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# TOXICITY OF HONEYBEE PROPOLIS AGAINST *Pectinophora gossypiella* (SAUND.), *Spodoptera littoralis* (BOISD.) AND *Aphis craccivora* (KOCH)

**Amer, Reda, A. M. and E. A. Nafea**
**Plant Protection Research Inst., Agric. Res. Center, Dokki. Giza. Egypt**

## ABSTRACT

Propolis is a natural resin produced by honeybees colonies in two kinds (Egyptian and Chinese propolis) were tested against some injurious pests i.e. eggs, newly hatched and 4th instars larvae of the pink bollworm, *Pectinophora. gossypiella* (Saund.), 4th instars larvae of the cotton leafworm, *Spodoptera. littoralis* (Boisd.) and the cowpea aphid, *Aphis craccivora* (Koch) adults and nymphs. The results showed that; the newly hatched larvae is considered the most susceptible stage of the pink bollworm, followed by 1, 2, 3 and 4-day old eggs especially 1-2 day old eggs. While, the fourth instar larvae were the least susceptible than the other tested stages of *P. gossypiella* to tested propolis preparations. Fourth instar larvae of the cotton leafworm, *S. littoralis* were treated by propolis and the mortality rates were recorded at 1, 2, 3, 5 and 7-day after treatments used by the tested preparations of propolis. The propolis exhibited toxicity effect on the 4th instar larvae of *S. littoralis* especially at 5-7 days after treatment. Adults and nymphs of the cowpea aphid, *A. crassivora* were affected and should high susceptibility to the toxicity of the propolis treatments.

Collectively, Egyptian honeybee propolis was more effective than Chinese one in all the treatments against the tested pests aforementioned.

## INTRODUCTION

In the recent years many efforts were done to reduce the environmental pollution resulting from the application of pesticides. Propolis is a natural resin produced by bees, to build their hives. It is made from the buds of conifer and poplar trees, beeswax, and other bee secretions. Propolis is commonly found in chewing gum, cosmetics, creams, lozenges and skin creams. It is frequently used in foods and beverages with the claim that it can maintain or improve health (Kooa *et al*., 2000). The Assyrians used propolis to heal wounds and tumors, while the Egyptians used it for mummification (Kartal *et al*., 2003). The chemical compounds of propolis are polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols, and ketones), terpenoids, steroids, amino acids, and inorganic molecules. Flavonoids are the main components that exert various biological activities and have been reported to inhibit the development of carcinogen. The anticancer activities of flavonoids may due to their apoptotic effect. Antibacterial, antifungal, antiviral, local anesthetic, anti-inflammatory, antioxidant, hepatoprotective, immunostimulating and cytostatic activities have been also described for propolis (Feyzan *et al.,* 2010). The medical applications of propolis led to an increase interest to its chemical composition. The propolis could be applied safely to the cultivated plant to

control phytopathogenic fungi (Peoplinijak *et al.,* 1982; Abdulsalam 1995 and El-Kafrawy, 2008).

At the present work, the propolis tested against some important injury pests i.e. the pink bollworm, cotton leafworm and cowpea aphid. The pink bollworm, *Pectinophora gossypiella* (Saunders) (Lepidoptera : Gelechiidae) that considered being one of the most injurious pests attacking cotton bolls in Egypt. The newly hatching larvae can penetrate flowers or bolls within 20-30 min. or within 2h. (Ingram, 1994). Infestations by the pink bollworm can cause severe losses in qualitively and quantitively of cotton yield. One of the commonest methods used to control the pink bollworm is by applications of insecticides; however, these applications cause destroying predators and parasites represented in the fields, can lead to outbreaks of secondary pests, as well as the development of resistance to the insecticides and in some instance, the insecticides applications may present a direct hazard to nearby humans and wildlife.

Cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera : Noctuidae) one of injurious pest can controlled by the tested propolis. The cotton leafworm is one of the major cotton pest in Egypt. The control programs of this pest in Egypt mostly depend on the use of various conventional insecticides. The overuse of insecticides eventually created many of the problems we have today as resistance and environmental pollution. In order to avoid the insecticidal hazards, there is a great need to develop alternative safe control agents with new modes of action. Among these agents is propolis usage for suppressing or controlling *S. littoralis* population was among the outstanding recent contribution to economic entomology.

Cowpea aphid was tested in the present work by the propolis. The cowpea aphid, *Aphis craccivora* (Koch) (Homoptera : Aphididae) is a key pest of many crops. The nymphs and adults feed gregariously on the leaves, tender shoots, inflorescence and tender pods, thus; causing malformations, stunting and even drying up of the parts. *A. craccivora* is also reported to be a vector of mosaic virus (Nayer *et al.,* 1976). The host plants range associated to cowpea aphid infestation is limited to Leguminosae, and this species has a cosmopolitan distribution. The majority of herbivores insect species are very selective feeders that choose their host plant base on visual, mechanical, and chemical stimuli (Bernays, 1998).

