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Evaluation of the efficiency of alcoholic propolis extract and Bacillus thuringiensis in the mortality rate of the third larval age of the great wax Moth Galleria melonella (L.)

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***Abstract*---**This study was conducted at the AL- Musayyib Technical College / Department of Biological-Control Techniques for the period from 1/10 /2021 to 15/3/2022. The study aimed to compare propolis extract and *B. thuringiensis* bacteria on the larvae of the major wax moth. The direct treatment of the larvae of the major wax moth and the food provided to them (natural wax) indicated that the organic extract (ethyl alcohol) of Propolis showed effectiveness in destroying the third larval age the major wax moth.The results showed that there were significant differences between the concentrations of the extract in the percentage killing percentages depending on the concentration and the duration of exposure, if the highest rate of the killing percentage in the concentration reached 3%, reaching 76.6% within a week of the treatment, while the lowest rate of killing at the concentration was 2%, as it reached 53.3% % during the first day of the transaction.Using different dilutions of the bacterial suspension of B. thuringiensis had a significant effect on the percentage of killings for the third larval age of the Great Waxworm after different exposure periods of the treatment. The interaction between the dilutions and the exposure time after the treatment showed. It gave the highest percentage of killing

rate, reaching 86% at diluted 10^{-2} after a week of treatment, while the lowest percentage of killing was 43.3% at diluted 10^{-6} during the first day of treatment. The study results indicated that the use of different dilutions of *B. thuringiensi* suspension significantly affected the percentage of killings of the third larval larvae of the Great Waxworm, *B. thuringiensi,* after different exposure periods of the treatment.

Keywords---propolis extract, Bacillus thuringiensis, Galleria melonella.

Introduction

The Great Wax Moth *Galleria melonella*, which infects honeybee colonies, is a dangerous pest widespread worldwide. The resulting losses are a significant obstacle to honey production processes due to the substantial damage caused by its larvae in the wax frames inside the cells. Its damage to bees and wax disks lies By digging their larvae tunnels inside the discs, which leads to damage to the wax (Kwadha , 2017).The use of pesticides inevitably leads to the emergence of resistance in the insect and poisoning of non-target organisms, which requires finding appropriate solutions to combat the pest, which calls for reducing the use of chemical pesticides and moving to the use of previous alternative control methods with modern and advanced techniques according to a safe mechanism for bees and the environment and within Integrated management programs to combat the great wax moth (Omran *et al.*, 2019). Many microbial pesticides are used to control insect pests. Perhaps the most widely used in this control is Bacillus thuringiensis (B. thuringiensis), which specializes in controlling larvae (Morandin *et al.,* 2016). Studies since the late 1940s have proven the microbial biological activity of Propolis against a wide range of fungi and bacteria, and it is considered as a cell disinfectant through its antibacterial effect (Rott, 2003).

The study aims to:

1. Study of the effectiveness of propolis extract in the third larval third age of the significant wax moth *Galleria mellonella*.
2. Studying the effect of propolis and bacteria extracts on the third larval age of the major wax moth *Galleria mellonella,* and comparing the results obtained.

Materials and Methods

Preparation of alcoholic propolis extract

Then 10 gm of Propolis was taken after cleaning it from dust and impurities, and placed in a thimble in the saxolite extractor, 200 ml of ethyl alcohol was added at a concentration of 99%, and the extraction of

the plant sample continued for 24 hours. Then the resulting solution was evaporated by a rotary evaporator at a temperature of 45-40°C. The sample was dried in an electric oven at a temperature of 45-40°C. To test the effect of the extract of the dry matter resulting from the extraction with the organic solvent, I followed (Al-Rabee' method, 1999) to prepare the required concentrations.

Insect collection and breeding laboratory

This study was conducted in the entomology laboratory - Department of Biological Control of the Technical College / Musayyib 2021-2022. Under laboratory conditions, wax infected with larvae of the significant wax moth ages and from weak apiaries and tire storage stores in Babil Governorate were obtained.

Sample collection

Ten soil samples were taken from agricultural lands from Babil Governorate after removing the surface layer of the soil to a depth of 3-5 cm and the difference in soil texture, taking care when taking samples from agricultural lands and making sure that *B. thuringiensis* preparations were not used in it by inquiring with the landowner. The following information was fixed on it (the area's name - the nature of the land - the date of taking the form), and then a code was given to each sample and kept in the refrigerator until. Soil samples were taken from agricultural lands after removing the surface layer of soil with a depth of 3-5 cm.

