Journal of Agronomy Research

Effect of Bio-Controlling Methods (Proplis and Bacteria) on the 3rd_larval Instar of *Galleria mellonella* (Lepidoptera: Pyralidae)

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Research Article

Open Access &

Peer-Reviewed Article

DOI: 10.14302/issn.2639-3166.jar-22-4389

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Keywords:

Bacillus thuringiensis, Ethanol, Kurstaki, Mortality, lethal effect, Soxhlet..

Received: Nov 30, 2022

Accepted: Jan 21, 2023

Published: Feb 18, 2023

Academic Editor:

Raj Kishori , CSIR - Central Institute of Medicinal and Aromatic Plants P.O. CIMAP, Lucknow, UP 226 015, India.

Citation:

Wegdan Abass Hussien, Abubaker Haroun Mohamed Adam, Siefaldinn Mahammed Khaeir, Abd Elbagi Elsayed Ali (2023) Effect of Bio-Controlling Methods (Proplis and Bacteria) on the 3rd_ larval Instar of Galleria mellonella (Lepidoptera: Pyralidae) Journal of Agronomy Research–4(4):24-29. DOI: https://doi.org/10.14302/issn.2639-3166.jar-22-4389

Abstract

The greater wax moth (Galleria mellonella L.) is one of the most destructive insect pests that threaten apiculture. Laboratory experiments were conducted in the Research Laboratory, College of Agricultural Studies, Sudan University of Science and Technology during the period from the 2nd_ of April to 29th_ of October 2021 to evaluate the lethal effect of Bee glue (proplis) and Bacillus thuringiensis var. Kurstaki (Btk) on the *G.mellonella*. Five concentrations (5%, 7.5%, 10%, 12.5% and 15%) of proplis extract and (0.62,1.25,2.5,5 and 10 mg/ml) of Bt were used for each proplis extract and Bt in a Completely Randomized Design. Powder ethanol extract of proplis at 15% concentration caused 83.3% mortality after 96 hrs of application, where as 10mg//ml concentration of Bt gave 93.3% mortality after 72 hrs of exposure. The statistical analysis revealed significant differences between all treatments and control. Additionally, all treatments (proplis/ /Bt) increased the mortality %.

Introduction

The greater wax moth (GWM) Galleria mellonella (Lepidoptera: Pyralidae) is the most serious pest of honeybee wax combs in stores. It can cause substantial losses to combs and frames of beehives (Kwadha, 2017) [1]. Initially, the larvae bore into the comb later, making silken threads into the middle of the comb and with the course of the time entire comb gets covered with the mass of silken webbing and faecal matter leads to a condition called "Galleriasis" (Laltia, 2017, Hosamani,2000) [2,3]. The wax moth larvae partly remove the cell capping of developing pupae leads to a condition known as "Bald Brood" which sometimes leads to the formation of adults with deformed legs and wings (Goodman, 2019) [4]. Stated that every year wax moth causes financial losses in the apiculture industry which has raised a serious concern among the apiculturists, scientists as well as the

general public (Kumar, et, al.; 2018) [5].

In the last three decades the control measures that are available for combs and other equipments not occupied by bees are physical methods involving exposure to heat (Charrière, 2002) [6]. Given the undesirable ecosystem outburst that ensued following excessive and continuous reliance on conventional pesticides (Carson, 2002):[7], there have been considerable research efforts to provide alternative "reduced-risk products". It has been focused here on the past and current management methods,and further highlight their merits and demerits which should form the basis of decision making for efficient control of GWM.

Because of the hazards of synthetic insecticides, recently the pesticidal effects of botanical extracts have been investigated by several researchers worldwide .(Imam, , et,al.; 2013. Jawalkar, et,al.; 2016) [8,9].

Propolis is a wax –like resinous substance collected by honey bees from tree buds or other botanical sources and used as cement to seal cracks and open spaces in the hive.Its colour varies from green to brown and reddish, depending on its botanical source. Propolis is normally composed of 30% wax, 50% resin,10% essential oils,5% pollen and 5% various organic residues (Silici, et, al.; 2007)[10]. Propolis is sticky at and above room temperature. At lower temperature it becomes hard and very brittle (Bankova, 2005) [11].

*Bacillus thuringiensis*_(Bt) is a Gram-positive bacterium that produces insecticidal crystal proteins (ICPs) during sporulation. ICPs are mostly Cry proteins an important component of Bt biopesticides and vital tools for insect control in transgenic crops. The identified Cry proteins have been classified into Cry1−Cry78 on the basis of amino acid sequence identity, among these Cry1, Cry2, and Cry9 proteins exhibit strong insecticidal activity against lepidopteran pests. Cry1 proteins have been widely applied in transgenic cotton, corn, and soybean to control lepidopteran pests over the last 22 years (Shan, et, al.; 2019)[12] .

The main objectives of this study was to examine the ethanolic extract of bee glue (Proplis) powder and *Bacillus thuringensis* var.kurstaki (Btk) against the 3rd larval instars of the Greater wax moth (*Galleria mellonella)*.