So, the aim of the present work is to determine the active ingredient (phenolic compounds) in two kinds of PEE (propolis ethanol extract). In addition to test the efficiency of Egyptian and Chinese propolis against some pests included eggs, newly hatched and $4^{th}$ instar larvae of the pink bollworm, *P. gossypiella,* $4^{th}$ instar larvae of the cotton leafworm, *S. littoralis* and the cowpea aphid, *A. craccivora* adults and nymphs.

## MATERIALS AND METHODS

**A- Honeybee Propolis preparation:**

Two propolis samples were used; the first sample was Egyptian propolis (E.) which collected by glass traps technique (Mohany, 2005) from honey bee colonies located in the apiary of Beekeeping Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza Governorate, Egypt. The second sample was Chinese propolis (C.) which imported from China and purchased commercially in Egyptian market.

**I- Preparation of propolis water extract (PWE) solution.**

(BWE) was prepared according the method of Ildeniz *et al.,* (2004) as follows:

Finelly ground propolis was extracted by maceration at room temperature, with occasional shaking in the proportion of 10 gm of (C and E) propolis to 100 ml of solvent (ethanol 80% v/v). Extracts were obtained after 7 days of maceration and filtered. The extracts obtained by ethanolic solution and incubated at room temperature until ethanol evaporated and the product obtained a honey-like consistence are referred to as PWE (Propolis water Extract).

**II- Identification of phenolic compounds in PEE by HPLC instrument.**

Identification of individual phenolic compounds of the two kind propolis ethanol extract (PEE) was performed on a HPLC instrument; 1 g sample was soaked in 20ml of ethanol (80%v/v) and filtered through 0.45µm filter membrane prior to HPLC analysis.

High Performance Liquid Chromatography Analytical (HPLC) was run on HPLC (JASCO, Japan), equipped with a pump (model PU-980) and a UV detector (UV-970). Separation was achieved on a hypersil BDS C18 (Thermo Hypersil-keystone, Germany) reversed-phase column (RP-18, 250 x 4.6 mm) with 5µm particle size, a constant flow rate of 0.7 ml $min^{-1}$ was used with two mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65 and solvent (B) 0.5% acetic acid in 99.5% acetonitrile, the system was run with a gradient program: 100% A (0 min); 0% B (0 min);100-50% A (50 min); 0-50% B (50 min), using an UV detector set at wavelength 254 nm. Phenolic compound of each sample were identified by comparing their retention times with those of the standard mixture chromatogram. The concentration of an individual compound was calculated on the basis of peak area measurements, and then converted to 1g phenolic /100g fresh weight. All chemicals and solvents used were HPLC spectral grade and obtained from sigma (st. Louis, USA) and Merck - (Munich, Germany chemical companies), 28 Components which presented the identical UV spectrum as standard compounds.

**B- Efficacy of propolis on some pests:**

Different dilutions of crude propolis water extract (PWE) were used to evaluate its activity on some pests i.e. 1-4 day old eggs, newly hatched and

fourth instars larvae of the pink bollworm, cotton leafworm larvae and cowpea aphid adults and nymphs.

**1- Pink bollworm, *P. gossypiella*.**

A laboratory strain of the newly hatched or fourth instar larvae and eggs stage of the pink bollworm, *P. gossypiella* (Saund.) was reared at Bollworms Department, Plant Protection Research Institute, Agricultural Research Center on semi artificial diet as described by Rashad and Ammar (1985). Rearing conditions were controlled at 27±1$^{o}$C and 65-75% RH.

**I- Egg stage:**

The immersion technique was used in this study. Five replicates were used from each concentration and each replicate contained batches of 1- 4 days old eggs. A piece of paper contained deposited eggs were immersed for 1min in each tested concentrations of 0.0625, 0.125, 0.25, 0.5, 1 & 2% of the propolis, another 5 replicates were immersed in water for the check. Each treated replicate/each concentration was placed in a clean tube (3x10 cm.) after water evaporated until hatchability was occurred under the controlled conditions (27±1$^{o}$C & 65-75%R.H); the dead and alive eggs were counted.

**II- Larval stage:**

Thin film technique was used as a method of application in the present work. Each Petri-dish was treated with 1.0 ml of the tested concentrations of 0.0625, 0.125, 0.25, 0.5, 1 & 2% of the propolis. The Petri-dish used as control was treated with water only. Twenty five of newly hatched or fourth instar larvae were exposed for one hour to the propolis film in each Petri-dish. The alive larvae from each treatment were transferred to clean vials containing artificial diet and maintained at 27$^{o}$C. Then the numbers of alive and dead larvae were counted at three days after treatment. Five replicates for each concentration and the control were done.

