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valuation The efficiency of Nanotechnology and biological activity of Nanoparticle for two plant extracts *Ricinus communis* and *Neriun oleander* against immature stages of Greater wax moth.*Galleria mellonella* (Lepidoptera: pyralidae)

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Abstract

The results of present study conducted on the Laboratory of advance entomology \ Department of biology \ Faculty of Education for Girls \ University of Kufa, during the years 2018-2019 for evaluating The efficiency of Nanotechnology and biological control and the effect of two plant extracts on the different immature stages of the greater wax moth *Galleria mellonella* (Lepidoptera: Pyrallidae). eggs, first and fourth larval instars. The cold aqueous extract of castor *Ricinus* communis and *Nerium oleander* leaves ,from which different concentrations (1,2,3 and 4) mg \ ml were prepared in the Laboratory**.** The experiments include the enhancement of these plant extracts of toxic nature with chemically prepared particles, silver nanoparticles, and conducting multiple experiments for the same instars of the insect, which was treated with aqueous plant extracts .The statistical results as well as the observation during different stages of the experiments were superior of these nanoextracts over plant extracts in the mortality of eggs and first and fourth latval instars. Experiments indicated that the mortality percentages when treated with aqueous leaves extract of castor were (76, 91, 96) % respectively while the same instars were treated with nano extract of castor was(83.3 ,92.3 ,98.6)% respectivly . The leaves extract of *N.oleander* was (86.6%, 96.6%, 99.4%) compared with (93.3, 100, 100) % respectively of the nanoparticle of the eggs and larvae of the first and fourth phases at 4 mg / ml. It also proved that the first instar was the most sensitive to the extract of the fourth instar, where the highest mortality percentages was (96.6, 100) % compared with (86.6, 93.3)% respectively of the first and fourth larval instars when treated with and nano of *N. oleander* and *R.communis* respectively.

Keyword : *Nanotechnology, Ricinus communis, Galleria mellonella, pyralida*

Introduction

G. mellonella is one of the most dangerous pests of honeybee communities around the world. It is known that the Great wax moth is harmful to stock beeswax and causes significant damage to beekeepers and financial losses each year, as well as damage to wax frames by feeding larvae and destroy the wooden parts in the cell. Great wax moths can also transmit pathogens such as bee-studded bees, foulbrood or bee-borne strain (Foulbrood), which is transmitted by great wax moth waste containing *Paenibacillus* bacteria in large numbers causing the disease (Owayss and Abd-Elgayed, 2007).

Researchers have become increasingly persistent in the search for alternative natural pesticides and obtained from plant extracts that do not harm the environment and have not caused the appearance of resistance in insects treated (Schmutterer 1988).

Some plants have been used as food contraceptives, insecticides, repellents, growth regulators or egg-laying constraints by affecting egg formation, growth and development (Metcalf 1967Al- Al-Sharook et al 1991 Bloszyk 1995 Kashab 1999). Present study included the use of plant extracts and enhanced with modern techniques such as nanotechnology and nanoscale experiment tagainst the different phases of the major wax moth *Galleria mellonella*.

Materials and methods

Collect a great wax moth

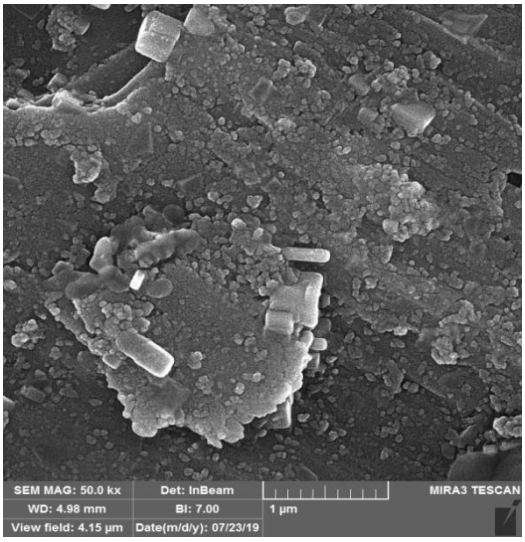
This study conducted in November 2018, the insect was raised in the Advanced Insect Laboratory of the Department of Biology Sciences / College of Education for Girls / University of Kufa. In a box of wood (26 × 23 × 55) cm 3 and placed the insect in complete darkness and at a temperature of 30 and relative humidity 10 ± 50% (Al-Waili, 2013) was raised laboratory moth, by placing pairs of insects (5 females + 5 males) adult insect. In sterile plastic bottles of 12 cm high and 6 cm diameter, containing a quantity of dark wax pieces for the purpose of feeding insect larvae Ribbons of cardboard length (10 cm) and width (5 cm) were placed inside the mating bottles to lay the insect eggs. For several generations before the experiment was carried out, the immature stages of the insect were continuously monitored and renewed after each generation.

