees have been the subject of studies due to their important role as an efficient pollinators, and producing of phytonutrients. itis have also been the subject of nutritional and therapeutic benefits for some disease conditions represented by honey, wax and Royal jelly and propolis and bee venom [1]. They are exposed to various of diseases, such as bacteria, viruses , and fungi diseases, parasitic diseases well as, and vertebral pests including frogs , lizards, and insect pests such as ants, wasps, major and small wax worms [2]. the mjor waxworm *Galleria mellonella* (Lepidoptera: Pyralidae), is one of the most important pests the insect that attacks honey bees combs, it is one of the most widespread insects in the world that, they are on the most harmful pests to honey bee colonies as a result feeding its larvae on wax combs and pollen stored in these combs [3]. As a result of the economic damage caused by the major wax worm, several methods have been used to control it, such as chemical repellents, physical methods and biological control, such as the use of Parasitodies [4].

Due to the necessary need to control this insect, chemical pesticides have been previously used on large areas, but for some reason including lack of specialization and the emergence of control to insects, and its negative effect on honey bees, environment and human health, as well as the high cost of production, prompted researchers to search for other alternative ways to control and reducing this insect. Terefore, the biological control was widely used, to control or reduce pests according to a specific strategy using natural enemies which are safe for humans, animals or crops [5]. One of this method is- using the bacteria *Bacillus thuringiensis* which is friend for environmental and considered the most important pathogen of insects. This is characterized by its toxic effects on some insects, and its toxicity due to production of crystals proteins [6].

In addition to the aforementioned control, there is another modern method, used of nanomaterials, as nanotechnology occupies a prominent position among the innovative methods for developing agricultural transactions and food production, This technology represents a new generation of techniques that can facilitate the control process by finding effective solutions regarding Cost-effectiveness and safety [7]. The nanoparticies varying in size,
1-100 nanometeres, as these particles possess new physical, chemical, and biological properties [8].
The current study aimed to compare the effectiveness of nanomaterials (Zinc oxide and Titanium dioxide), used for the first time, with the activity of *B. thuringiensis* spores in some biological aspects of the major wax worm *G.mellonella*.

Materials and Methods

It incubated in incubator at 30 ± 1 °C and 70 ± 5% R.H, the from was constantly renewed after each generation.

1-The source of insects and their breeding method

The major wax worm Galleria mellonella L. (Lepidoptera: Pyralidae) was collected from infected wax from some indigenous apiaries scattered in some areas in Nineveh Province.

2-Nanomaterials

Zinc oxid nano particlec a white powder,was purchased from the company: (International Trading Oma Authorized Partner Of Sigma Aldrich\Germany), nanozinc oxide was used at a size of 5 nm according to manufactures instruction.
In addition to using Titanium Dioxide nanoparticles (TiO2) from Sigma-Aldrich Company, China, as it was in the form of crystals in the form of white powder, the particles measured less than 25 nanometers and the concentrations were (5000, 1000, 500, 100) ppm was for the two.

3-B. thuringiensis Spores

Alocal isolated of bacterial spores was obtained from the plant protection laboratories of the College of agricultureThe concentrations were 10^6,10^7 , $10^8$and 10^9cells/ml of D.W. and the
experiment was conducted as follows:

1-Egg stage

Eggs were picked at the age of one day from new emeryed adults and treated with the materials under study. In addition to the control treatment (treatment with D.W.) by treating the eggs superficially, in this experiment a micro applicator was used to cover the surface of the egg with 0.1 µl per egg [9] and three replicates were used for each treatment in each replicate of 25 eggs. Concentration of the used concentrations. The treated eggs were placed in plastic cups, their nozzles covered with a tampon, and then placed in the incubator at 30 ± 1 °C and a relative humidity of 70 ± 5%.
The following is calculated:
1-Eggs incubation period of the eggs
2-% Haching
3-Duration of the larval stage and the percentage of death
4-Duration of the pupal stage and the percentage of death
5-Number of emerging insects
6-Sex ratio

Statistical analysis

The results were statistically analyzed using the SAS computer program, with the complete random design (C.R.D.) used as a factorial experiment, then the differences between the means were tested using Duncan's multi-range test below the level of probability of 5% [10].