We are preparing the bacterial suspension for the purposes of biological evaluation of the insect. The method (Stahly *et al.*, 1991) was used to prepare the bacterial suspension.

Culture media

All cultural media were prepared according to the instructions of the supplied company. Nutrient agar, MR-VP, Ammonium salt sugars, Medium baseT3 Agar.

Microscopic diagnosis

The phenotypic traits were examined based on what was mentioned (Thiery and Frachon, 1997).

Dyes and reagents

Gram stain, basic fuchsin dye, 0.5. sodium ascent solution

Biochemical test

The Biochemical test included: Motility test, Methyl red test, Sugar Fermentation test, Voges – Proskauer test, and Mannitol, and catalase test.

Basal fuchsin dye

The examination was carried out for colonies kept inside the incubator for a period of (3 days - a week) and for positive colonies with Gram stain only to observe the presence of spores and crystals by preparing sterile and dry glass slides after they were marked with the number of the colony to be examined and a small drop of water was added in the middle of the slide, and a small part of the colony was taken by Flame sterilized metal lube, It was gently distributed and mixed with a drop of water and passed over a light flame several times to dry, left to dry at room temperature until it dried, then dipped in the basic fuchsin dye for 3-5 minutes, washed with distilled water, left to dry, and then examined with an oil lens 100x .

Statistical analysis

The statistical program Statistical Analysis System -SAS (2012) was used in data analysis to study the effect of different factors (concentration and time) according to a factorial experiment (5 x 3) on the studied traits according to a completely randomized design-CRD, and the significant differences between the means were compared. By the Least Significant Difference (LSD) test.

Results and Discussion

The results of the statistical analysis are indicated in the table (1), which includes the direct treatment of the larvae of the major wax moth and the food provided to them (natural wax), as it was shown that the organic extract (ethyl alcohol) of Propolis showed effectiveness in destroying the third larval age (third)of the Wax Moth in vitro . Laboratory great wax, The results indicate that there are significant differences between the concentrations of the extract in the percentage of killing, at a probability level of 5%, depending on the concentration and the duration of exposure. The killing rate at the 2% concentration reached 53.3% during the first day of the treatment, 60.0%. The interaction achieved the highest fatality rate of 76.6% at the 3% concentration within a week, while the comparison treatment did not give any killing rate of 0.0.

Table (1) shows the effect of alcoholic propolis extract on the third larval age of the Great Wax Moth *Galleriamelonella* (first day, third day, one week) of treatment

Concentration	The percentage of mortality in the third larval age			the average
	First day	the third day	First day	
2%	53.3	63.3	63.3	60.0

2.5%	66.6	66.6	66.6	66.6
3%	73.3	76.6	76.6	75.5
Comparison	0.0	0.0	0.0	0.0
the average	64.4	69.0	69.0	---
LSD values: cocentration: *6.89 , Time: 6.13 Ns , intraction: *11.51 , (P≤0.05) *.				

Bees use it to protect the colony and maintain the health of bees, as it resists abnormal conditions by reducing pathogens and defending against many animals and microbes. Through the results reached by (Al-Jurani *et al.,* 2002), the treatment of the third-stage larvae of the Great Waxworm with the oil extract of the Eucalyptus plant led to the destruction of all larvae at the two concentrations (5,10.)%. And the cinnamon plant in an effect of reducing larval weight, prolonging larval life, and increasing the cumulative percentage of larval death and for the first and third ages of the larvae of the Great Waxworm in general and all dilution, and its effect increased with increasing concentration. The study also proved the sensitivity of the first larval stage to compounds extracted from the propolis and cinnamon plant.

Cultivational characteristics of *B.thuringiensis*

The cultivar properties of the bacteria growing on the culture media for their growth were shown, which consisted of Nutrient Agar, Ammonium salt sugars, and T3 Agar. After the end of the incubation period, the colonies that were seen with the naked eye were white to yellowish-orange, small, round, and mucous, as several culture dishes were selected, and their results were compared. As shown in Table (2).

Table (2) Microscopic shapes of *B. thuringiensis* . primary isolate colonies

Colonial symbol	microscopic description
A	- white - diffuse - wavy and mucous
B	-Bright yellow - small - round - smooth
C	-White - diffuse - radial and coarse
D	-Bright orange - small - round and smooth
E	- white - small - rounded and mucous

The results of the statistical analysis in Table (3) indicated that the use of dilutions with *B. thuringiensis* had a significant effect on the percentage killing of the third larvae age of the Great Wax worm after different exposure periods,at the level of probability. 5% .The results of the interaction, according to the dilution and the duration of exposure after treatment, showed that the highest percentage of killing was 86.0% when diluted 10^{-2} after a week of treatment, while the lowest percentage of killing was 43.3% when diluted 10^{-6} during the first day of treatment. While the comparison treatment did not give any killing rate of 0.0 .

