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Bioactivity Of Bacillus Thuringiensis ,Conocarpus And Oleander Extracts Against The Wax Worm Galleria Mellonella L

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Abstract

This study was conducted in the entomology laboratory of the Department of Bio-control /AL- Musayyib Technical College and under laboratory conditions. To investigate the Biological activity of Bacillus thuringiensis and plant extracts against the wax worm Galleria mellonella . 110 soil samples were collected from different areas in Babylon governorate, and the presence of B. thuringiensis was investigated in the examined soil samples . As the number of samples that contained B. thuringiensis bacteria was 67 samples out of the total samples that were examined.

The results of the current study revealed that alcoholic and aqueous extract of Oleander had a clear effect on the biological activity of the larval great wax worm . as the concentration (0.75 mg/L^{-1}) significantly outperformed all the concentration used in the research experiment and recorded the highest rate of (74.9 %). The results showed that alcoholic and aqueous extract of Conocarpus had a clear effect on the biological activity of the larval great wax worm. The concentration (0.75 mg/L^{-1}) revealed high average by Effect on the mortality of the larval great wax worm was (73.3 %) . When using different concentrations of the B. thuringiensis and testing its effectiveness on the larval great wax worm . The concentration (10^{-2}) showed high average by Effect of using bacterial concentrations on the mortality of the larvae of the great wax worm was (76.7 %) .

Key word : Bacillus thuringiensis , wax worm, Galleria mellonella , Conocarpus , Oleander.

Introduction

The great wax worm Galleria mellonella L is one of the most important pests that infect bees in many countries of the world. Its damage is represented by digging tunnels for its larvae in the waxy foundations, watching thickly covered with silk threads made by the larvae, which leads to impeding the movement of bees and their activity in watching the infection by recording in the rest of the tires. The migration of whole hive bees (Warhust and Goebe, 1995) . The pest is very harmful to stored tires and generally spreads in temperate climates and continues its activity almost throughout the days of the year, and the insect spreads in weak cells that have been weakened by chemical pesticides, lack of food, weakness of the queen (Prasad and Nilofer, 2010). The control of stock insect pests relied heavily on the continuous use of manufactured chemical insecticides and fumigators, despite the effectiveness of pesticides in eliminating pests, but the continuous use led to serious defects, including the

emergence of resistance in them against the chemical pesticides used in the control in addition to the deadly effect of non-target organisms as well as the The risks of environmental pollution resulting from its use (Tapondjou et al., 2002).

Plant powders and extracts contain chemically active substances against many insect pests by preventing egg-laying or repellent to them and inhibiting feeding as well as limiting the growth and development of larvae or limiting the fertility of adults (De Sousa et al., 2005).Oleander is used as an effective plant pesticide against many agricultural pests and rodents, as the plant parts contain effective compounds such as Oleandriginin and Neriin, which cause damage by inhibiting plasma in addition to sodium, potassium and glycosides (Bandaram et al ., 2010))

In addition to the Capris plant Conocarpus lancifolius, which belongs to the family Combretaceae, there is a need for more research and studies that should address the biological aspects in terms of diseases that affect it, insects, fungi, etc. and the impact of all this on biodiversity in the environments in which it is grown (EI-Juhany and Ibrahim, 2005). Biological control includes the use of pathogen products such as bacteria, fungi, viruses, rickettsia, protozoa and nematodes in pest control known as microbial resistance. Among all microorganisms, Bacillus thuringiensis , a gram-positive bacillus, has the ability to produce protein crystals, which are responsible for its toxicity to insects. These bacteria produce many virulence factors such as vegetative insecticidal, Delta-endotoxine, and the diversity in the production of these factors is very different between serotypes of B. thuringiensis and sometimes it differs between isolates belonging to the same serotype (Travis and Maureen, 2003). As a result of the widespread spread of this insect in most governorates of Iraq in cells and in stores and for most months of the year due to the environmental suitability of the sheep to it and due to the increasing economic value of the damages that occur to tires due to the infection of the great wax worm Proceeding from the importance of this pest and for the purpose of arriving at modern control methods that are safe for humans and the environment as alternatives to chemical pesticides, which can be integrated with each other and be effective to reduce infection and reduce damage, this study was conducted, which aims to:

1- Biological efficacy of Bacillus thuringiensis against the wax worm Galleria mellonella .

