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Influence of Some Honey Bee Products and Plant Oils on the Greater Wax Moth, Galleria mellonella L. (Lepidoptera: Pyralidae)

Mahmoud M.A.* and Y.A. Abdel-Rahman

Plant Protection Department, Faculty of Agriculture, Al-Azhar University, Assiut, Egypt E. mail: MahmoudAli.49@azhar.edu.eg

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ABSTRACT

The current study revealed the effect of some honey bee products viz., pollen, honey, propolis and bee wax and some plant oils on the greater wax moth, Galleria mellonella L. (Lepidoptera: Pyralidae). Results show that the females produced from the larvae which had fed on bee wax mixed with propolis gave the highest mean of egg, larval, pre-pupal, pupal and development periods which were 7.4 ± 0.40 , $34.2 \pm$ $0.66, 6.8 \pm 0.86, 13.2 \pm 0.66$ and 61.1 ± 1.21 days, respectively. The longest mean of pre-oviposition was 3.2 ± 0.37 days, and the shortest means of oviposition and post-oviposition period was 4.4 ± 0.51 and 1.0 \pm 0.0 days, respectively. The shortest means of male and female longevity were 9.8 ± 0.49 and 8.4 ± 0.75 days, respectively. While the lowest means of the weight of each larva and pupa were 105.2 ± 5.47 and 71.0 ± 2.43 mg, respectively. Also, results show the effect of clove, garlic and rosemary oils on larval mortality of G. mellonella. The highest mean percentage of larval mortality was for clove oil (68.33 ± 3.33%), followed by garlic oil, 51.66 ± 1.66 . The lowest mean percentage of larval mortality was for rosemary oil (38.66 \pm 4.41%) at a concentration of 3% after 7 days of treatment, respectively.

INTRODUCTION

The greater wax moth Galleria mellonella L. (Lepidoptera: Pyralidae) is a notorious pest of honey bee colonies, and the larvae cause no direct damage to bees at any stage of their life (Hanumanthaswamy, 2000). The presence of financial losses every year from G. mellonella because of significant economic losses in the beekeeping industry by feeding on honeycombs (Charriere and Imdorf, 1999). Adults hide in dark places during the day and fly at night (Gillard, 2009). It blooms in warm areas and poor ventilation that honey bees do not protect (Williams, 1997; Ellis et al., 2013). The warm temperature promotes the rapid development of this month, so it is found in temperate, tropical and sub-tropical beekeeping areas (Chandel et al., 2003; Mohamed et al., 2014). Adults do not feed because they have atrophy in the mouth, but the damage happened during the larval stage due to the voracious feeding of the larvae which leads to the destruction of the honeycomb (Awasthi and Sharma, 2013; Ellis et al., 2013; Kwadha et al., 2017). Larvae cause great damage to beeswax combs that the bees have left unattended; resulting heavy economic losses for beekeepers (Van Engelsdorp et al.,

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2010). Also, the larvae feed on pollen, honey, and other protein substances (Nurullahoglu and Susurluk, 2001). Adults and larvae can also transmit serious bee pathogens, for example foul brood (Owayss and Abd-Elgayed, 2007). This pest has been biologically studied and can complement its development on various types of food (Coskun et al., 2006; Abou-Shaara, 2017). The effects of diet and nutrient quality on egg incubation period and development of G. mellonella were also studied (Ozer, 1962; Abdel-Naby et al., 1983; Mohamed 1983; Gulati and Kaushik 2004). The use of chemical agents to prevent or control insect infestation was the main method of protection against the wax moth, as it is the simplest and most effective way to deal with pests of stored products (Hidalgo et al., 1998). However, insecticides have serious defects such as the return and resistance of pests, deadly effects on non-target organisms, the risk of user contamination, food residue, and environmental pollution (Tapondjou et al., 2002). Hence, there is an urgent need to develop safe alternatives to control this insect. Great efforts by many researchers have focused on plant-derived materials, which may be useful as commercial insecticides (Sreekanth, 2013; Sharma et al., 2014). One alternative to synthetic insecticides is natural compounds, such as essential oils. Essential oil and its components are shown to be an effective source of botanical pesticides (Tripathi et al., 2002).

The present study aimed to assess the effect of some honey bee products on the biology of *G. mellonella*. Also, the effectiveness of some vegetable oils against greater wax moth larvae, as these oils seem safe for humans and the environment, as well as easy to use and cheap.

MATERIALS AND METHODS

Insect Rearing:

The greater wax moth, *G. mellonella* used in this study originated from the larvae collected from naturally-infested wax combs, which were obtained from infested beehives in Assiut Governorate, Egypt. The collected samples were transferred into black plastic bags and were moved to the laboratory in the Department of Plant Protection, Faculty of Agriculture, Al-Azhar University, Assiut Governorate. Infested wax combs were cut into suitable pieces then were placed in clean plastic containers (vol. 2 l) covered with perforated covers and let so as a culture to supply female and male adult moths for egg production.