Table (3) Effect of different dilutions of *B. thuringinsis* suspension on the preparation of the death of the third larval age of *G. melonella* major wax moth during (first day, third day, one week) of treatment

Concentration	preparation of the mortality of the third larval age			the average
	First day	the third day	a week	
10^{-2}	76.6	83.3	86.0	82.1
10^{-3}	66.6	70.0	80.0	71.0
10^{-5}	53.3	63.3	70.0	62.3
10^{-6}	43.3	50.0	56.6	50.0
Comparison	0.0	0.0	0.0	0.0
Average time period	60.0	66.6	73.2	---
LSD values: cocentration: *8.21 , Time: 7.30 Ns , intraction: *13.61 , (P≤0.05) *.				

During spore formation, many strains of *B. thuringiensis* produce crystalline proteins (inclusions) commonly used as a biological agent for pest control. . The results of the effect of different dilutions of the suspension of *B. thuringiensis* on the larvae of the largest waxworm showed that this bacteria had an apparent impact on the destruction of the larval stages of the worm, according to the dilution used in the experiment. By reviewing the studies and research in this field, 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) by completing a bioassay for the suspended bacteria *B. thuringiensis* on the Great Waxworm, it was observed that the larvae decreased appetite and food consumption as a result of infection with bacteria, in addition to the appearance of black spots or areas on the body of the infected larvae.

These symptoms are similar in most Lepidoptera larvae when infected with *B. thuringiensis*. After its death, its color changes from light to dark brown and then black. The larvae began to die 24 hours after feeding, and the killing rate ranged after 72 hours (67.6 and 67.3%) in some bacterial isolates.

Conclusions

We conclude from the study that propolis extract concentrations and *Bacillus Thuringia is* have an apparent effect on the third larval age of the Great Wax Moth, and the first ages were more sensitive than the last larval age.

References

Al-Alan Muhammad, Nour Al-Din Zahir, Adel Al-Menoufi, Nabil Al-Ahmad, and Mahaba Ghannam. (2018). Isolation of local strains of bacteria. Bacillus spp and its efficacy test against *Galleria mellonella* L larvae in vitro. The Syrian Journal of Agricultural Research 5(2): 229-235.

Al-Jourani, Reda Sakab, Saadi Hassan Sabr, and Tamadur Marwan Abdel-Jabbar. (2002). The effect of the oil extract of eucalyptus on the eggs and larvae of the great waxworm Galleria mellonalla. L. (Lepidoptera: Pyralidae) Journal of the College of Education for Girls, (4) 13: 738-730.

Kwadha,.C. A., Ong`amo, G. O., Ndegwa, P. N., Raina S. K., & Fombong, A. T. (2017) The biology and control of the greater wax moth, G. mellonella Insect,8(2),61.

Morandin, L. A.; Long, R. F. &Kremen, C.(2016). Pest control and pollination cost-benefit analysis of hedgerow restoration in a simplified agricultural landscape. J. Econ. Entomol. 109: 1020–1027.

Narvis Rott et al., Encyclopedia of Honeybees, Translated by: Dreadnway 1997. I 2003, Glory of the University Foundation for Studies, Publishing and Distribution, p.: 419-423.

Omran ,. Eman Musa and Anaa, Daoud, Hussein and Muhammad Alwan (2019). Integrated control of the major wax moth (Galleria mellonella L (Pyralidae: Lepidopera), Plant Protection Department, College of Agriculture, University of Basra, 1-9 p.

SAS. (2012) . statistical Angalysis system, user's Guide . ststistical .Version , 1th ed . SAS . Inst .Inc . cary .N.C.USA.

Stahly , D.P.; Andrews R.E. and yousten, A.A.(1991) .The genus Bacillus-insect pathogens. In:Balows A.truper HG,D workin M,Harderw. Qschleifer K.H ed. The prokaryotes , 2nd ed.New york , Basel, springer –ver lag , vol II,PP,1697 – 1735.

Thiery . A . and Frachon , E. (1997) . Identification, Isolation, culture and preservation of entomopathogenic bacteria in: Manual of Techniques in insect pathology: Academic press: 54-77.