Results and Discussion

In this experiment, the eggs of the major wax worm G. mellonella were treated with concentrations 100, 500, 1000, 5000 GV of Zinc oxide nanomaterials (ZNO) and Titanium dioxide (TiO2). The eggs were also treated with spores of B. thuringiensis at concentrations 10^6,10^7 , $10^8$and 10^9 cells/ml D.W. to compare the effectiveness of these materials in some biological aspects of the worm G.mellonella The egg stage:
Table (1) shows that treatment with it nanomaterials Zinc oxide nanomaterials, titanium dioxide and B. thuringiensis spores has a different effect on the egg stage. The results in the above table showed that the egg incubation period was 10.667 days, at control treaatmant while it increased significantly in the Zinc oxide treatment, reaching 17.667day. There was no significant difference between it and the titanium dioxide treatment at the concentration of 100 ppm, (17.333 days), while it decreased insignificantly at the concentration 5000 ppm in each of the Zinc oxide treatment. Zinc and Titanium dioxide reached 15.667 days in both materials.
In general, we note from Table (1) that the incubation period was the shortest in B. thuringiensis spores treatments and amounted to 12.333 days at concentration 10^9 cells/ml D.W., and it reached 15,000 days at concentration 10^6cells/ml D.W. There was a significant difference between them, all treatments were significantly different from the control treatment. It can be said that the incubation period is inversely proportional to the concentrations of the materials used.
[26] Also emphasized the role of nanomaterials in controling the different stages of insects, as they work on tissue lysis and programmed cell death by breaking down protein, fat and DNA of insects. We also note from Table (1) that the treatment with the substances under study affected the percentage of eggs hatching of the Major Waxworm G. mellonella, as we find that the percentage in general has decreased significantly in all treatments compared to the control treatment.
The lowest percentage of hatched eggs was 26.663 % in the treatment of spores of B. thuringiensis bacteria at concentration 10^6 cells/ml of D.W. While the lowest percentage of hatching in the Zinc oxide treatment was 30% at a concentration 100 ppm, and the lowest hatching percentage was in the Titanium dioxide treatment 28% at a concentration 5000 ppm and there was no significant difference between the three treatments above, except that all of them differed significantly from the control treatment, which amounted to 83.600%
We note from Table No. (1)also that the treatment with Zinc oxide and B. thuringiensis spores for hatched eggs was a direct relationship, as the higher the concentration, the higher the hatching rate. While in the Titanium dioxide treatment, there was an inverse relationship, the higher the concentration, the lower the percentage of eggs hatching.
[11] confirmed that Zinc oxide had a positive effect in reducing the hatchability of eggs of the southern cowpea beetle C. maculatus, which gave a positive response with an increase in the concentration of Zinc oxide nanoparticles.
The genotoxicity of nanoparticles is caused by the formation of reactive oxygen species (ROS) either by the particles themselves by inducing cellular responses or by stimulating target cells, and ROS results in the formation of OH which is one of the
species harmful to insect DNA [12].

2- Larval and pupal stage

The results shown in Table (2) showed the effect of Zinc oxide and Titanium dioxide nanomaterials used at concentrations of 5000, 1000, 500, and 100 PPM, in addition to the effect of B. thuringiensis spores used at concentrations 10^6,10^7 , $10^8$and 10^9cells/ml of D.W. over the duration of the larval and pupal stages in the treatment of eggs of the major waxworm G.mellonella

The results indicate that there are significant differences in the duration of the larval stage treated with different concentrations compared to the control treatment, as the control treatment amounted to 25.667 days and it differed significantly with the highest duration of the larval stage in Titanium dioxide at concentration 5000 ppm, duration was 39.333 days, which differed significantly at the same concentration of Zinc oxide, the larval duration was 32.000 days,

In general, notice Table (2) that the duration of the larval stage was the shortest in the treatment of spores of B. thuringiensis bacteria, reaching 29.667, 29.333 at concentrations 10^6,10^7 cells/ml D.W., respectively, and there was no significant difference between the duration, it was 28.667 and 28.000 days at concentrations 10^8 and 10^9 cells/ml of D.W., respectively, and there was no significant difference between them also.

