Academic Journal of Entomology 16 (3): 141-146, 2023
ISSN 1995-8994

DOI: 10.5829/idosi.aje.2023.141.146

Evaluation Activity of Honeybee Propolis Extract for Controlling Some Harmful Economic Insects at Qalyubia Governorate, Egypt

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Abstract: The study was conducted to evaluate the activity of 'ethanolic extract of propolis' (EEP) against 2nd instar larvae of four economic insect's pests; *Pieris brassica*, *Pieris rapae*, *Pectinophora gossipella* and *Spodoptera littoralis*. The results show that, EEP was effective against all treated insects at 5, 10 and 20% concentrations. The mortality percent of treated larvae increased with increasing of EEP concentration. Also, propolis extract at 20% concentration record the highest mean accumulative mortality rate of 7 days post treatments for tested insects were 31.6%, 35.53%, 52.60 % and 45.46 % for *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis,* respectively. While the propolis extract at 5% concentration record the lowest mean accumulative mortality rate of 7 days post treatments for tested insects were 11.77%, 11.20%, 17.13 % and 15.25 % for *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis,* respectively. So, ethanolic extract of propolis can be used as safe alternative to chemical insecticides. This approach considered as environmentally friendly approach in controlling insect pests.

Key words: Pieris Brassica · Pieris Rapae · Pectinophora Gossipella And Spodoptera Littoralis · Propolis Extract.

INTRODUCTION

Without insecticides, 50% or more of the major crops could be lost due to insects' pests in the USA [1]. Use of insecticides for controlling the divesting pests caused several environmental problems such as pollution besides the negative effects on the other beneficial insects. These problems derived the scientists for finding more safe methods in controlling these pests [2]. Propolis ethanolic extract is a safe alternative to synthetic insecticide. It is environmentally friendly with no adverse health risks.

Propolis is a resinous substance collected by worker bees (*Apis mellifera*) from the bark of trees and leaves of plants [3]**.** The insecticidal activity of the propolis has been examined as it causes inhibition of larval development against honey bee pests like varoa and greater wax moth [4].

Unfortunately, there are very limited literatures on the acaricidal or insecticidal activity of the proplois. Propolis contains substances of plant origin, and is reported to have medicinal, insecticidal, antimicrobial and phytotoxic properties. The different components of propolis are produced by plants in order to avoid infection of injured plant parts and help ward off and kill insects or mite pests [5]. Some authors suggested that some flavonoid components of propolis have insecticidal or at least insect static effects [6]. Johnson *et al*. [7] and Erturk and Guler [8] determined the physical and chemical composition of propolis from samples collected from North American of and tested their bioactivity against larvae of the greater wax moth *Galleria mellonella* L..

Propolis extracts evaluated as control agents against leafminer which considered one of the most serious pests all over the world because it has a wide host range,

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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 [9]. Recent years have seen a significant global drive towards sustainable and ecological agricultural practices. This change is due to growing concerns about the harmful effects of synthetic pesticides and chemical fertilizers on ecosystems and human health [10]. Researchers and farmers are exploring alternative methods to increase crop productivity and ensure the long-term viability of agricultural systems. One such natural solution that is gaining more and more attention is propolis [11]. Because propolis is a multifunctional material used by bees in the construction and maintenance of their hives.

This study aimed to evaluate the insecticidal activity of propolis extract as a safe material against some harmful economic insects such as *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis*.

MATERIALS AND METHODS

In this study, the larvae of the target pests were collected from the infested field during summer and winter seasons 2022 from the area of Qalyub, Qalyubia Gov., Egypt. The leaves of cotton contained the eggs of each target insect pest were collected from the field in cloth bags and transported to the laboratory. A stock culture of each target pest was grown in the laboratory by introducing the infested leaves, with eggs of each target pest, into a plastic cage measuring (60x60) cm and kept at photoperiod 12:12 LD. After eggs hatching fresh leaves of each plant host (cotton & cabbage) were introduced into the cage for feeding the hated larvae and maintain considerable number of target larvae for further evaluation. All collections were held in the laboratory at Department of Pests and Plant Protection, National Research Centre, Egypt, to record the identification of the adult insects. The taxonomy was described according to the available of literature.

Preparation of Propolis Extract: Propolis samples obtained from Beekeeping Research Dept. Plant Protection Research Institute, Agricultural Research Centre, Dokki, Egypt. The hand-collected propolis was stored in a brown bottle and away from exposure to light until further processing. Propolis extract was prepared as described by Embaby *et al.* [12], Özdemir *et al.* [13]. Propolis extract was prepared by adding 100 g of the collected propolis to 900 mL completed to 1000 ml of 70% ethanol to give 10% ethanolic extract of propolis (EEP) which extracted and heating for evaporating ethanol (at 50°C for 5 hours) and agitating. Water was then added. To optimize purification, centrifugation at high speeds was proposed. All samples were centrifuged at 3000 rpm for 25 minutes. The supernatant was stored overnight at ambient temperatures, filtered through filter paper (Whatman no. 1) and stored at ambient temperatures in a bottle and kept at 4ºC in dark storage until use. The tested concentrations were prepared by making a dilution of 5, 10 and 20 of propolis extract ml per 100 ml of sterilized water.