**2- Fourth instar larvae of the cotton leafworm, *S. littoralis*:**

A laboratory strain of fourth instars larvae of the cotton leafworm, *S. littoralis* (Boisd.) was reared at Leafworm Department, Plant Protection Research Institute, Agricultural Research Center on castor oil leaves. Rearing conditions were controlled at 27±1$^{o}$C and 65-75% RH.

Dipping technique was used at the present work. Castor oil leaves dipped in the tested propolis concentrations of 0.0625, 0.125, 0.25, 0.5, 1 & 2%. The control was done by castor oil leaves dipped in water only. Five replicates/ concentration were used. The leaves were kept until water evaporated. Starving larvae were transferred to glass jars (11x22 cm). Each jar contained 25 fourth instars larvae as a replicate and maintained under 27$^{o}$C. Then the numbers of alive and dead larvae were counted at 1, 2, 3, 5 &7 days after treatment.

**3- Adults and nymphs of the cowpea aphid, *A. craccivora*:**

A laboratory strain of adults and nymphs of the cowpea aphid, *A. craccivora* (Koch) were reared at Sucking and Piercing Department, Plant Protection Research Institute, Agricultural Research Center on *Ficia faba* beans leaves. Rearing conditions were controlled at 27±1$^{o}$C and 65-75% RH.

Dipping technique was used at the present work according to Dennehye, *et al.* (1983). The slides were prepared by sticky bands and put the adult or

nymph aphids on the sticky surface. The slides were dipped in the tested propolis concentrations of 0.0625, 0.125, 0.25, 0.5, 1 & 2% for 20 second. Five replicates/ concentration were used. Each slide contained 10 aphid adults or nymphs of the cowpea aphid/replicate. The slides which used as control was dipped in water only and maintained under 27$^{o}$C. Then the numbers of alive and dead larvae were counted at 24 hour after treatment under binocular focus.

$LC_{50}$ & $LC_{90}$ values were measured by software computer probane. The efficiency of different insecticides could be measured by using Sun 's equation (1950) as follows:

$$\text{Toxicity index} = \frac{LC_{50}\ (LC_{90}) \text{ of the compound A}}{LC_{50}\ (LC_{90}) \text{ of the compound B}} \times 100$$

Where A: is the most effective compound.

B: is the other tested compound.

## RESULTS AND DISCUSSION

**A- Separation of phenolic compounds in two kind of propolis ethanol extract (PEE) by HPLC**

Phenolic compounds from PEE soluble in ethanol 80% were subjected to HPLC separation. Table (1) showed that there were 62 and 66 separated compounds in Egyptian PEE, and Chinese PEE, respectively and 25 compounds were identified by comparison with authentic samples (RT) while the remaining part was unknown. Moreover, the most interesting fact was that the E.PEE is more rich in phenolic compounds than compounds in C.PEE. Four phenolic compounds namely; Pyrogallic acid, Gallic acid, Vanillin and Eugenol did not detected in both kind of propolis.

**A- Toxicity of tested propolis against some pests:**

**1- Pink bollworm, *P. gossypiella*:**

**I- Egg stage:**

Batches of the pink bollworm, *P. gossypiella* eggs were tested in four ages 1, 2, 3 and 4- day old eggs to evaluate their susceptibility to the toxicity of both Egyptian and Chinese propolis. Data in table (2) show that Egyptian propolis was more potency against pink bollworm eggs than Chinese propolis. Concerning the $LC_{50}$ and $LC_{90}$ values; the toxicity of both Egyptian and Chinese propolis decreased with the increase of the aged eggs. The $LC_{50}$ values of Egyptian propolis against eggs at 1, 2, 3 and 4- days old were 1.114, 1.781, 2.959 and 7.006% and at $LC_{90}$ values were 28.26, 46.97, 76.43 and 104.85%, respectively. The correspondent $LC_{50}$ values of Chinese propolis were 2.843, 3.222, 5.5562 and 10.42%, and at $LC_{90}$ levels were 34.25, 56.99, 84.13 and 116.6%, respectively. Based on the toxicity index values, the toxicity of Chinese propolis ranged between 39.2 and 67.24% according to $LC_{50}$, and between 82.4 and 90.8% relative to $LC_{90}$ as toxic as the toxicity of the Egyptian propolis, respectively.