2- Using Conocarpus and Oleander extracts extracts and comparing their effectiveness with the effect of bacteria on the wax worm Galleria mellonella .

Materials and methods

This study was conducted in the entomology laboratory of the Department of Bio-control / AL- Musayyib Technical College and under laboratory conditions. The larvae of the great wax worm were obtained from one of the weak apiary cells in the Al-Mahaweel area. In temperature 5±30 c and a relative humidity of 55±70% As concentrations of extracts were used 0.25, 0.50, 0.75 for each of oleander and capris (aquatic and alcoholic), the plants were obtained from the gardens of the University of Baghdad and the plant was washed with running water and then distilled water well to remove the dust and dust covering the surface of the plant, the leaves were left to dry at a temperature The chamber, taking into account its constant stirring to prevent rotting, then grind the dry parts with an electric grinder and keep the powder in dry packages, It was placed in the refrigerator at a temperature of 4 °C until its use, the boiled water extract was prepared according to the method of Harborne ,(1984) to prepare the water extract, as 20 gm of each dry sample of the different plant parts was taken

and placed in a conical flask of 500 ml size and 200 ml of distilled water was added at a temperature 20-25 c The samples were placed in a horizontal vibrator (GFL model 2015 model) for half an hour at a medium speed, the samples were left to last for an hour, then filtered with three layers of gauze to separate the solid plankton, then the sedimentation was carried out using a centrifuge at 3000 speed for 15 minutes to separate the plankton. The filtrate was concentrated in a rotary evaporator and dried in an oven at 45°C. The dry matter was used in preparing the different concentrations of the extracts. As for the alcoholic extract, it was prepared according to the method described by Desmukh and Borle ,(1975) with some modifications as follows:

The extraction was carried out by placing 50 grams of the powder of the two plants used in the experiment in the extraction vessel (Thumble), which was placed in the continuous extraction device - Soxhlet, and using (250) milliliters of ethyl alcohol at a temperature of 80 °C. The extraction process continued for 8 hours, the solvent was evaporated using a device Rotary evaporator under vacuum pressure at a temperature of 45°C, and completed drying of the extract in an electric oven at a temperature of 37°C for 10 hours to obtain a dry powder, which was kept in the refrigerator until use.

Treatment of larval instars with alcoholic and aqueous extracts

Treatment of larval instars with different concentrations of alcoholic and aqueous extracts of oleander and capers 10 larvae were taken for each stage of the first and second instar larvae, with three replications for each concentration, and sprayed directly on the larvae with alcoholic and aqueous extracts at concentrations (0.25, 0.50, 0.75) mg/ml . In addition to the comparison treatment in which only the distillate was used, it was then kept in breeding bottles that were tightly closed by a piece of boring cloth and a rubber band with some wax for feeding and left under laboratory conditions of 30±5 C and relative humidity of 55±70% (Yan, 2004 and Algacyer, 2002).

Effect of different treatments of Bacillus thuringinsis on the larvae of the great wax worm

The larvae were treated in two ways: the first was to spray the larval instars (the first and the second) separately, and the other was to treat the food, both separately, as three replicates were used for each treatment and ten larvae per replicate.

first treatment

The larvae were sprayed in a volume of 1 ml by means of a sterile hand sprayer with a capacity of 40 ml vertically and at a distance of 15 cm from the biocide, each separately.

Second treatment

Sterilized Dark wax Set Sterilized Transcriptase 0.5, Reset for Larval Rearrangement, 1 Repurchase, Rearrange Larva, Transcript, Transcript, Transcript, 0.5ml for 0.5 Weight and 1ml for each, Mediated, Paper, Arrange, Capacity 400ml for each iterator. Mechanism of formal larvae, formation and death ratios calculation.