Materials and methods

Larval instars of *G. mellonella* were collected from local honey bee apiary located at Khartoum State, Shambat area during the period from the 2nd_ of April to 29th_ of October 2021.The infested honey bee wax combs contained all stages of insect development were used to establish the laboratory stock culture for further studies. The larvae were reared on an artificial diet (Metwally, et, al.; 2012)[13]. The moths were placed and allowed to reproduce in the laboratory with a temperature of 31±1°C, 66.28% RH and 12L: 12D photoperiod (These were the average conditions for all farther experiments) and placed in a closed aquarium tank (9.2x16x9.2 cm), covered with muslin cloth and brought to the laboratory for mass rearing. Early larval instars were reared in groups of 100 larvae in plastic cages 19 cm in diameter covered with muslin cloth and fed on bee wax. The 3rd larval instars were reared separately in plastic cups 5 cm in diameter and 7 cm in height to avoid cannibalism. The bottom of each cup was filled with bee wax for pupations. Upon emergence, the adults were transferred to glass cages30*30*30 cm covered with muslin cloth and fed on 10% sugar solution (Jallow, et, al.; 2001) [14]. The glass cages contained a comprised folded paper sheets for the deposition of eggs. The rearing process continued until a sufficient number of homogenous

populations of larvae were collected for the experiments.

The bee glue powder was collected from AL Baraka Componay for bee products and honey. One hundred and fifty (150)g of prepared bee glue powder were extracted with absolute ethanol using soxhlet apparatus, extraction continued for six hours, and the ethanol solvent was removed off the crude extract by rotary evaporator (Elnour, 2014) [15]. Five (5) concentrations (5%, 7.5%, 10%, 12.5% and 15%) were used.

Biotect 9.4 % WP commercial formulation containing Bacillus thuringiensis kurstaki from (Organic Biotechnology Co., First industrial zone , El Noubareya, El Beheira, Egypt were used in five (5) concentrations (0.62,1.25, 2.5, 5, and 10 mg/ml). The obtained data were statistically analyzed according to analysis of variance(ANOVA).Duncan's Multiple Range Test was used for means separation using Statistix 8.Also the data were subjected to probit analysis using SPSS16.0software to get LC50 and LC90.

Results and discussion

Results

It was observed that the *Bt* have recorded the highest mortality percentage which was (100%) at the biggest concentration (10%) after 96 hours (the longest exposure period) and then the bee glue at the concentration (15%) gave mortality percentage (83.3%).All treatments were significant different from control

The results shown in Table (2) and Fig (1) proved that all Bt concentrations generated a significantly ($p < .001$) higher mortality percentage than the control throughout the experimental period. It should be noted that the percent mortality increases with the increase of both concentration and exposure period. The lowest lc50 value recorded by Bt (-0.78), followed by proplis (3.1).

Discussion

In traditional beekeeping, many drugs and insecticides such as acaricides, fumigants, antibiotics are used for both bee pests and diseases such as *Varroa destructor* (Varroa mite), *Acarapis woodi* (tracheal mite), *Galleria mellonella* (Greater wax moth), American foulbrood, European foulbrood, *Nosema* etc. (Mert, 2007)[16]. Unconscious use of drugs leads to the resistance of disease factors, damage to beneficial microorganisms for bees and colonies, as well as residue problems in products obtained from beekeeping. For these reasons, alternative treatments or control methods are needed, the present study serve in the same issue so that the result of this study showed that all tested concentrations of ethanolic extract of the bee glue powder (*Proplis)* in addition to *Bacillus thuringiensis* had a positive lethal effect against 3rd larval instar *G.mellonella* and generated significantly higher mortality percentage than control throughout the experimental period. The result also indicated and demonstrated at the same time that the percentage of mortality increases with the increase of both concentration and exposure period.

The results in this study demonstrate that proplis or (bee glue) ethanolic extract at the concentration (15%) gave mortality percentage (83.3%) after 96 hours , the present study result agreed with (Muslem, 2012) [17] that the extract of phenolic proplis gave a high mortality percentage for the last instars of the greater wax moth. This result also similar to (Lucia, et,al.; 2011) [18] who reported that the solvent extracts of proplis samples from Brazil and Bulgaria activity different ethanolic extracts against the larger grain borer, Prostephanus truncates (Horn) in maize grains . Also this results agreed with (Lucia, et, al.; 2011)[19] who stated that Propolis ethanolic extracts was effective against red spider mites (*Tetranychus* spp.), which attack tomatoes.

