Egyptian Journal of Plant
Protection Research Institute
www.ejppri.eg.net

Effect of propolis extracts and *Bacillus thuringiensis* on leafminer fly *Liriomyza sativae* (Diptera: Agromyzidae)

Amal, E. Marouf; Ghada, E. Abd-Allah and Marwa, M. Shalaby
Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

ARTICLE INFO
Article History
Received:12 / 4/2021
Accepted: 27/ 5 /2021

Keywords

Liriomyza sativae, tomato crop, propolis, *Bacillus thuringiensis* and control.

Abstract:

This study aimed to evaluate the effect of water, ethanol extracts of propolis, and *Bacillus thuringiensis* on larvae of tomato leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae). This pest is considered one of the most serious pests all over the world because it has a wide host range and may promote pathogen transmission followed by loss of crop quality and yield. Due to the problems of chemical pesticides to all organisms and the environment, natural control replaced pesticides. Microbial control with *B. thuringiensis* is one of the effective methods used in pest control. Also, propolis extracts are one of the natural control methods which is used recently in pest control. In this study, a water propolis extract achieved a high mortality rate against *L. sativae* with LC_{50} of 4628.002 ppm followed by BT with LC_{50} of 7110.849 ppm, then propolis ethanolic extract was less effective material with LC_{50} of 9288.848 ppm.

Introduction

Tomato (*Lycopersicon* spp.) is economically one of the most important vegetables (Polston and Anderson, 1999). Leafminer flies (Diptera: Agromyzidae) are phytophagous insects distributed worldwide, with approximately 2500 species being described (Spencer, 1990 and Winkler *et al*., 2009). These leafminers are characterized by the development of eggs and larvae inside the leaves, leading to the formation of mines in the leaf parenchyma that reduce photosynthesis, increase premature leaf drop and promote pathogen transmission, subsequently compromising crop quality and yield (Johnson *et al*., 1983; Parrella, 1987 and Matteoni and Broadbent, 1988). International trade of ornamental and vegetable crops facilitates their dispersion because the eggs and larval mines are not always visible in the host. *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) is polyphagous and cosmopolitan, with an increased distribution and agricultural importance. Pesticides produced from natural products have been recently attracting the attention of many scientists to avoid the problems caused by synthetic compounds. They are deeply interested in their chemical constituents and biological properties (Abou-Yousef *et al.*, 2010).

Propolis is generally known as the “bee glue”, which is a generic name that refers to the resinous substance accumulated by the bees from different types of plants. The word “propolis” is derived from Greek to mean defense for “pro” and city or community for “polis”, or in other words the beehive

(Castaldo and Capasso , 2002). Propolis functions in sealing holes and cracks and for the reconstruction of the beehive. It is also used for smoothing the inner surface of the beehive, retaining the hive's internal temperature (35 °C), preventing weathering, and invaded by predators. Furthermore, propolis harden the cell wall and contributes to an aseptic internal environment. Propolis generally becomes soft and sticky upon heating (Shehu *et al.* , 2016). It also possesses a pleasant smell. Propolis and its extracts have numerous applications in treating various diseases due to its antiseptic, anti-inflammatory, antioxidant, antibacterial, antimycotic, antifungal, antiulcer, anticancer, and immunomodulatory properties.

Bacillus thuringiensis (BT) is an aerobic gram-positive endospore-forming bacterium which is a part of the family Bacillaceae and it is widely used in agriculture as a biological pesticide (Aronson *et al*., 1986; Höfte and Whiteley, 1989 and Feitelson *et al.*, 1992).

The hypothesis of the present study was to evaluate the influence of natural and microbial control methods with propolis extracts and *B. thuringiensis*, respectively, as control agents against leafminer which considered one of the most serious pests all over the world because it has a wide host range, followed by loss of crop quality and yield. As well as reducing the use of chemical pesticides that cause a lot of hazards to all organisms and the environment.