The results were corrected according to the Abbott equation (Abbott , 1952) .

Corrected depreciation percentage = (transaction to decimation % -transaction to decimation %) / (transaction to decimation % -100) x 100.

Statistical analysis

The statistical program (SAS , 2012), (statistical analysis) was used in analyzing the data, indicating the different treatments (isolates) in the studied traits, the larval death rate, and the significant differences between the means were compared with the LSD test .

Result and discussion

Cultivational characteristics of B. thuringiensis.

110 soil samples were collected from different areas in Babylon governorate, and the presence of B. thuringiensis was investigated in the examined soil samples . According to the results shown by the study, it was found that the method, which used an inhibitor of bacterial spores, which is sodium acetate, was more selective in isolating bacteria, as the number of samples that contained B. thuringiensis bacteria when using this method was 67 samples out of the total samples that were examined from the total samples . The cultivar characteristics of the bacteria growing on the culture media for their growth were revealed, which consisted of Nutrient Agar, Ammonium salt sugars and T3 Agar. It was found after the end of the incubation period that the colonies that were seen with the naked eye are white to yellowish-orange, small, round and mucous colonies, as several culture dishes were selected and their results were compared. As shown in table (1)

Table (1) Microscopic shapes of primary isolate colonies

colony symbol	microscopic description
A	white - diffuse - wavy and mucous
B	Bright yellow - small - round – smooth
C	white - diffuse - radial and rough
D	Bright orange - small - round and smooth
E	White - small - round and mucous

Phenotypic characterization of the crystalline protein of B. thuringiensis.

The results of the current study showed through microscopic examination of 67 bacterial isolates to investigate the shapes of crystalline protein using a 100× oil lens. 5 shapes of crystalline protein for these bacteria were distinguished: spherical, spherical and cubic , cubic, bipyramidal and oval as shown in table (2). Determining the percentage of crystalline forms present in the local isolates. The results showed that the spherical shape was the most common among the isolates, as it recorded the highest percentage of (28.3 %), while the lowest percentage was for the bipyramidal crystalline protein with a percentage of (13.4 %).

Table (2) the percentage of crystalline forms of B. thuringiensis present in local isolates.

Crystalline protein shape	Total number of isolates	percentage%
Spherical	19	28.3
Spherical and cube	15	22.3
Cubic	12	17.9
Oval	12	17.9
Bipyramidal	9	13.4

Total	**67**	

Effect of alcoholic and aqueous extract of oleander on the life of the larval stages of the great wax worm

The results of the current study concluded that **alcoholic and aqueous extract** had a clear effect on the biological activity of the larval stages of the great wax worm. The results show in table (3) the clear effect of different concentrations of **oleander** on the destruction of the larvae of the great wax worm

Table 3: Effect of using alcoholic and aqueous extract of Oleander on the mortality of the larvae of the great wax worm

Concentrations mg/L^{-1}	alcoholic extract		aqueous extract		average
	first phase	second phase	first phase	second phase	
0.25	66.6	73.3	46.6	43.3	57.5
0.50	76.6	80.0	60.0	56.6	68.3
0.75	90.0	86.3	63.3	60.0	74.9
control	0.0	0.0	0.0	0.0	0.0
average	77.7	79.8	56.6	53.3	---
LSD values: Concentration: 7.05 *, Extract: 7.05 *, Interference: 13.48 *					

When using different concentrations of the bacterial suspension of B. thuringiensis and testing its effectiveness on the larval stages of the great wax worm in table (4) .

Table 4: Effect of using Bacillus theuringnisis on the destruction of the great wax worm larvae

Concentration spore/ml	direct spray	Mix with food	the average
10^{-2}	70.0	83.3	76.6
10^{-3}	63.3	80.0	71.6
10^{-5}	56.3	73.3	64.8
Control	0.0	0.0	0.0
the average	63.2	78.8	---
LSD values:Concentration:6.941*,Spraying & Mixing: 4.682*,Overlap: 10.03*			

The results of the current study concluded that alcoholic and aqueous extract of carpis had a clear effect on the biological activity of the larval stages of the great wax worm. The results show in table (5) the clear effect of different concentrations of alcoholic and aqueous extract of carpis on the destruction of the larvae of the great wax worm.