**Table (1): Composition of the phenolic compounds of E.PEE and C.PEE generated by HPLC.**

| Phenolic compounds | C.PEE | E.PEE: |
|---|---|---|
| Phenol | 0.03757 | 0.15968 |
| Pyrogallic acid | 0.00000 | 0.00000 |
| Resorcinol | 0.00111 | 0.00000 |
| Salicylic acid | 0.01572 | 0.71680 |
| para hydroxy benzoic | 0.00918 | 0.01160 |
| Protocatechuic acid | 0.02966 | 0.05460 |
| Gallic acid | 0.00000 | 0.00000 |
| Vanillin | 0.00000 | 0.00000 |
| *p*-Coumaric acid anhydride | 0.00125 | 0.00000 |
| Coumarine | 0.00588 | 0.00000 |
| Caffeic Acid | 0.00000 | 0.01077 |
| 3,5-Dimethoxybenzyl alcohol | 2.66410 | 0.00000 |
| trans-Cinnamic acid | 0.32582 | 0.03864 |
| Eugenol | 0.00000 | 0.00000 |
| ferulic acid | 0.00156 | 0.19355 |
| Quercetin | 0.00000 | 0.09811 |
| Pinocembrin | 0.00000 | 2.37000 |
| Chrysin | 0.67039 | 0.53290 |
| Galangin | 1.40139 | 1.35100 |
| 3.5 dihydroxy isoflavone | 0.05460 | 0.00000 |
| Pinostrobin | 0.00000 | 1.46600 |
| Genistein | 0.00990 | 0.08740 |
| Catechines | 0.08650 | 0.12132 |
| Acacetin | 1.38320 | 0.11000 |
| Daidzein | 0.00199 | 0.25447 |

**E.PEE: Egyptian Propolis Ethanolic Extract.**
**C.PEE: Chinese Propolis Ethanolic Extract.**

**Table (2): Toxicity of propolis against different egg ages of *P. gossypiella.***

| Tested Propolis | $LC_{50}$ (%) 95%Confidence limits | $LC_{90}$ (%) 95%Confidence limits | Toxicity index | |
|---|---|---|---|---|
| | | | $LC_{50}$ | $LC_{90}$ |
| **1-day old egg** | | | | |
| Egyptian | 1.114<br>0.846±1.635 | 28.26<br>23.344±38.86 | 100 | 100 |
| Chinese | 2.843<br>1.235±3.942 | 34.25<br>26.50±42.44 | 39.2 | 82.5 |
| **2-day old egg** | | | | |
| Egyptian | 1.781<br>1.344±2.797 | 46.97<br>35.56±59.82 | 100 | 100 |
| Chinese | 3.222<br>2.422±3.910 | 56.99<br>44.82±63.67 | 55.3 | 82.4 |
| **3-day old egg** | | | | |
| Egyptian | 2.959<br>2.252±4.333 | 76.43<br>65.58±83.15 | 100 | 100 |
| Chinese | 5.562<br>3.445±8.567 | 84.13<br>69.99±89.42 | 53.2 | 90.8 |
| **4-day old egg** | | | | |
| Egyptian | 7.006<br>3.863±12.286 | 104.85<br>92.474±114.85 | 100 | 100 |
| Chinese | 10.42<br>8.567±12.12 | 116.6<br>107.5±124.4 | 67.24 | 89.9 |

As described in the same table, the tested propolis in both Egyptian and Chinese preparations were more potenty against 1-2 day old eggs than 3-4 day old eggs, it may be explained the mode of action of the propolis on the pink bollworm eggs and mentioned its affect on ATPase enzymes in addition to their effect on central nervous system. Also, the embryo were killed just after egg hatching. In this field of study (Babu *et al.*, 1996) stated that different esterases started to accumulate in 1- day old eggs and increased gradually with 3rd and 4th days which did not sufficient affected by propolis.

**II- Newly hatched larval stage:**

As shown in table (3), the newly hatched larvae were considered the most stage to the tested propolis where $LC_{50}$ value was 0.175% for Egyptian propolis and 0.251% for Chinese propolis. The Egyptian propolis is more potent compound than the Chinese one. Based on the toxicity index at $LC_{50}$ and $LC_{90}$ values, the toxicity of Chinese propolis was 69.7 according to $LC_{50}$ and was 91.1 according to $LC_{90}$ as toxic as the toxicity of Egyptian propolis.

**III- Fourth instar larval stage:**

The 4th instar larvae of the pink bollworm were affected by propolis preparations as illustrated in table (3). The Egyptian propolis had potent effect on the fourth instar larvae where the $LC_{50}$ was 19.58% and its toxicity index was taken 100 units for both of $LC_{50}$ and $LC_{90}$ values. While, Chinese propolis had lower potency than Egyptian propolis where the $LC_{50}$ was 25.82% and its toxicity recorded 60.6 and 77.2 based $LC_{50}$ and $LC_{90}$ as toxic as the toxicity of Egyptian propolis, respectively. Etebari *et al.* (2007b) observed that protein level was continued to decrease, cholesterol was increased, alkaline phosphatase activity was decreased, acid phosphatase was increased and ATPase was increased in silkworm larvae due to pyriproxyfen used.

