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Histological Studies Using Honeybee Propolis Extract for Controlling Med-Fly, *Ceratitis capitata* (Wiedemann) to Protecting Apricot Fruit in Egypt

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Abstract: This aim of the present work was to study the histological effects of different concentrations of ethanolic extract of honeybee propolis (EEP) and control larval instares of Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae) in order to protect apricot fruit in Egypt. Results indicated that, *C. capitata* was identified and classified as the major invasions Apricot fruits. EEP) was effective against all treated larva instars at concentrations 7%, 12% and 17% . Mortality percent of treated larvae was increased with the increasing of the EEP concentrations. EEP at 17% was more active and record the highest mean mortality rate of 43.82% for treated larvae compared with 7% and 12% EEP those record 21.15% and 26.36% mortality respectively. Histological of mid-gut in treated *C. capitata* larvae showed epithelial cells lined mid gut were necrosis, lost their brush borders as well as peritrophic membrane was intact. This approach is considered as environmentally friendly approach in contrast to physical and biological techniques. This is the first time EEP against *C. capitata* (Wiedemann) in Egypt.

Key words: Apricot Fruits · Infestation, Fruit Flies · Propolis Extract · Histology

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are among the most important insect pests affecting fruit crops and fruiting vegetables worldwide. This family includes over 4,000 species, approximately 1,400, of which attack soft fruits. The four major groups of fruit pest tephritids, in the subfamily Dacinae such as *Ceratitis capitata* (the Mediterranean fruit fly), *Bactrocera zonata* (peach fruit fly), *Bactrocea cucurbitae* (the melon fly), *Bactrocera dorsalis* / *Bactrocera invadens* (the oriental fruit fly / the African invader fly), and *Bactrocera olea* (olive fruit fly) are of considerable agricultural concern in the tropics and sub-tropics. They are still causing excessive damage in horticulture production almost everywhere in the world. The damage caused by fruit flies could be direct due to infestation or indirect due to loss of export market through quarantine restrictions and transmission of phyto-pathogenic microbes [1, 2].

The Mediterranean fruit fly C. *capitata* (Wiedemann) (Diptera: Tephritidae), is among the world's most economically damaging pest species. *Ceratitis capitata* infests more than 250 species of fruits and vegetables. It is responsible for direct economic losses in fruit production and quarantine costs, and is the focus of considerable and costly detection, control and/ or eradication programs [3-5]**.** *Ceratitis capitata* can infest 100 percent of susceptible fruit such as apricots, nectarines, peaches and mandarins and to a lesser extent, fruits such as apples and pears [6]. *Ceratitis capitata* is one of the world's most destructive fruit pests. Because of its wide distribution over the world, it is ranked first among economically important fruit fly species. Its larvae feed and develop on many deciduous, subtropical, and

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tropical fruits and some vegetables. Although it may be a major pest of citrus, often it is a more serious pest of some deciduous fruits, such as peach, pear, and apple. The larvae feed upon the pulp of host fruits, sometimes tunneling through it and eventually reducing the whole to a juicy, inedible mass. Some areas have had almost 100% infestation in stone fruits. Harvesting before complete maturity also is practiced in Mediterranean areas generally infested by this fruit fly. *Ceratitis capitata* can be transported from one part of the world to some distant place in a matter of hours, which greatly complicates efforts to contain it within its present distribution. Once it is established, eradication efforts may be extremely difficult and expensive. In addition to reduction of crop yield, infested areas have the additional expense of control measures and costly sorting processes for both fresh and processed fruit and vegetables. Some countries maintain quarantines against the *C. capitata* which could jeopardize some fresh fruit markets if it should become established [7]. *Ceratitis capitata* can be infested apricot (*Prunus armericana* Marsh; Family: Rosaceae) causing direct economic losses in fruit production. So**,** the overall objective of this study was focused on the invasions of apricot fruit flies' morphology detection, identification and taxonomy.

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 [8]. 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 [9]. Propolis is a brownish, resinous wax-like material that is produced by bee workers and subsequently mixed with bee saliva and wax. Bees use this material to cover holes and crevices and narrow the entrance to the hive thus maintaining the environment in the hive and preventing air from flowing into the hive [10]. Propolis consists of 50 % resin, 10 % essential oils, 30 % beeswax, 5 % pollen, and 5 % other organic compounds [11]. Honeybees use propolis to defend their colonies against microbial pathogens and it can also protect them against other pests [12]. These effects suggest that propolis could be useful in protecting crops from arthropod and insect pests. The aim of study to evaluate propolis extract to protecting apricot fruits against invasions insect and developing more environmentally friendly, and efficient treatments in controlling *C. capitata*.