Materials and methods

1. Insect rearing:

Tomato leaves carrying larvae of *L. sativae* were collected from the unsprayed farm of Agricultural College, Mansoura University, Dakahlia, Egypt. The leaves were kept in jars at 27 ± 2 °C and 65 ± 5% RH. The colony was maintained for two generations before the beginning of the tests. Then, the leaves with the newly hatched larvae of *L. sativae* were placed in plastic Petri dishes (10 cm diam). Each dish was covered with muslin for aeration and tomato leaves were put on the bottom of the dish (Madahi and Sahragard, 2012). Whenever the leaves appeared discoloured, they were replaced with fresh ones.

2. Bacterial strain used, source and applications:

Bacillus thuringiensis (4QSTR1) was obtained from the Center of *Bacillus* Genetics Stock, Biochemistry Department, Ohio University, Columbus, U.S.A. *Bacillus thuringiensis* was preserved on Luria-Bertani (LB) medium, containing: 0.5% NaCl, 0.5% yeast extract, and 1% tryptone and pH 7.0 (Sambrook *et al*., 1989). *B. thuringiensis* was grown in Petri dishes. The spores were collected from L.B agar plates, then washed three times with ice-cold distilled water. Pellets (spores and crystals) were responded in small volumes of distilled water. The bacterial crystals and endospores were prepared according to the method described by Karamanlidou *et al*. (1991).

3. Preparation of propolis extracts:

Propolis, which used in this work was collected. then kept in the dark until processing. The procedure was described by Alencar *et al*. (2007) with some modifications. Twenty g of finely ground propolis was added to different solvents (Water and 95% ethanol) to a final volume of 100 ml. The mixtures were protected from light, with moderate shaking during 24 h, at room temperature, and left to rest overnight. These mixtures were filtered through Whatman filter paper No.1. The mixtures were an ethanolic extract of propolis (EEP) and water extract of propolis (WEP) (Sabrien *et al*., 2016).

4.HPLC separation of flavonoids and phenolic compounds:

HPLC analysis was conducted in the laboratories of Food Science and Technology Institute, Giza, Egypt. An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) was used to identify and quantify the flavonoids and phenols of propolis (Shuai *et al*., 2014).

5. Spray application:

Tomato leaves which containing larvae of *L. sativae* were used for the application. Four concentrations were used as well as four replicates for each concentration. Ten larvae for each replicate were used to estimate the mortality line. Different concentrations were sprayed directly on the larvae. The concentrations used were 5000, 10 000, 15 000, and 20 000 ppm. The percentage mortality was recorded after one, three, five, and seven days and the data were corrected relative to control mortality (Abbott, 1925). LC_{50} values were determined using the probit analysis statistical method of Finney (1971). The equation of Sun (1950) was used to determine LC_{50} index

Toxicity index for LC_{50} =

(LC_{50} of the most effective compound/ LC_{50} of the least effective compound) × 100

6. Field experiment:

LC_{90} for each material was used for this experiment. A field experiment was conducted on tomato plants at Aga, Dakahlia Governorate, Egypt. All cultural practices for tomato plants were followed according to the instruction laid down by the Egyptian Ministry of Agriculture.

A complete randomized block design with three replicates was adopted in this experiment and the treatments are as follows :

1. Control treatment (Tap water)
2. LC_{90} BT.
3. LC_{90} propolis water extract .
4. LC_{90} Propolis ethanolic extract

Regular samples of larvae were collected and examined to record the percent of reduction in larval infestation, according to Hendrson and Tilton formula (1955) after 3, 5 and 10 days of spraying by assigning treatments according to the formula:

Reduction (%mortality) = [1- (C_b/ C_a X T_a/ T_b)] X 100

Where:

C_b= number of alive pest individuals in control before treatment.

C_a= number of alive pest individuals in control after treatment.

T_a= number of alive pest individuals after treatment.

T_b= number of alive pest individuals before treatment.

Results and discussion

1. Effect of *Bacillus thuringiensis* and propolis extracts on the mortality rate of *Liriomyza sativae* larvae:

In Table (1), results indicated that the water extract of propolis was the most effective material than the other materials, the ethanolic extract of propolis and BT, against larvae of leafminer *L. sativae* with different concentrations. At the higher concentration, 2000 ppm, the mortality rates were 76.67, 73.33, and 70 ppm for water extract of propolis, ethanolic extract of propolis, and BT, respectively.