**Table (3): Toxicity of propolis against the newly hatched and fourth instar larvae of *P. gossypiella*.**

| Tested Propolis | $LC_{50}$ (%) 95%Confidence limits | $LC_{90}$ (%) 95%Confidence limits | Toxicity index | |
|---|---|---|---|---|
| | | | $LC_{50}$ | $LC_{90}$ |
| **Newly hatched larvae of the pink bollworm** | | | | |
| Egyptian | 0.175<br>0.1135±0.2552 | 5.916<br>2.691±8.223 | 100 | 100 |
| Chinese | 0.251<br>0.1746±0.3618 | 6.496<br>3.0692±8.242 | 69.7 | 91.1 |
| **4th instars larvae of the pink bollworm** | | | | |
| Egyptian | 19.58<br>5.723±15.92 | 90.84<br>82.145±112.32 | 100 | 100 |
| Chinese | 25.82<br>16.28±29.44 | 117.7<br>100.4±122.5 | 60.6 | 77.2 |

**2- Cotton leafworm, *S. littoralis* larvae:**

Table (4) show that fourth instar larvae of cotton leafworm were less susceptible to propolis concentrations than the other tested pests. The $LC_{50}$ were 22.99 and 27.82% when the 4th instars larvae treated by Egyptian and Chinese propolis, respectively at 1- day post treatment. Concerning the toxicity index, the Egyptian propolis was given 100 units for both $LC_{50}$ and $LC_{90}$ values. While, the Chinese propolis had potency effect close the Egyptian compound, the toxicity index values of Chinese propolis were 82.6 and 86.9 according to $LC_{50}$ and $LC_{90}$; respectively (table 4).

At 2- day after treatment, the $LC_{50}$ values were 18.14 and 23.64% in case of treating the 4th instars larvae of *S. littoralis* with Egyptian and Chinese propolis, respectively. Whereas, the $LC_{90}$ values were 110.72 and 140.4%, respectively. On the other hand, the Egyptian propolis had toxicity index = 100 units according to $LC_{50}$ and $LC_{90}$, the toxicity at recorded Chinese propolis 76.7 and 78.9% as toxic as the toxicity of Egyptian propolis, respectively.

The med-lethal dose decreased to reach 6.447% when the fourth instar larvae of cotton leafworm were treated by Egyptian propolis at three day old after treatment, while; the correspondent $LC_{50}$ value of Chinese propolis was 10.22%. Based on the toxicity index, the Egyptian propolis was given 100 units according to the $LC_{50}$ and $LC_{90}$ levels, while; Chinese propolis toxicity of 63.1 and 86.1 at $LC_{50}$ and $LC_{90}$, respectively as toxic as the toxicity of Egyptian propolis.

At five day post-treatment, the larvae became more susceptible to the propolis than the tested times aforementioned. The $LC_{50}$ values were 2.588 and 5.869% in Egyptian and Chinese propolis treatments; respectively, but the Egyptian propolis was still the most potent than the Chinese one. The Egyptian propolis had toxicity index =100 according to $LC_{50}$ and $LC_{90}$, while; toxicity values of Chinese were 44.1 and 81.90 as toxic as the toxicity of Egyptian propolis at $LC_{50}$ and $LC_{90}$, respectively.

The susceptibility of the fourth instar larvae of cotton leafworm were increased at the seventh day after larva treatment by the tested propolis. The $LC_{50}$ levels were 0.535 and 0.948% in case at the 7th day post-treatment of treating the 4th instars larvae with Egyptian and Chinese propolis, respectively. Also, the $LC_{90}$ values were lower on the cotton leafworm compared with other periods, $LC_{90}$ values were 17.25 and 22.22% when the larvae treated by Egyptian and Chinese propolis, respectively as shown in table (4). Renuga and Sahayaroj (2009) found significantly reduction in total protein after 24, 48, and 72 h treated the third and fourth instars larvae of *Spodoptera litura* Fabr by spinosad. Also, Nathan *et al.* (2005) found a decrease activity of alkaline & acid phosphatase and ATPase when *S. litura* larvae were fed on a diet of castor leaves treated with azadiractin and nucleopolyhedrosis virus.