MATERIALS AND METHODS

Apricot fruit samples was obtained from field-infested apricot (*Prunus armericana* Marsh; Family: Rosaceae). Two hundred fruit samples were collected from apricot trees and ground in the infested field during spring and summer seasons 2021 from the area of Al-Ammar, Toukh, Qalyubia Gov., Egypt then checked and larvae were placed in plastic containers and brought to the laboratory at Department of Pests and Plant Protection, National Research Centre, Egypt, to identify the adult insects (150 individuals). Collected fruit flies were identified based on the morphological characteristics by using taxonomic keys [13-16]. A digital camera (Samsung) was used to take photos and to produce fully-focused images [17].

Ethanolic Propolis Extract Preparation: Propolis samples obtained from Beekeeping Research Dept. Plant Protection Research Institute, Agricultural Research Centre, Dokki, Egypt. Propolis extract was prepared as described by Özdemir *et al.* [18] and Embaby *et al.* [19]. Propolis 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 methanol (at 50°C for 5 h) and agitating. Water was then added. To optimize purification, centrifugation at high speeds was proposed. All samples were centrifuged at 3000 rpm for 25 min. The supernatant was stored overnight at ambient temperatures. The supernatant was further filtered through filter paper (Whatman no. 1) and stored at ambient temperatures in a bottle**.** The final solution was termed 'ethanol extract of propolis' (EEP) to produce a final solution from various propolis samples**,** kept at 4ºC in dark storage until use. The propolis extracts were prepared by making a dilution of the 7, 12 and 17 ml per 100 ml of sterilized water**.**

Larval Treatment: Ten 2nd larval instar (5.0 cm diameter) was treated by topical application in Petri dish per replicate /concentration. Three concentrations were used 7.0%, 12.0% and 17.0 %. Five replicates per concentration were used. Also, 10 larvae treated by diluted ethanol. Mortality and virulence due to EEP as a bio-pesticide were evaluated against the 2nd larval instar. The tested larvae were checked every day to record the number of dead larvae and to add fresh food material if required.

Statistical Analysis: The data were subjected to analysis of variance (ANOVA) and the means were compared by LSD test at 0.05 levels, using SAS computer program [20]. Pathogenicity analysis was done according to Gomez and Gomez [21].

The data were analyzed using Probit analysis [22] to calculate LC_{50} & LC_{90} values.

Histological Investigation: Tissues of live treated larvae and untreated *C. capitata* larvae were fixed in 10% formalin, embedded in paraffin and then sectioned at 5 mμ thickness. Slides were stained with hematoxylin and eosin for histopathological examination according to Bancroft and Stevens [23].

RESULTS AND DISCUSSION

Identification of Collected Insect Infested Apricot Fruits Description: Fruit flies may be detected as larvae in fruits. *Ceratitis capitata* female lays their eggs in apricot fruits, larvae feed on apricot pulp Fig. 1. Egg color of the *C. capitata* is usually white to a creamy and elongate. Attacked fruit will often have puncture marks made by the female's ovipositor. Sometimes there may be some tissue decay or secondary rot around these marks. Rotting of the underlying tissue causes a depression on the surface as external symptoms of fruit fly infested apricot fruits as result of infested apricot fruits causing fruit decay Fig. 2. When cutting infested fruit, fully grown larvae flex and 'jump' repeatedly when removed from fruit as internal symptoms of fruit fly infested apricot fruits Fig. 1. Larvae appeared white creamy, cylindrical, a legless, pointed head, somewhat curved and flattened. Infested fruits should be placed in a container in which emerging larvae can pupate. When all the larvae have emerged from the fruit or if any sign of mould appears the sawdust should be sieved and the puparia collected. Cylindrical capsule pupa with dark reddish brown were occurred. The flies develop their full body coloration and normal shape. The adult color is yellowish with brown and have two wings