Larval Treatment: The leaf-dipping technique was used. Serial concentrations of the propolis extract were prepared. For each concentration, leaves of cotton were dipped for 30 seconds then allowed to dry at room temperature. Treated leaves were introduced to 2nd instar of each pest species for 24h after that mortality counts were recorded.

RESULTS

1- Identification and Classification of Collected Insects: The taxonomy of collected pests largely on the morphology of the larvae and adults after examined were identified and description of species as shown in Table 1. Four species of insects were collected from the infested field and identified. These insects belonged to Lepidoptera order. Four economic insects were identified as shown in Table 1.

2- Activity of Propolis: Effect ethanol extract of propolis,, (EEP) on mortality percent of *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis* larval instars at 3 different concentrations, were recorded in Table 2 and Figs. (1-3). Table 2 presents the cumulative mean mortality

Table 1: List of collected insect treatments

Treatment	Common name	Scientific name	Order
1	Cabbage worm	*Pieris brassica*	Lepidoptera
2	Cabbage White Butterfly	*Pieris rapae*	Lepidoptera
3	Cotton bollworm	*Pectinophora gossipella*	Lepidoptera
4	Cotton leaf worm	*Spodoptera littoralis*	Lepidoptera

Table 2: Cumulative mean mortality percent of larvae treated by ethanolic propolis extract at different concentrations.

Propolis concentrations	Days	Accumulative mean Mortality (%)			
		Pieris brassica	*Pieris rapae*	*Pectinophora gossipella*	*Spodoptera littoralis*
5%	1	0.0	0.0	0.0	0.0
	2	0.0	0.0	12.0	6.66
	3	16.33	12.0	22.33	20.00
	4	17.33	13.66	23.66	23.00
	7	25.20	30.33	27.66	26.60
Mean± SE		11.77±0.33^{c}	11.20±0.33^{c}	17.13±0.58^{c}	15.25±0.9^{c}
Control		0.0	0.0	0.0	0.0
10%	1	0.0	0.0	9.66	10.00
	2	0.0	0.0	19.66	27.00
	3	20.66	25.3	28.33	35.33
	4	25.33	33.66	36.0	44.00
	7	35.0	45.33	47.0	61.66
Mean± SE		16.20±0.6^{b}	20.86±0.33^{b}	28.13±0.33^{b}	35.60±0.33^{b}
Control		0.0	0.0	0.0	0.0
20%	1	0.0	0.0	30.66	17.00
	2	18.0	20.0	42.33	30.00
	3	35.0	32.33	50.33	44.33
	4	40.0	55.33	68.33	57.33
	7	65.0	70.0	71.33	78.66
Mean± SE		31.6±0.33^{a}	35.53±0.9^{a}	52.60±0.58^{a}	45.46±0.6^{a}
Control		0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}	0.0±0.0^{d}
L.S. D at 0.05		0.941422246	1.2153708935	1.4380462344	1.630591161
F Value		1939.8***	1520.7333***	2519***	1582.3333***

The means sharing the same small letter within a column are not statistically different at the 5% level of probability

Fig. 1: All treated larvae of *Pieris brassica* are stopped feeding

Fig. 2: Symptoms of malformed larval of *Pieris brassica* and damaging pupa

Fig. 3: Larvae of *Pieris brassica* were stopped eating and malformed.

percentages of larvae exposed to various concentrations of propolis extract over time. The data indicates a clear dose-dependent relationship, with higher concentrations of propolis leading to significantly higher mortality rates in all tested insect species. This suggests that propolis could be a promising natural insecticide, particularly for *Pi. brassicae* and *Sp. littoralis*. Data presented that, a bio-pesticide propolis (EEP) was effective against all tested larvae when treated with 5, 10 and 20% concentration after dipping respectively. Continue increasing of propolis concentration was found to increase the mortality percent of all tested larvae. On the other hand, the mortality percent increased with increasing the time period/day with all tested larvae. This study reveals that, the ethanol extract of propolis at higher concentration (20%) had high toxic action which record the highest activity of mortality percent compared with other concentrations. Propolis extract at all concentration was found to accelerated larva development stage. The abnormally higher rate of development may lead to malformed and immature individuals. All treated larvae were found to stopped eating, slow moving and appeared symptoms of malformed larval and pupa.

Higher mortality percent was recorded with *Pi. brassica* followed by *P. rapae* respectively. Moderate mortality percent was recorded with *Pe. gossipella* while, less mortality percent was occurred with *Sp. littoralis*. Complete mortality percent which gave hundred percent was recorded with *Pi. brassica* when treated with 5 and 10% concentration of EEP after 5 days from dipping and at 20% of EEP after 4 days from dipping of the larvae *Pi. brassica*. Complete mortality percent was recorded with *Pi. rapae* when treated with 20% concentration of EEP after 7 days from dipping.