The results also showed the effect of the interaction between the concentrations of the substances used and the duration of the pupal stage for the treatment of eggs of the major wax worm G.mellonella, as shown in Table (2), that there were significant differences between the treatments, and all of them differed significantly from the control treatment, which amounted was 9.333 days, while the longest period of the stage reached virginity in Zinc oxide at concentrations 500,100 ppm was 22.333 and 21.333 days, respectively, and there was no significant difference between them. the treatment of B. thuringiensis spores, show the longest period 20.000 days at concentration 10^6 cells/ml D.W., followed by treatment with concentration 10^7 cells/ml of D.W. as it reached 19,000 days and there was no significant difference between the two treatments.

We also note from(Table 2) that treatment with Zinc oxide (ZnO) and spores of B. thuringiensis had an inverse effect with the concentration, as the higher the concentration, the shorter the pupal longivity.

We note from the same table that in the treatment of Titanium dioxide TiO2, cause shortest period of the pupal stage at concentration 100 ppm was 16.333 days, which differed significantly with control treatment and the longest duration of the pupal stage. Here it can be said that the higher concentration, cause longer the pupal stage. The longest period in the Titanium dioxide treatment (20.000 days) at the concentration 5000 ppm ,and in all the concentrations used in the Titanium dioxide treatment, the duration of the pupal stage was significantly different from the control treatment.

[13] confirmed that the size of nanoparticles is close to the size of cellular proteins, so nanoparticles have the ability to cross some barriers of biological systems, and the optional permeable cell membrane has the ability to control the movement of large or small molecules inside and outside the cell, this led to obtaining the aforementioned results.

3- Mortality of the larval and pupal stages

Results shown in (Table 3) that the effect of Zinc oxide nanoparticles (ZnO) and Titanium dioxide (TiO2) nanoparticles at concentrations 100, 500, 1000 and 5000 ppm in addition to the effect of B. thuringiensis spores used at concentrations 10^6,10^7 , 10^8 and 10^9 cells/ml of D.W. in percentage mortality in the larval and pupal stages that emerged from of treating the eggs of the major waxworm G. mellonella,

The results indicate that there are significant differences in the percentage of mortality of members of the larval stage in the treatment of the egg stage G. mellonella, where the show in table (3) that the highest percentage of mortality in the larval stage was 82.100% at concentration 10^6 cells/ml w. D of B.thuringiensis spores and it was 78.450 % at a concentration of 5000 ppm of Zinc oxide nanoparticles, they differed significantly from the control treatment which amounted to 9.510%.

We note from the results in (Table 3) the effect of the interaction between concentration, stage and the substance used, had a clear significant effect on the percentage of mortality in the larval stage, as the percentage of mortality in the larval stage in Zinc oxide was 16.320, 18.980, 21.743 and 78.800% at The concentrations 100, 500, 1000 and 5000 ppm, respectively, while the mortality rate was 16.980, 20,717, 41.663 and 74.540% in the larval stage in Titanium dioxide treatments. respectively, and the same concentrations in the previous treatment of Zinc oxide. While the mortality rate of B.thuringiensis spores treatment was 82.100, 63.320, 62.500 and 25.233% at concentrations 10^6,10^7 , 10^8, 10^9 cells/ml D.W..

Respectively, and based on these results, it was observed that the mortality rate of the members of the larval stage in the treatment of the egg stage of the nanomaterials used increases with increasing concentrations.