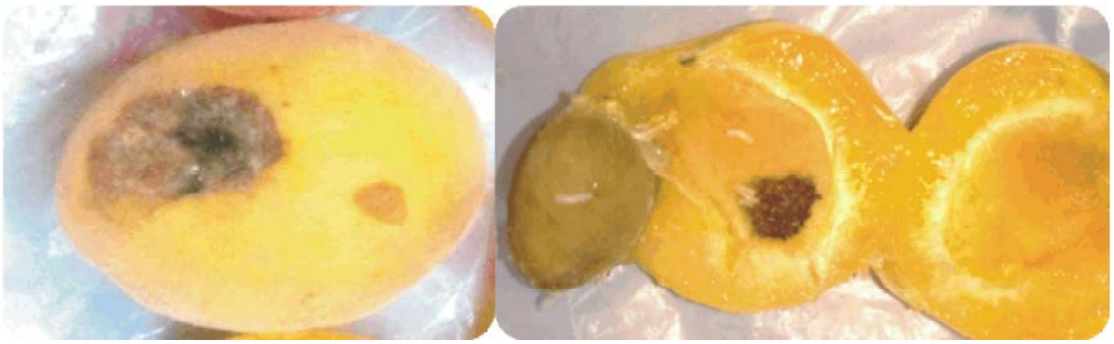

Fig. 1: External and internal symptoms of *Ceratitis capitata* as a result of primary infestation.

Fig. 2: Secondary rotting infection of the underlying tissue as a result of infested apricot fruits causing fruit decay.

Table 1: The mean mortality percent of Med fly *Ceratitis capitata* (Wiedemann)larvae treated by Propolis extract at different concentrations (as a bio-pesticide)

		Accumulative mean mortality %							
Treatment	Conc.	1 days	2 days	3 days	4 days	5 days	6 days	7 days	Mean ± SE
Propolis	7%	15.28	15.33	15.81	23.53	25.15	26.15	26.82	21.15±0.33^{c}
	12%	16.33	16.77	18.25	25.15	35.15	36.15	36.77	26.36±0.58^{b}
	17%	30.10	30.86	34.70	45.50	48.55	55.55	61.51	43.82±0.33^{a}
Control					0.0 ±0.0 ^{d}				
L.S. D at 0.05					1.2153708935				
F Value					2290.8667***				

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

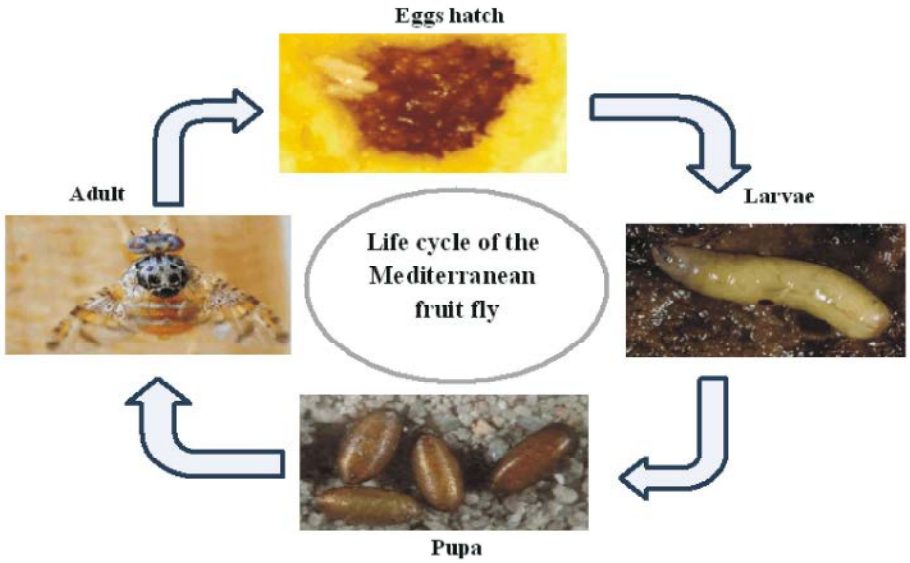

Fig. 3: Life cycle of the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann)

typical of fruit flies and the morphological characteristics that allow to distinguish them easily. It's similar to *C. capitata* (Wiedemann) belongs to the Order Diptera, characterized by the presence of only one pair of wings. The thorax is creamy white to yellow with a characteristic pattern of black blotches as shown in Table 1.