2. Efficiency and toxicity index of the tested materials (Water propolis extract, ethanolic propolis extract, and *Bacillus thuringiensis*) against larvae of *Liriomyza sativae*:

However, Table (2) and Figure (1) showed that propolis water extract was the most effective material with LC_{50} 4628.002 ppm and LC_{90} 78279.872 ppm. Then BT was effective with LC_{50} 7110.849 ppm and LC_{90} 98172.253 ppm. The propolis ethanolic extract was less effective material than the other tested materials with LC_{50} 9288.848 ppm and LC_{90} 51399.335 ppm.

Data in Tables (3 and 4) demonstrated reduction in *L. sativae* after 3, 5 and 10 days that treated with propolis water extract, propolis ethanolis extract and BT. comparing with control.

Table (1): Corrected mortality % of larvae of *Liriomyza sativae* treated with *Bacillus thuringiensis* and propolis extracts under laboratory conditions at 27 ± 2 °C and 65 ± 5% RH.

Treatments	Conc. (ppm)	Mortality after treatments %				Total Mortality %
		One day	Three days	Five days	Seven days	
Propolis water Extract	5000	3.33	26.67	10	13.33	**53.33**
	10000	3.33	33.33	20	3.33	**60**
	15000	6.67	30	16.67	16.67	**70**
	20000	16.67	43.33	13.33	3.33	**76.67**
Propolis ethanolic extract	5000	3.33	10	13.33	6.67	**33.33**
	10000	3.33	13.33	20	3.33	**40**
	15000	3.33	43.33	10	6.67	**63.33**
	20000	10	40	20	3.33	**73.33**
BT	5000	3.33	10	3.33	10	**26.67**
	10000	10	16.67	3.33	16.67	**46.67**
	15000	10	20	23.33	10	**63.33**
	20000	3.33	50	10	6.67	**70**

Table (2) : Efficiency of propolis extract and *Bacillus thuringiensis* against *Liriomyza sativae.*

Treatments	Conc.	Corrected mortality%	LC_{50}	LC_{90}	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/LC_{50}
Propolis water extract	5000	53.33	4628.002	78279.872	1.043	100	16.91
	10000	60					
	15000	70					
	20000	76.67					
Propolis ethanolic extract	5000	33.33	9288.848	51399.335	1.725	49.823	5.53
	10000	40					
	15000	63.33					
	20000	73.33					
BT	5000	26.67	7110.849	98172.253	1.124	65.084	13.81
	10000	46.67					
	15000	63.33					
	20000	70					

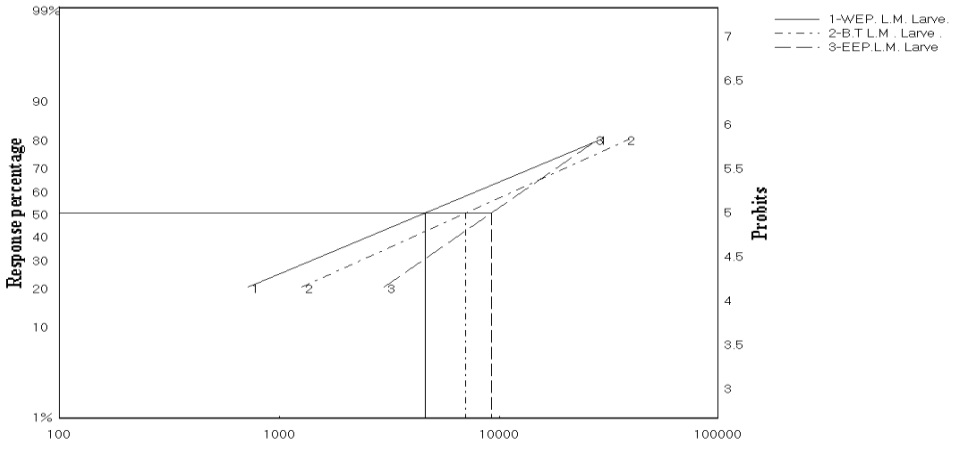

Figure (1): LC-P line for propolis extracts and *Bacillus thuringiensis* of *Liriomyza sativae.*