Table 5: Effect of using alcoholic and aqueous extract of Conocarpus on larval mortality great wax worm larvae.

Concentrations	alcoholic extract	aqueous extract	average

mg/L^{-1}	first phase	second phase	first phase	second phase	
0.25	76.6	66.6	53.3	46.6	60.7
0.50	80.0	70.0	63.3	60.0	68.3
0.75	83.3	73.3	66.6	70.0	73.3
Control	0.0	0.0	0.0	0.0	0.0
Average	79.9	69.9	61.0	58.8	---
LSD values: Concentration: 7.39*, Extraction: 7.39*, Overlap: 13.85*					

Discussion

110 soil samples were collected from different areas in Babylon governorate, and the presence of B. thuringiensis was investigated in the examined soil samples . As the isolates differed according to the geographical location of the area and the nature of the surrounding environmental conditions and the type of soil through the two methods that were used to isolate bacteria from soil samples to choose the best method for isolation **.** By reviewing the sources and research of previous studies, it was found that the results of the current study agree with a study conducted by the researcher (Apaydin , 2004) in Turkey and the researcher (Obeidat ,2008) in Jordan they found that the spherical shape of crystals is the most prevalent. While the current results did not agree with what was found by (Jameel , 2007) in Iraq and Keshavarzi, that the bi-pyramidal form is the most prevalent.

The results of the effect of different concentrations of B. thuringiensis suspension on the larvae of the largest waxworm showed that this bacteria had a clear effect on the destruction of the larval stages of the worm . it was found that the results of our current study are in agreement with many previous studies. In a study conducted by (Al-Alan et al ., 2018) confirming a bioassay of B. thuringiensis on the great wax worm, a decrease in the appetite and food consumption of the larvae was observed as a result of infection with the bacteria, in addition to the appearance of black spots or areas on the body of the infected larvae, and these symptoms are similar to those of larvae. Wings when infected with the bacteria B. thuringiensis. After its death, its color changes from light to dark brown and then black. The larvae began to die 24 hours after the start of feeding, and the killing rate ranged after 72 hours (67.6 and 67.36%) in some bacterial isolates, and the death of larvae decreased in all isolates after 20 hours, while the effect of some isolates was 96 hours.

The reason can influence the effect of Conocarpus and Oleander alcoholic and aqueous extracts, in the destruction of the first and second larval stages of the great wax worm insect to the sensitivity of the larvae to valid materials or plant extracts, making it a reason for the effect of chemicals in the moulting hormones ((Jaipal et al., 1983).

In addition, the cause of the larvae's death may be due to some plant compounds, as they have a role in the death of the epithelial cells lining the middle digestive canal of the insect feeding on these compounds, and since these cells are responsible for secreting digestive enzymes that remove the action of these compounds, thus leading to the killing of the insect (Bowers, 1984).

The study conducted by Rashid, (2018) in which boiled water extract of the wormwood plant was used on the first and third larval stages of the Great Waxworm, showed that the highest percentage of death was (6.6%) at the concentration of 50 g/L/100 g/L after three days, while the lowest percentage of death was (3.3%) at concentration 25 after one day of treatment for the first phase. As for the third phase, the highest percentage reached 26.6% g/L at concentration 100 g/L after three days of treatment, while the lowest percentage was 10% at concentration 25 g/L after A day has passed since the transaction

Conclusions

1 - Presence of Bacillus thuringiensis in soil samples examined from different regions in Babylon Governorate. As the bacterial isolates differed according to the geographical location of the area, the nature of the surrounding environmental conditions and the type of soil.
2- Conocarpus and Oleander extracts had a clear effect on the biological activity of the larval stages of the great wax worm.
3- The use of different concentrations of B. thuringiensis had a significant effect on the destruction of the larvae of the great wax worm.

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