These results are consistent with what was observed by [14] that the effect of TiO2 nanomaterial against the first, second and third larval stages of the house fly M.domistica, the mortality rate was 86.6, 70.0 and 53.3%, respectively.

As for the percentage of mortality in the pupal stage, the results (Table 3) indicate that there are clear significant differences in the percentage of mortality of the members of the pupal stage in the treatment of

the egg stage of the insect G. mellonella, where the table shows that the highest percentage of mortality in the pupal stage was in the treatment of spores B. thuringiensis reached 100.00, 80.153, 86.143 and 65.073% at concentrations of 10^6,10^7 , $10^8$and 10^9 cells/ml D.W.,respectively which differed significantly with control treatment, which was to 8.517 %, while the lowest percentage of mortality in the pupal stage was 15.277% at the concentration of 100 ppm of Zinc oxide nanoparticle, which differed significantly from the control treatment which amounted to 8.517%.

While the lowest percentage of mortality in the pupal stage of Titanium dioxide was 16.177% at the concentration of 100 PPM, while the treatment with concentrations of 500, 1000 and 5000 ppm for TiO2 reached 20.453, 50.863 and 73.417%, respectively, and there were significant differences between them in addition to their significant difference from the control treatment.

[15] confirmed the effect of Zinc oxide nanoparticles of size 5 and 100 nm and silver Ag nanoparticles of size of 100 nm on the lethal effect on the growth and development of the southern cowpea beetle C. maculatus, as chickpea seeds were treated with nanoparticles, and the results showed a high rate of mortality in the larval stage, was 98.0% at a concentration of 1000ppm of Zinc oxide nanoparticles, while the rate of mortality in the pupal stage was 60% at a concentration of 500ppm.

4- Adult stage

Table (4) shows that the treatment of G. mellonella eggs with different concentrations of Zinc oxide nanomaterials (ZNO), Titanium dioxide (TiO2) and B. thuringiensis spores on the average number of emerging insects and sexual ratio had a clear effect.

We note from the table(4) that the average number of emerging insects was 0.0% in the treatment with spores of B. thuringiensis at the concentration 10^6 cells/ml of D.W., while the highest mean number of the emerging insects was 73.3% at the concentration of 100 ppm in the nano Zinc oxide treatment Both treatments differed significantly from the control treatment, which amounted to 96.0%

The interaction effect between concentration and substance was clear, and significant differences were also found between them. The nano Zinc oxide treatment, the average number of emerging insects was 73.3, 53.3, 30.0 and 26.6% insects at concentrations 100, 500, 1000 and 5000 ppm respectively, and there significant differences among them, while in the treatment of Titanium dioxide for the same concentrations, the number of emerging insects reached 63.3, 56.6, 53.3 and 26.6% insects, respectively.

We also notice from the table(4) the effect of treating B. thuringiensis spores on the average number of emerging insects at concentration 10^6,10^7 , $10^8$and 10^9 cells/ml of D.W., which were 0.0, 23.3, 40.0 and 50.0% insects, respectively, and there was a significant difference between these treatments.

[16] pointed out the reason for the death of the different stages of insects due to the fact that nanoparticles can cause abnormalities or abnormalities in chromosomes and damage the DNA, which leads to cell damage and death.

Table (1) Effect of treating *G. mellonella* eggs with different concentrations of nanomaterials (Zinc oxide and Titanium dioxide) and *B. thuringiensis* spores in the incubation period and % of hatched eggs.

material	concentration	incubation period/day	%for hatched eggs
Zinc oxide	100	17.667 A	**30.000 c**
	500	17.000 Ab	**42.200 bc**
	1000	16.333 abc	**48.867 b**
	5000	15.667 abc	**76.667 b**
Titanium dioxide	100	17.333 a	**53.300 b**
	500	16.333 abc	**51.667 b**
	1000	16.000 abc	**46.633 b**
	5000	15.667	**28.000**

		bc	**c**
	10^6	15.000	**26.000**
		bc	**c**
	10^7	14.333	**28.663**
bacteria spores B.thuringiensis		bc	**c**
	10^8	13.667	**30.333**
		bcd	**c**
	10^9	12.333	**53.330**
		bcd	**b**
control		10.667	**83.600**
		d	**a**