DISCUSSION

Researchers and farmers are looking to discover alternative ways to increase crop productivity and ensure the long-term sustainability of agricultural systems. The fascinating world of propolis and its utilization in agriculture due to its chemical composition and the bioactive substances responsible for its biological properties including its composition in terms of chemical constituents such as flavonoids, phenolic compounds, terpenoids and other bioactive substances responsible for its biological activity.

The study was focused to see the effect of propolis on *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis* larvae. Results show that, a natural product, “ethanol extract of propolis” (EEP) had effective against larvae of all tested insect pests when treated with 5, 10 and 20% concentration. Continue increasing of propolis concentration were found to increase the mortality percent of larvae of all tested isects. Also, the mortality percent was increased with increasing the time period/day. Higher concentration of EEP (20%) has high toxic action which record the highest activity compared with other concentrations.

All treated larvae were found to stopped eating, slow moving and symptoms of malformed larval and pupa were appeared. Similar results were obtained by Imdorf *et al.* [15] who reported that, the use of propolis as an insecticide may help us to minimize the problem environmental pollutions as result of synthetic insecticide applications. It also helps to reduce the constantly increasing problem of insecticide resistance development. Since propolis is a complex natural product, having different components with various modes of action is unlikely or very slow. This may suggest propolis extract

at higher concentration accelerated larva and/or pupa development stage/stages. The abnormally higher rate of development may lead to malformed and immature individuals. Garedew *et al.* [16-18] and Ararso and Legesse [19] concluded that, "ethanol extract of propolis" at 8 and 10 % w/v were the most toxic causing 90% and 80% mortality against young wax moth larvae. These results indicate higher concentrations were more toxic. Treatment of 10% (w/v) propolis extract resulted in 100% of mite Varroa *destructor* mortality regardless of a treatment time. This result is in agreement with the micro-calorimetric toxicity results obtained on larvae, pupae and adults of the yellow meal worm *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Simone-Finstrom and Spivak [20] stated that, as with *Formica paralugubris* ants, it is likely the presence of propolis in a honey bee colony may reduce the investment in the innate immune response by acting as an external immune defense mechanism. Assegid [21] Pastagia and Patel [22]and Asmaa M. Fawzy [23] stated that pupal stage lasts for 12 to 19 days. Propolis accelerates the development of the larval/pupal stage of *Galleria mellonella*. The unusually higher rate of metamorphosis may lead to malformed and immature individuals. The sixth and seventh larval instars were reported to be more sensitive to treatments with propolis concentrations of 10% propolis that was resulted in 100% mortality of seventh larval instars. The abnormally higher rate of development may lead to malformed and immature individual. On the other hand, earlier adult emergence was observed in treatments of higher concentrations. This may suggest propolis extract at higher concentration accelerated larva and/or pupa development stage/stages. The abnormally higher rate of development may lead to malformed and immature individuals. Asmaa M. Fawzy [23] reported that, Propolis extract at higher concentrations caused significantly higher mortality to wax moth larvae than the lower concentrations and untreated controls 24 h after treatment the larvicidal action of propolis increases with the concentrations. However, the larvae of wax moth responded similarly to all concentrations 48 h but significantly more larvae up to 90% were killed in propolis treated than the controls. This may suggest propolis extract at higher concentration accelerated larva and/or pupa development stage/stages. from different concentration of propolis, 8 and 10 % w/v were the most toxic causing 90% and 80% mortality. El-Helaly and Saye [24] Propolis tested as UV- protectants additives of *Sp. littoralis* nucleopolyhedro virus (SpliNPV) suspension. The obtained result demonstrated that certain propolis isolates gave better protection than cacao.

CONCLUSION AND RECOMMENDATIONS

According to these results, ethanolic propolis extract had effective against 2nd larva instars of the insects: *Pi. brassica*, *Pi. rapae*, *Pe. gossipella* and *Sp. littoralis*. It is excellent insecticides activities. This approach is considered as environmentally friendly approach in contrast to physical and biological techniques. The cost of this process is very important in choosing the cheapest and the most effective method in controlling these pests. So that, the use of propolis as an insecticide may help us to minimize the problem environmental pollutions as result of synthetic insecticide applications. It also helps to reduce the constantly increasing problem of insecticide resistance development.

Propolis is a complex mixture of different naturally-occurring constituents with more than 300 constituents identified to date, which includes phenolic acid, terpenes, cinnamic acid, caffeic acid, aromatic aldehydes, alcohols, amino acids, fatty acids, vitamins (A, B1, B2, B3, and B7), several esters, minerals, essential oils, and flavonoids (flavones, flavonols, and flavanones) [14, 25, 26].

Further research is needed to determine the optimal application rate and the specific mechanisms of action underlying propolis' insecticidal properties.

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