The obtained data were confirmed by Yoav *et al.* [5] who reported that, fruit is damaged by the wound made by the female as she lays eggs in the fruit. That wound and the development of larvae in the fruit cause fruit shedding and decay. According to Thomas *et al.* [7], adults are slightly smaller than a house fly and have picture wings typical of fruit flies. The color is yellowish with brown tinge, especially on abdomen, legs, and some markings on wings. The thorax is creamy white to yellow with a characteristic pattern of black blotches. Dorsocentral bristles are anterior of the halfway point between supraalar and acrostichal bristles. They can be distinguished fairly readily from any of the native fruit flies of the New. The egg is very slender, curved, 1 mm long, smooth and shiny white. Larvae are white with a typical fruit fly larval shape, i.e. cylindrical maggot-shape, elongate, anterior end narrowed and somewhat recurved ventrally, with anterior mouth hooks, and flattened caudal end. The pupa is cylindrical, 4 to 4.3 mm long, dark reddish brown, and resembles a swollen grain of wheat. There is a wide brownish yellow band across the middle of the wing. The apex of the wing's anal cell is elongate. There are dark streaks and spots in the middle of wing cells in and anterior to the anal cell. The males have black pointed expansion at the apex of the anterior pair of orbital setae. The females have yellow wing pattern and the apical half of the scutellum being entirely black. The female's extended ovipositor is 1.2 mm long.

Life Cycle: *Ceratitis capitata* can overwinter as adults, as eggs and larvae (in fruit), or as pupae in the ground and adults again Fig. 3. *Ceratitis capitata* female lays their eggs under the skin of the fruit in infested apricot fruits which is just beginning to ripen. Damage in the

early stages is difficult to be detected, as the oviposition holes are very small. Changes in the color of the fruit skin and soften areas of the fruit, due to rotting, are later visible. Several larvae develop inside a fruit, feeding on apricot pulp, facilitating the decomposition of plant tissue by invading secondary microorganisms**.** Fully-grown larvae leave the fruit and jumps to the soil, where they pupate**.** It overwinters in the ground as pupa within a puparium. Newly emerged adults look for food, that is needed for egg maturation.

Similar results were obtained by Broughton [6] and Broughton [24] who reported that, the Med-fly larvae are white with a flat, pointed head. The larvae feed on the flesh of the fruit, causing it to decompose. When fully grown, larvae stop feeding and jump out from the fruit or leave the fruit, burrowing into the soil to pupate. Thomas *et al*. [7] found that, a *C. capitata* female laid one to 10 eggs in an egg cavity 1 mm deep. The number of eggs found at any time in the reproductive organs is no indication of the total number of eggs an individual female is capable of depositing, as new eggs are being formed continually throughout her adult life. Eggs are deposited under the skin of fruit that is just beginning to ripen, often in an area where some break in the skin already has occurred. When the eggs hatch, the larvae promptly begin eating, and at first tunnels are formed, but may keep close together in feeding until nearly full grown. Ripe fruit is likely to be juicier, and such fruits often are associated with a high mortality of eggs and young larvae. Pupae carry the species through unfavorable conditions, such as lack of food and water, and temperature extremes. Larvae leave the fruit in largest numbers at or just after daybreak and pupate in the soil or whatever is available. Adults emerge in largest numbers early in the morning during warm weather and emerge more sporadically during cool weather. Lack of fruit for three to four months reduces the population to a minimum.

Activity of Propolis: The study in Table (1) revealed that increasing concentrations of propolis extract led to a significant increase in the mortality of *C. capitata* larvae. At a concentration of 7%, the mean mortality rate was 21.15%, while at a concentration of 17%, it reached 43.82%. These findings suggest a strong correlation between the concentration of propolis and its insecticidal efficacy against larvae. The data indicates that EEP has potential activity to be a promising natural insecticide for controlling *C. capitata* population. . The observed dose-dependent mortality rates suggest that propolis could be a valuable tool in integrated pest management strategies, offering a more environmentally friendly alternative to synthetic pesticides. The control group exhibited a significantly lower mortality rate compared to all treatment groups, confirming the insecticidal effect of propolis. Our results agree with that of Sanad and Mohanny [25] stated that; Egyptian propolis 4% gave only 40%. A natural product propolis, having different components with various modes of action is unlikely or very slow. Propolis extract at all concentration was found to accelerated larva and/or pupa development. The abnormally higher rate of development may lead to malformed and immature individuals**.** All treated larvae were found to stopped eating, slow moving and symptoms of malformed larval and pupa were appeared.