Numbers with letters that are significantly different from each other at a probability level of 5%, according to Duncan's multiple range test

Table (2)Effect of treating G.mellonella eggs with different concentrations of Zinc oxide nanomaterials (ZnO), Titanium dioxide (TiO2) and spores of B. thuringiensis in the duration of the larval and pupae stages.

material	concentration	Stage duration/day	
		larval	**pupae**
	100	35.667	**22.333**
		b	**a**
	500	35.000	**21.333**
Zinc oxide		bc	**a**
	1000	34.977	**20.000**
		bc	**ab**
	5000	32.067	**19.333**
		cd	**ab**
	100	36.177	**16.333**
		ab	**b**
	500	37.533	**17.667**
Titanium dioxide		ab	**ab**
	1000	38.567	**18.333**
		ab	**ab**
	5000	39.333	**20.000**
		a	**ab**
	10^6	29.667	**20.000**
		d	**ab**
	10^7	29.333	**19.000**
bacteria spores B.thuringiensis		d	**ab**
	10^8	28.667	**17.667**
		de	**ab**
	10^9	28.000	**17.000**
		de	**b**
control		25.667	**9.333**
		e	**c**

Numbers with letters that are significantly different from each other at a probability level of 5%, according to Duncan's multiple range test.

Table (3) Effect of treating G. mellonella eggs with different concentrations of Zinc oxide nanomaterials (ZnO), Titanium dioxide (TiO2) and B. thuringiensis spores in% on mortality in the larval and pupae stages.

material	concentration	%to kill in stage	
		larval	pupae
Zinc oxide	100	16.320 ed	**15.277 fg**
	500	18.980 ed	**17.187 fg**
	1000	21.743 ed	**26.110 f**
	5000	78.80 a	**64.880 d**
Titanium dioxide	100	16.980 ed	**16.177 fg**
	500	20.717 ed	**20.453 fg**
	1000	41.663 c	**50.863 e**
	5000	74.540 ab	**73.417 cd**
bacteria spores B.thuringiensis	10^6	82.100 a	**100.00 a**
	10^7	63.320 b	**86.143 b**
	10^8	62.500 b	**80.153 bc**
	10^9	25.233 d	**65.073 d**
control		9.510 e	**8.517 g**

Numbers with letters that are significantly different from each other at a probability level of 5%, according to Duncan's multiple range test

Table (4) Effect of treating G. mellonella eggs with different concentrations of Zinc oxide nanomaterials, ZnO, Titanium dioxide TiO2, and B. thuringiensis spores on the number of emerging insects and sexual ratio.

material	concentration	%of emerging insects	sex ratio ♀ : ♂
Zinc oxide	100	73.3 b	**1:2**
	500	53.3 bc	**1≃ 1.2**
	1000	30.0 c	**2:1**
	5000	26.6 c	**1.6≃1**
Titanium dioxide	100	63.3 b	**1≃1.4**
	500	56.6 bc	**1~1.4**
	1000	53.3 bc	**1:1**
	5000	26.6 c	**1.6≃1**
bacteria spores	**10^6**	0.0	**0:0**

B.thuringiensis		d	
	10^7	23.3 c	1.6≃1
	10^8	40.0 bc	1:2
	10^9	50.0 bc	1≃1.1
control		96.0 a	1:1

Numbers with letters that are significantly different from each other at a probability level of 5%, according to Duncan's multiple range test

Plate: Adults of G. Mellonella which had been treated at egg stage with different concentrations of two nanoparticles and bacteria spores

Control(egg)

Control(adult)

Mild corrugaed(ZnO/1000)

Pupae stage with different emergence (TiO2/5000

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