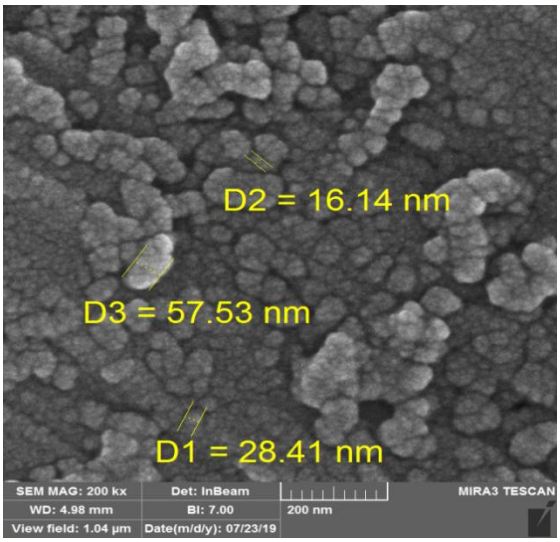
The first phase larvae were collected immediately after hatching by a soft brush and placed in a petri dish and sterilized with a wax amount. The nanostructure extract was sprayed by hand sprayer at a height of 20 cm, vertically and in concentrations (1.2.3.4) mg / ml. Each of the three replicates of each concentration and transferred 10 larvae per dish and then took notes and information pertaining to this phase from the date of the experiment and the concentration used on each of the three dishes used as well as prepared dishes for control laboratories in the form of three replicates sprayed with distilled water only and fed on wax Untreated with an extract and placed at a laboratory container temperature Heating continuously and was follow up after (96,72,48) hours. In the same way the larvae of the first stage were treated with nanofiltra extract and the same method was applied for the treatment of the fourth stage larvae which determined their age depending on the size of the larvae.

Preparation of nanoscale extracts

The silver nanoparticles were prepared with *R. communis* and *N.oleander* extracts, weighing (4 g) of castor in 100 ml of distilled water at 30 degrees, stirring for 20 minutes and filtered several times using a centrifuge (10 minutes) and then with a filter of 0.4 nm.

Then a volume of 5 mg / ml of the prepared silver nitrate solution was prepared and prepared in the nanotechnology laboratory at the University of Babylon College of Pharmacy. The previous method used in the preparation of aqueous plant extracts and complete the volume to 100 ml by adding distilled water and adjusting PH to 10 after the addition of NaoH and note the color change immediately and then heat the solution to 80 degrees with constant stirring for 20 minutes in the water bath with a thermometer to monitor the temperature. The solution was then cooled and filtered using a large syringe and a 0.4 nm filter to make sure that the nanoparticles were crossed only to conduct experiments and to test the efficacy at different life stages of the insect. The extract was then diluted with 10 mL of distilled water to obtain the concentrations (1.2.3. and 4) using the dilution law.V1 × C1 = V2xC2





Results and discussion

Nanoscale extracts

Contact effect of different nanoparticle concentrations of castor leaves R.communis and rudimentary *N. oleander* on the destruction of *G. mellonella* eggs

The results of Table (1) showed that there was a significant difference at the concentration of 4 mg / ml, while the (1.2.3) mg/ ml concentrations did not show any significant differences with the highest mortality and 4 mg / ml concentration (98.6%, 98% and 96.6%). After (96, 72.48) hours respectively of treatment time, compared with the control factor, which recorded 2% of the losses after 96 hours. Also, it was found from the table that there was a direct correlation between the concentrations used and the mortality rates, while the lowest mortality rates were observed at the 1 mg /ml concentration (95.4%, 88.6%, 80%) after (96, 72, 48) hours, respectively, compared with the control trwatment which did not record any mortality rate

Table (1) Contact effect of different concentrations of silver Nanoparticle of *R.communis* leaves on the eggs of the major wax moth

48hours	72 hours	96hours	Concentration mg / ml
Percentage	Percentage	Percentage	
0	0	2	Control
80	88.6	95.4	1
88.6	93.4	98.6	2
90	95.4	98.6	3
96.6	98	98.6	4
8.2	9.3	7.2	LSD 0.05

While the results of statistical analysis of Table (2) to the presence of a direct correlation between the rates of mortality of eggs treated in contact with different concentrations of cold water extract of the leaves of *N.oleander*, where the highest rate of mortality at the concentration of 4 mg / ml was (100%, 96%, 90% after (96, 72, 48) hours respectively of the treatment time compared with the control treatment

where the mortality rate reached 2%, where the statistical results confirmed the presence of significant differences at the concentration (3 and 4 mg / ml) while the (1.2) mg/ ml concentration was not recorded The table indicated that the lowest mortality rates were recorded at the first concentration (96%, 80.6%, 80%) after (96, 72, 48) hours. And the time of the transaction, respectively. Attributed the death of embryos inside the eggs or not hatching to the toxic effects of these extracts and their impact on protoplasm, causing the death of the fetus inside the egg (: Rokestin, 1978 ,Al-Rubaye, 1999)

Table (2): Effect of contact with different concentrations of leaves of *N.oleander* nanoparticles on the destruction of eggs of the great wax moth