Table 1. Lethal effect of Bacillus thuringiensis and proplis against 3rd larval instar G.mellonella (Shambat-Khartoum-

Treatments	Conc. (%)	Means mortality (%)			
		Exposure time (hrs.)			
		24	48	72	96
Bt (mg/ml)	**0.625**	16.7 (4.1)g	33.3 (5.8)fg	46.7 (6.9)de	63.3(8.0)ef
	1.25	26.7 (5.2)ef	43.3 (6.6)de	53.3 (7.3)cd	73.3 (8.6)cde
	2.5	43.3 (6.6)bcd	50.0 (7.1)cd	66.7 (8.2)b	80.0 (9.1)bc
	5	53.3 (7.3)b	63.3(8.0)ab	66.7 (8.2)b	93.3 (9.7)ab
	10	70.0 (8.4)a	76.7(8.8)a	93.3 (9.7)a	100.0 (10.0)a
proplis	5	16.7(4.1)g	26.7(5.2)g	40.0(6.4)e	56.7(7.6)f
	7.5	23.3(4.8)fg	36.7(6.1)ef	46.7(6.9)de	63.3(8.0)ef
	10	33.3(5.8)de	46.7(6.9)cde	56.7(7.6)c	70.0(8.4)de
	12.5	36.7(6.1)cd	56.7(7.6)bc	66.7(8.2)b	76.7(8.8)cd
	15	46.7(6.9)bc	63.3(8.0)ab	73.3(8.6-)b	83.3(9.2)bc
Control	-	(0.7000)h	(0.7000)h	(0.7000)f	(0.7000)g
C. V. %		9.8	7.4	4.7	4.6

* Means followed by the same letter (s) are not significantly different at ($p < .001$).

* Means between brackets are transformed according to $\sqrt{(X+0.5)}$

*C. V. = Coefficient of Variation.

Table 2. LC values for ethanolic extracts of t bee glue and Btk against 3rd larval instar of G. mellonella after 96 hrs of exposure(Shambat-Khartoum-Sudan2021).

Plant extract	LC* values (%) and 95% Confidence limits (Lower – Upper)		
	LC50	LC90	Chi- square $\chi 2$
Propolis	3.1(-20.7 – 6.7)	19.3(14.6 -54.3)	0.03
Bt	-0.78(-5.0 -0.6)	4.1(2.9 – 7.4)	0.2
LC = Lethal Concentration			

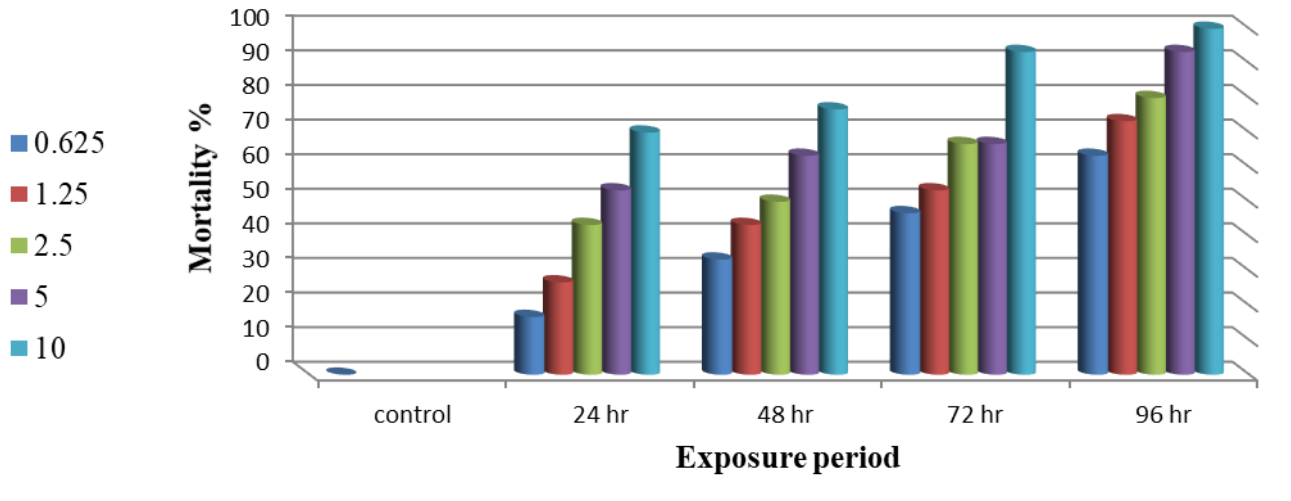

Figure 1. Lethal effect of Bacillus thuringiensis against 3rd larval instar G.mellonella

The results also revealed that all Bt concentrations represent a significantly higher mortality percentage ($p < .001$) than that of the control throughout the experimental period, and LC50 value of Bt generate in this study was - 0.78 mg/ml. Whereas (Kareru, et. al.; 2012)[20] found that LC_{50} of *Btk* on nettle caterpillar *Euprosterna elaeasa* was 1.25 mg/ ml. In addition the result showed morphological malformation, color of the larvae turns brown until it becomes black, the live of larvae are very short in addition to th slow movement and have soft texture. The present results are also in line with Elhaj, et. al.; 2021)[21] who found that Bt at all concentrations were better than other extractions against greater wax moth *Galleria mellonella* .Also the above mentioned result agreed with AL-Jassani [22] who indicated that the sub –lethal concentration led to a mortality percentage of 100% on wax comb stored with greater wax moth when treated with commercial preparation of bacteria at a concentration of 4.78 gram/liter.

Conclusion and recommendations

The obtained results clearly proved that the proplis ethanolic extracts at 15% and 10mg/ml ***Bt*** have insecticidal activity against 3rd larval instar of *G. mellonella.* Further studies are neded to use different concentrations of Proplis (Bee glue) and *Bacillus thuringiensis* (Bt) in field conditions.

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