**Table (4): Toxicity of propolis against 4th instars larvae of *S. littoralis*.**

| Tested Propolis | $LC_{50}$ (%) 95%Confidence limits | $LC_{90}$ (%) 95%Confidence limits | Toxicity index | |
|---|---|---|---|---|
| | | | $LC_{50}$ | $LC_{90}$ |
| **1-day after treatment** | | | | |
| Egyptian | 22.99<br>15.77±35.59 | 75.59<br>67.70±83.59 | 100 | 100 |
| Chinese | 27.82<br>21.24±38.45 | 86.98<br>78.67±99.87 | 82.6 | 86.9 |
| **2-day after treatment** | | | | |
| Egyptian | 18.14<br>15.23±22.43 | 110.72<br>92.34±130.5 | 100 | 100 |
| Chinese | 23.64<br>18.87±28.65 | 140.4<br>128.7±155.6 | 76.7 | 78.9 |
| **3-day after treatment** | | | | |
| Egyptian | 6.447<br>5.017±8.699 | 99.39<br>89.48±105.1 | 100 | 100 |
| Chinese | 10.22<br>6.642±15.44 | 115.4<br>95.99±128.3 | 63.1 | 86.1 |
| **5-day after treatment** | | | | |
| Egyptian | 2.588<br>1.425±3.858 | 57.26<br>48.12±61.98 | 100 | 100 |
| Chinese | 5.869<br>3.467±8.486 | 69.93<br>58.22±81.18 | 44.1 | 81.9 |
| **7-day after treatment** | | | | |
| Egyptian | 0.535<br>0.352±0.836 | 17.25<br>15.87±20.81 | 100 | 100 |
| Chinese | 0.948<br>0.624±1.423 | 22.22<br>17.44±27.95 | 56.4 | 77.6 |

**Table (5): Toxicity of propolis against adults and nymphs of the cowpea aphid, *A. crassivora.***

| Tested Propolis | $LC_{50}$ (%) 95%Confidence limits | $LC_{90}$ (%) 95%Confidence limits | Toxicity index | |
|---|---|---|---|---|
| | | | $LC_{50}$ | $LC_{90}$ |
| Egyptian | 0.282<br>0.1858±0.4346 | 5.987<br>2.828±8.489 | 100 | 100 |
| Chinese | 0.792<br>0.529±1.387 | 16.093<br>10.50±28.68 | 35.6 | 37.2 |

**C- Cowpea aphid, *A. crassivora*:**

Adults and nymphs stages of the cowpea aphid, *A. crassivora* treated by both tested propolis, Egyptian and Chinese as illustrated in Table (5). $LC_{50}$ and $LC_{90}$ values were 0.282 and 5.987% in case of adults and nymphs of the cowpea aphid treated by Egyptian propolis. The correspondent values of Chinese propolis were 0.792 and 16.09%, respectively. Toxicity index of Egyptian propolis was given 100 units according to both $LC_{50}$ and $LC_{90}$. Also, the toxicity index values of Chinese propolis were 35.6 and 37.2% compared with the Egyptian one.

Generally, the natural compound of propolis in both kinds; Egyptian and Chinese can be used to control the most important injury pests i.e eggs, newly hatched and 4th instar larvae as harmful stages of the pink bollworm, *P.*

*gossypiella*. Also, fourth instar larvae of the cotton leafworm, *S. littoralis* and adults and nymphs of the cowpea aphid, *A. crassivora.*

It could be mentioned that efficacy of propolis is close to the toxicity of the biocides compounds of Protecto (*Bacillus thuringiensis*) and Biover (*Beauvaria bassiana*) against the newly hatched larvae of *P. gossypiella* (Saund.) as reported by Amer (2006), Amer & El-Nemaky (2008) and (Prasad and syed, 2010). Also, Malarvannan, *et al.* (2010) found the same result nearly against the cotton leafworm, *Spodoptera litura* (Fabricius) and Saranya*, et al.* (2010) against the cowpea aphid, *A. crassivora* (Koch).

In addition, toxicity of propolis interesting near from mineral and plant oils that have efficient results against eggs and newly hatched larvae of the Pink bollworm in laboratory and field experiments (Hewady *et al.*, 1993) and Rofail *et al.* (2000) against cotton leafworm (Badr *et al.,* 1995). Khan Khattak and Ur-Rashid (2006) reported that plant neem oil at 1.5 and 2% reduced the population of spotted bollworms and American bollworms up to 168 hours after spray. Neem oil at 2% remained effective up to 336 hours after spray as significantly lower number of bollworms larvae settled on bolls. In addition, Ratnadass, *et al.* (2009) showed that physic nut oil (*Jatropha curcas*) had insecticidal activity near from propolis against *H. armigera* and plant bugs.

It could be mentioned that propolis can be used as a factor in the Integrated Pest Management Programme for controlling of different economic pests (Peoplinijak, *et al.,*1982; Abdulsalam*,* 1995 and El-Kafrawy, 2008) without plant, human and animals harm.

## REFERENCES

Abdulsalam, K.S. (1995): Bioactivity of propolis extracts against certain soil borne fungi. Alex. J. Agric. Res., 40 (3): 305-313.

Amer, R.A. (2006 a): Combination of gamma irradiation with *Bacillus thuringiensis* (Kurs.) and the synergistic effect of two bioinsecticid mixture for controlling the pink bollworm, *Pectinophora gossypiella* (Saund.) in cotton bolls. J.Egypt.Ger.Soc.Zool., 51: 1-13.