48hours	**72 hours**	**96hours**	**Concentration mg / ml**
Percentage	**Percentage**	**Percentage**	
0		**2**	**Control**
80	**80.6**	**96**	**1**
80	**90**	**96**	**2**
86.6	**96**	**100**	**3**
90	**96**	**100**	**4**
1.2	**1.7**	**4.4**	**LSD 0.05**

The results of Table (3) indicated that there were significant differences that appeared at the concentration of 4 mg / ml where the highest mortality rates were recorded (92.3%, 77.6%, 67.6%) after (92, 72, 48) hours of treatment time respectively. Compared with the control factor, which recorded a mortality rate of 2%. The (1.2.3) mg/ ml concentrations did not record any mortality. It also showed that the lowest mortality rates were at the 1 mg/ ml concentration (76.6%, 52.3%, 44.3%) after (96, 72, 48) hours of treatment time respectively. The larvae of the first phase and the concentrations used

Table (3) Effect of Nanoparticle of R.communis against first larval instar mortality rates

48 hours	**72 hours**	**96hours**	**Concentration mg / ml**
Percentage	**Percentage**	**Percentage**	
0	**0**	**2**	**Control**
44.3	**52.3**	**76.6**	**1**
54.3	**66.6**	**83.3**	**2**
65.6	**74.3**	**83.3**	**3**
67.6	**77.6**	**92.3**	**4**
4.4	**4.7**	**5.1**	**LSD 0.05**

The results of table (4) and according to statistical analysis that there are significant differences at the concentration of 4 mg / ml where achieved the highest rate of loss at this concentration (83.3%, 66.6%, 51%) respectively at the passage (96, 72, 48) hours of time Treatment respectively compared with the control treatment , which did not record any mortality rate and also showed the presence of a direct correlation between the mortality rates obtained and concentrations used and the lowest percentage of

mortality seen at the 1mg /ml concentration reached (66.6%, 51% 36.6%) respectively at the passage (96, 72, 48) hours of treatment respectively

Table (4) Effect ratios percentage mortality Nanoparticle of *R.communis* extracts on in the fourth larval instar of great wax moth

48 hours	**72 hours**	**96 hours**	**Concentration mg / ml**
Percentage	**Percentage**	**Percentage**	
			Control
36.6	**51**	**66.6**	**1**
40	**60**	**77.6**	**2**
51	**60**	**77.6**	**3**
51	**66.6**	**83.3**	**4**
3.3	**4.1**	**5.3**	**LSD 0.05**

The results of Table (5) showed that there were significant differences that appeared at the concentration of 4 mg / ml where the highest mortality rates reached (100%, 87.6%, 77.6%) respectively after (96, 72, 48) hours of treatment time respectively. The coefficient of control treatment which recorded depreciation ratios of 2%. There was a positive correlation between the concentrations used and the percentages of depreciation as well as showed that the lowest percentages of loss occurred at 1ml/ml concentration (77.6, 66.6, 51) % compared with the control treatment , which did not record any loss.

While the results of table (6) in the effect of the extract of nanoparticle leaves in the larvae of the fourth stage to the presence of a direct relationship between the rates of destruction and concentrations used as well as the results of the statistical to the presence of significant differences appeared at the concentration of 4 mg / ml while the first concentrations did not record any significant differences were recorded The highest mortality rates at the concentration of 4 mg / ml were (93.3, 83.3, 71)% after (96, 72, 48) hours of treatment time, respectively, compared with the control treatment which did not record any rate of destruction as well as the lowest rates were seen. The decay was obtained at the concentration of 1 mg / ml where it reached (66.6, 57.6, 44.3)% and also after (96, 72, 48) respectively of the time of treatment and For successive comparison with the control treatment , which did not record any percentages of destruction.

Table (5) Effect of mortality of Nanoparticle Extract of *N.oleander* Leaves against first larval instar of great wax moth

48hours	**72 hours**	**96hours**	**Concentration Mg/ml**
Percentage	**Percentage**	**Percentage**	
		2	**control**
51	**66.6**	**77.6**	**1**
67.6	**76.6**	**83.3**	**2**
74.3	**83.3**	**100**	**3**
77.6	**87.6**	**100**	**4**
3.4	**3.1**	**3.6**	**LSD 0.05**

Table (6) Infectious Ratio of Infectious Effect of Nano Extract of *N.oleander* Leaves against the fourth larval instar of great wax moth

48 hours	72 hours	96hours	Concentration Mg/ml
Percentage	Percentage	Percentage	
0	0	0	Control
44.3	57.6	66.6	1
60	67.6	76.6	2
67.6	74.3	92.7	3
71	83.3	93.3	4

theresults showed that the nanoparticles extracts had high mortality rates in the eggs and larvae of the first and fourth phase. The role of these particles may be due to their impact on the process of solidity or tanning (Melanization) where Cathrin et al. (2011) found that sub-lethal doses of silver nanoparticles when exposed to the fruit fly insect *Drosophila melanogaster* have It affected the hardness of the body wall, and the cause of the larval death exposed to silver nanoparticles can be explained by its high susceptibility to the penetration of Qiutecl and interfering with the decomposition and other physiological processes and thus its effect on the duration of growth of various larval phases (Naresh etal; 2012).

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