Our results disagreed with the study of Garedew *et al.* [26-30] concluded that, EEP 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 [31] 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. Garedew *et al.* [29], Assegid *et al.* [32], Pastagiaand Patel [33] and Asmaa *et al.* [34] stated that 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. The high rate of abnormal development may lead to malformed and immature individuals. Asmaa *et al.* [34] and Zewdu and Gemechis [35] reported that, propolis extract at higher concentrations caused significantly higher mortality to wax moth larvae than the lower concentrations. The larvacidal action of propolis increases with the concentrations. However, the larvae of

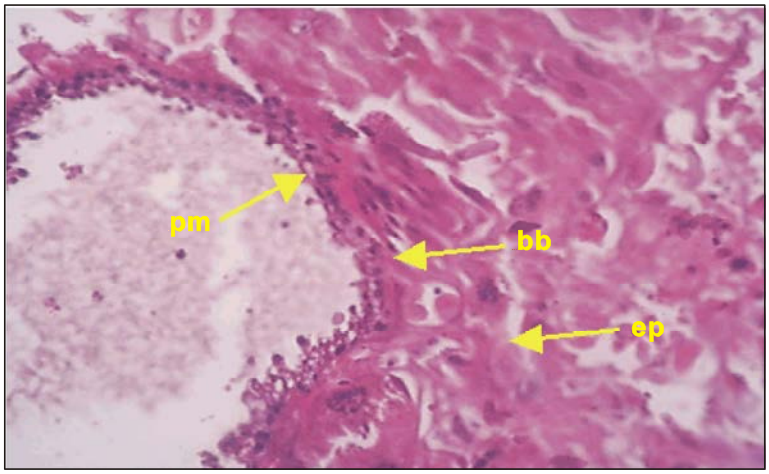

Fig. 4a: Photomicrograph of T. S. in the midgut of normal larvae of *Ceratitis capitata* (200X).

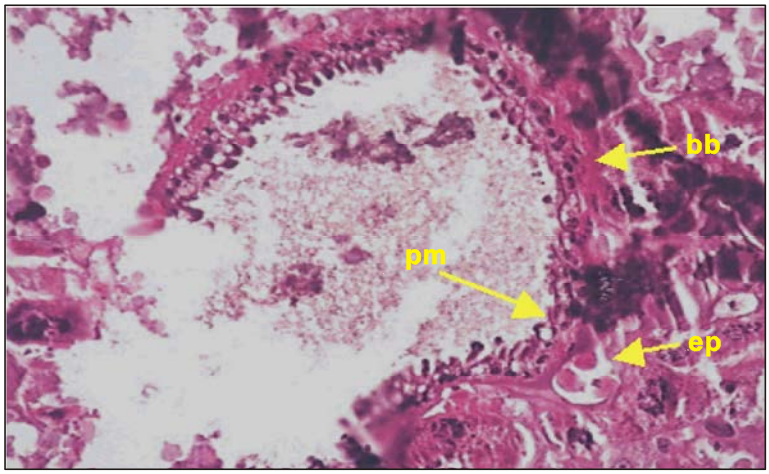

Fig. 4b: Photomicrograph of T. S. in the midgut of *Ceratitis capitata* larvae treated with propolis (200X).

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 high concentration accelerated larva and/or pupa development. From different concentration of propolis, 8 and 10 % w/v were the most toxic causing 90% and 80% mortality. These results indicate higher concentrations were more toxic. These results were in agreement with Amal *et al.* [36] who proved that propolis extracts suitable for developing a biological process and can be used successfully in integrated pest management (IPM) program to control larvae of tomato leafminer *Liriomyza sativae* Blanchard (Diptera: Agromyzidae). Ethanol extracts of propolis also showed toxic activity to Pink bollworm *Pectinophora gossypiella* larvae; cotton leaf worm *Spodoptera littoralis* and cowpea aphid *Aphis craccivora* [37] and Tomato *leafminer Liriomyza sativae* [36-39]. These results can state that propolis has general biological activities as insecticides activity.

Histological Changes in Treated Larvae: Histological examination of the midgut in Fig. 4a (revealed the normal structure of *Ceratitis capitata*. In contrast, Fig. 4b (depicted the midgut of *C. capitata* larvae killed by propolis. Microscopic analysis of the treated insects' midgut sections revealed various pathological changes, including necrosis in some epithelial cells (ep), loss of the brush border (bb) in others, and damage to the peritrophic membrane.

CONCLUSION AND RECOMMENDATIONS

Propolis extract had effective against Mediterranean fruit fly Med-fly *C. capitata* (Wiedemann). The use of

propolis as a Biocontrol Agent may help us to minimize the environmental pollutions as result of synthetic insecticide applications. It also helps to reduce the constantly increasing problem of insecticide resistance development. Ethanol extract of propolis can be used as an alternative to chemical insecticides against *C. capitata*, if applied during the development of larvae . This approach is considered as environmentally friendly approach in contrast to chemical and biological techniques.

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