Amer, R., A. and El -Nemaky, I .H. (2008): Effect of Some Biocides on the Biological and Prediction Parameters of the Pink Bollworm, *Pectinophora gossypiella* (Saund.) (Order:Lepidoptera-Family:Gelechiidae). *Egyptian Journal of Biological Pest Control,* 18(1), 2008, 61-70. Proceeding of 2nd Arab Conference of Applied Biological Pest Control, Cairo, Egypt, 7-10 April 2008.

Babu, R.; Murugan, K. and Vanithakumari, G. (1996): Interference of azadirachtin on the food utilization efficiency and midgut enzymatic profiles of *Helicoverpa armigera*. Indian J Environ Toxicol, 6: 81-84.

Badr, N.; El-Sisi, A.G. and Abdel- Meguid, N. (1995): Evaluation of some locally for mulated petroleum oils for controlling cotton leafworm, *Spodoptera littoralis* (Boisd.). J. Agric. Sci. Mansoura Univ., 20 (5): 2557-2563.

Bernays, E.A. (1998): The value of being a resource specialist: Behavioral support for a neural hypothesis. American Naturalist, 151:451-464.

Dennehye, J.J.; Grannett, J. and Leigh, T.F. (1983): Relevance of slide-dip and residual bioassay comparisons to detection of resistance in spider mites. J. Econ. Entomol., 76: 1225-1230.

El-Kafrawy, A.A. (2008): Effect of propolis on damping off disease of cucumber in protected cultivation. Egypt. J. Agric. Res., 86(1): 15-25.

Etebari, K.; Bizahamnia, A.R.; Sorati, R. and Matindoost, L. (2007b): Biochemical changes in haemolymph of silkworm larvae due to pyriproxyfen residue. Biochem Physiol., 88(1): 14-19.

Feyzan, O.K.; Vatanasever, H.S.; Sorkan, K.; Gurhan, S.I.; Turkoz, E.; Gencay, O. and Salih, E. (2010): Inhibitory Effects of Propolis on Human Osteogenic Sarcoma Cell Proliferation Mediated by Caspase Patway. Kafkas Univ. Vet. Fak. Derg., 16(3):397-404.

Hewady, M.; El-Sisi, A.G. and Omar, A. (1993): Pesticidal efficiency of local petroleum oil fractions against two developmental stages of the bollworms, *Pectinophora gossypiella* and *Earias insulana*. Egypt. J. Appl. Sci., 8(7) 494-502.

Ildenize, B.S.; Cunha, A.; Alexandra, C.H.; Sawaya Fabio, M.; Mario, T.; Shimizua Maria, C. Marcucci, C.; Flavia, T.; Drezza, A.; Giovanna, S.; Poviaa Patriciade, O. and Carvalhoa, A. (2004): Factors that influence the yield and composition of Brazilian propolis extracts. J. Braz. Chem. Soc., 15 (6): 964-970.

Ingram, W.R. (1994): *Pectinophora* (Lepidoptera: Gelechiidae). In "Insect pests of cotton". PP. 107-149. Edited by G.A. Matlews and J.P. Tunstall, Wallingford CAB International.

Kartal, M.; Yld, Z.S.; Kaya, S.; Kurucu, S. and Topcu, G. (2003): Antimicrobial activity of propolis samples from two different regions of Anatolia. J Ethnopharmacol., 86: 69-73.

Khan Khattak, M. and Rashid, M. (2006): Evaluation of neem (*Azadirachta indica* Juss) oil, neem seed water extracts and baythroid TM against bollworms and egg parasitoid, *Trichogramma chilonis*. Pak. Entomol. 28 (1): 5-10.

Kooa, H.; Gomesa B.P. and Rosalen, P.L. (2000): In vitro antimicrobial activity of propolis and *Arnica Montana* against oral pathogens. Arch Oral Biol., 45: 141-148.

Malarvannan, S.M.; Murali, P.D.; Shanthakumar, S.P.; Prabavatly, V.R. and Sudha, N.A. (2010): Laboratory evaluation of the entomopathogenic fungi, *Beauvaria bassiana* against the tobacco caterpillar, *Spodoptera litturalis* (Noctuidae: Lepidoptera). Journal of Biopesticides, 3: 126-131.

Mohany, K.M. (2005): Investigations on propolis and bee venom produced by two hybrids of honeybee with reference to a new device for bee venom collection. Ph.D, Faculty of Agriculture, Cairo Univ.

Nathan, S.S.; Kalaivani, K. and Chung, P.G. (2005): The effects of azadirachtin and nucleopolyhedrovirus on midgut enzymatic profile of *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Pestic Biochem Physiol., 83(1): 46-57.

Nayar, K.K.; Ananthakrishnan, T. N. and David, B. V. (1976): General and Applied Entomology. Tata Mc Graw- Hill Publishing Company Ltd, New Delhi. PP: 199.

Peoplinijak, S.D.; Maysinger, D. and Jalsenjak, I. (1982): Effect of propolis extracts on some fungi. Scientia Pharmaceutic., 50 (2): 165.