Table (3): Effect of assigned treatments on infestation reduction percentage of *Liriomyza sativae* after 3, 5 and 10 days of treatments

Treat.	1st replicate			2nd replicate			3rd replicate			4th replicate			Treatment efficiency		
	Pest number		Red. %	Pest number		Red. %	Pest number		Red. %	Pest number		Red. %	Pest number		Total Red. %
	Before	After		Before	After		Before	After		Before	After		Before	After	
After 3 days															
Propolis water extract	11	0	100	11	4	80	11	4	80	11	4	80	11	3	**85**
Propolis ethanolic extract	11	4	80	11	4	80	11	4	80	11	4	80	11	4	**80**
BT	4	3	58.75	4	3	58.75	4	3	58.75	4	3	58.75	4	3	**58.75**
Control	11	20	------	11	20	------	11	20	------	11	20	--------	11	20	------
After 5 days															
Propolis water extract	6	0	100	6	2	84.7	6	0	100	6	2	84.7	6	0	**92.35**
Propolis ethanolic extract	11	6	75	11	6	75	11	6	75	11	6	75	11	6	**75**
BT	4	0	100	4	0	100	4	0	100	4	0	100	4	0	**100**
Control	11	24	------	11	24	------	11	24	-------	11	24	-----	11	24	-----
After 10 days															
Propolis water extract	6	1	93.45	6	1	93.45	6	1	93.45	6	1	93.45	6	1	**93.45**
Propolis ethanolic extract	11	2	92.86	11	2	92.86	11	2	92.86	11	2	92.86	11	2	**92.86**
BT	4	2	80.36	4	2	80.36	4	2	80.36	4	2	80.36	4	2	**80.36**
Control	11	28	-------	11	28	-------	11	28	-------	11	28	------	11	28	------

Table (4): Mean of total reduction percentage of *Liriomyza sativae* infestation.

Treatments	Mean reduction of 1st scan	Mean reduction of 2nd scan	Mean reduction of 3rd scan	Mean of total reduction
Propolis water extract	85	92.35	93.45	90.3
Propolis ethanolic extract	80	75	92.86	82.6
BT	58.75	100	80.36	79.7

As described in Sabrien *et al.* (2016), the spores appeared as a dark-staining body. *Bacillus thuringiensis* (4QSTR1) produced cry proteins that appeared as stained crystals. There is a relationship between the insecticide activity and the crystal morphology of *B. thuringiensis* (Maeda *et al*., 2000). The results indicated that the water extract of propolis was the most effective material than the other materials, ethanolic extract of propolis and BT, against larvae of leafminer, *L. sativae* with different concentrations. In the higher concentration, 2000 ppm, These results were in agreement with Zewdu and Legessa (2016) Evaluate the insecticidal effect of propolis against larvae of lesser wax moth *Achroia grisell* concluded that the extract of propolis at higher concentrations is a powerful contact toxicant against young wax moth larvae. High-performance liquid chromatography equipped with a

diode array detector was used to separate flavonoids and phenolic compounds from samples with varied matrixes. It was used to identify and quantify the flavonoids and phenolic compounds in propolis. These results were in agreement with Sabrien *et al*. (2016) who proved that The resultant appeared that chlorogenic was the most identified phenolic compound (120.62 µg/100 g dry weight) and the acacetin was the major identified flavonoid component in propolis (647.53 µg/100 g dry weights) (Shuai *et al*., 2014). These results can state that propolis has general biological activities as insecticides activity These results were in agreement with Sabrien *et al*. (2016) who proved that water extract of propolis was the most effective material against *Tetranychus urticae*. Also, Talha *et al*. (2019) proved that microbial control using BT was effective and safe in controlling most pests. Sabrien *et al*., 2016 , also, proved effectiveness of water propolis extract against pests.

In the present investigation, bioinsecticidal effect of *B. thurinogiensis* cry toxin, propolis extracts against larvae of *L. sativae* were studied. The results revealed that they would be suitable for developing a biological process and can be used successfully in IPM program to control leafminer *L. sativae* .

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