Prasad, A. and Syed, N. (2010): Evaluating prospects of fungal biopesticide, *Beauvaria bassiana* (Balsamo) against *Helicoverba armigera* (Hubner): An Eco safe strategy for pesticidal pollution. Asian. J.EXP. Biol.Sci., 1(3): 596-601.

Rashad, M.A. and E.D. Ammar (1985): Mass rearing of the the spiny bollworm, *Earias insulana* (Boisd.) on semi artificial diet. Bull.Soc.Ent.Egypt, 65: 239-244.

Ratnadass, A.; Togola, M.; Cisse, B. and Vassal, J. (2009): Potential of sorghum and physic nut (*Jatropha curcas*) for management of plant bugs (Hemiptera: Miridae) and cotton bollworm (*Helicoverpa armigera*) on cotton in an assisted trap-cropping strategy. SAT Journal, (7): 1-7.

Renuga, F.B. and Sahayaroj, K. (2009): Influence of botanicals in total head protein *Spodoptera litura* (Fab.). J Biopestic., 2(1): 52-55.

Rofail, M.; Nada, M.; El-Sisi, A.G. and Rashad, A. (2000): Time of spraying some natural oils as a limiting factor for controlling cotton bollworm, *Pectinophora gossypiella* (Saunders). Egypt. J. Agric. Res., 78 (4): 1499-1507.

Saranya, S.; Ushakumari, R.; Sosamma, J. and Babum, P. (2010): Efficacy of different entomopathogenic fungi against cow pea aphid, *Aphis crassivora* (Koch). Journal of Biopesticides, 3: 138-142.

Sun, Y. P. (1950): Toxicity index on improved method of comparing the relative toxicity of insecticides. J. Econ. Entomol., 43: 45-53.

## سمية مركب بروبوليس نحل العسل على دودة اللوز القرنفلية ***Pectinophora gossypiella*** **(Saund.) ودودة ورق القطن *Spodoptera littoralis* (Boisd.) ومن اللوبيا *Aphis craccivora* (Koch)**

**رضا عبد الجليل محمد عامر و عماد الدين أحمد عبد الحميد نافع**

**معهد بحوث وقاية النباتات – مركز البحوث الزراعية - دقى - جيزة – ج.م.ع**

تم تحضير البروبوليس كأحد المنتجات الطبيعية للنحل فى صورة مستخلص مائى لنوعين من البروبوليس (البروبوليس المصرى و البروبوليس الصينى) وتم إختبارهم ضد بعض الآفات الضارة ومنها بيض ويرقات الفقس الحديث ويرقات العمر الرابع لدودة اللوز القرنفلية *Pectinophora gossypiella* (Saund.) وكذلك يرقات العمر الرابع لدودة ورق القطن *Spodoptera littoralis* (Boisduval.) بالإضافة للأطوار البالغة وحوريات من اللوبيا *craccivora* (Koch.) *Aphis*.

تعتبر يرقات الفقس الحديث لدودة اللوز القرنفلية *P. gossypiella* أكثر الأطوار حساسية للبروبوليس يليها فى ذلك بيض عمر يوم - يومين – ثلاثة – أربعة أيام وخاصة بيض عمر 1 – 2 يوم يعتبر أكثر أعمار البيض حساسية لسمية البروبوليس. بينما يرقات العمر الرابع كانت أقل أطوار دودة اللوز القرنفلية حساسية لسمية البروبوليس.

تم معاملة يرقات العمر الرابع لدودة ورق القطن *S. littoralis* بالبروبوليس وأخذت النتائج بعد مرور يوم – يومان – ثلاثة – خمسة – سبعة أيام على التوالى بعد المعاملة. أشارت النتائج الى أن أعلى سمية للبروبوليس كانت بعد 5 و 7 أيام من تاريخ المعاملة.

أظهرت النتائج المتحصل عليها أن الأطوار البالغة وحوريات من اللوبيا *A. crassivora* كانت الأكثرحساسية لسمية البروبوليس مقارنة بأطوار البيض والعمر اليرقى الرابع لدودة اللوز القرنفلية والعمر اليرقى الرابع لدودة ورق القطن.

عموما يعتبر البروبوليس المصرى أكثر سمية مقارنة بالبروبوليس الصينى ضد الآفات المختبرة السابق ذكرها.

مما سبق يمكن إستخدام مركب البروبوليس كأحد عناصر الإدارة المتكاملة للآفات الرئيسية التى تصيب المحاصيل الإقتصادية نظرا لأنه من المركبات الطبيعية والآمنة على النظام البيئى.

**قام بتحكيم البحث**

**أ.د / على على عبد الهادى** — **كلية الزراعة – جامعة المنصورة**

**أ.د / عبد العزيز ابو العلا خضر** — **مركز البحوث الزراعية**