Effect of Some Honey Bee Products on Some Biological Aspects of G. mellonella:

Pair of emerged moths (male and female) were kept each in a plastic container (250 ml) for egg collecting. To support egg-laying, folded paper scraps were placed inside the containers and females were allowed to lay eggs. Daily observations were made on eggs for hatching and newly hatched larvae were collected. Five replicas were made for each tested diet, each comprised twenty newly hatched larvae of the same age (< 24 h old) which were picked out and were placed in clean plastic containers (250 ml) and were provided with 30 g of one of the following diets: (a) pollen (corn) collected by a honey bee 3% mixed with bee wax (w/w); (b) honey 3% mixed with bee wax (w/w); (c) propolis 3% mixed with bee wax (w/w) and (d) bee wax only. Observations were made daily, larval duration and pre-pupael duration were calculated, this action was continued until larvae reached the pupal stage, the period between egg hatching and pupation was recorded as a larval period. Ten pupae each were selected from larvae that pupated on the same day representing one replica. Observations were made daily, the period between the formation of pupae till the adult emergence was considered as a pupal stage period.

In order to show the effect of some honey bee products on the weight of larvae and pupae of *G. mellonella*. Five replicas were made for each tested diet, each comprised

ten newly hatched larvae (< 24 h old) which were placed in petri dish (9 cm) containing 15 g of each diet. At the end of each period, larvae and pupae were weighed by an electric balance.

Ten replicas for each tested diet, each comprised a pair of newly emerged adults of *G. mellonella*, were placed in plastic containers 250 ml and tightly covered with muslin cloth secured with rubber bands. Folded paper scraps were placed in the containers to support egg-laying. The paper scraps were removed once a day and were kept in plastic containers for the determined incubation period. The pre-oviposition, oviposition, and post-oviposition periods were subsequently assessed. The adult longevities were also calculated following adult death.

Effect of Some Plant Oils on Mortality of G. mellonella:

To determine the effect of Clove oil (*Syzygium aromaticum* L.), garlic oil (*Allium sativum* L.) and rosemary oil (*Rosmarinus officinalis* L.) against 4th larval instar of *G. mellonella* at different time intervals. A piece of bee wax was placed in petri dish (9 cm), then was sprayed with 1 ml of 1.5 and 3% concentration of each oil was prepared in acetone and control ones had acetone only. After that, twenty of larvae of *G. mellonella* were placed in petri dish. Five replications were made for each concentration of all the treatments. Percentage mortality was recorded after 3, 5 and 7 days after treatment, while mortalities were calculated and corrected according to Abbott's formula (1925).

% Corrected mortality =
$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100$$

% Mortality larvae =
$$\frac{\text{No. of dead larvae}}{\text{No. of introduced larvae}} \times 100$$

The experiments were arranged in a completely randomized design (CRD). All cultures and experiments were conducted in an incubator that was set to 30 ± 2 °C and 70 \pm 5 % R.H. in darkness.

Statistical Analysis:

Collected data were subjected to the Analysis of Variance (ANOVA) using the Statistical Analysis System (SAS) at a 5% level of significance. The mean differences were separated using Least Significant Difference (LSD) and showed as means \pm SE. Shapiro-Wilk's W test was done for the assumption of normality in which the test was insignificant.

RESULTS

Effect of Some Honey Bee Products on the Immature Stages of *G. mellonella*: Incubation Period:

Data in Figure (1) show that the mean incubation periods of eggs laid by adult females of G. mellonella fed on bee wax mixed with pollen or honey or propolis by 3% separately during larval stage were 4.4 ± 0.24 , 5.8 ± 0.58 and 7.4 ± 0.40 days, respectively. While the mean incubation period of eggs laid by adult females fed on bee wax only during the larval stage was 5.6 ± 0.75 days.

Larva Period:

Results showed that the mean of the longest larval duration was 34.2 ± 0.66 days for larvae that had fed on bee wax mixed with propolis. The mean larval duration decreased to 20 ± 0.54 and 23.8 ± 0.86 days for larvae that had fed on bee wax mixed with pollen or honey each separately, respectively. While the mean larval duration of larvae that had fed on bee wax only was 27.0 ± 0.63 days (Fig. 1).

Pre-pupal and Pupal Period:

Results show that the means of pre-pupal and pupal duration were 6.8 ± 0.86 and 13.2 ± 0.66 days respectively, for larvae that had fed on bee wax mixed with propolis. Whereas the means of pre-pupal duration were 2.4 ± 0.23 and 3.0 ± 0.31 days and the mean of pupal duration were 8.0 ± 0.77 and 9.6 ± 0.51 days, for larvae which had fed on bee wax mixed with pollen or honey separately, respectively. While the means of the pre-pupal and pupal duration were 4.4 ± 0.40 and 11.2 ± 0.80 days respectively, for larvae that had fed on bee wax only (Fig. 1).

Total Developmental Period:

Results show that the means of total developmental period days of G. mellonella were 35.0 ± 1.05 , 42.2 ± 1.24 and 61.1 ± 1.21 days, for larvae which had fed on bee wax mixed with pollen or honey or propolis separately, respectively. While the mean of total developmental period days for larvae that had fed on bee wax only was 48.2 ± 1.28 days (Fig. 1).

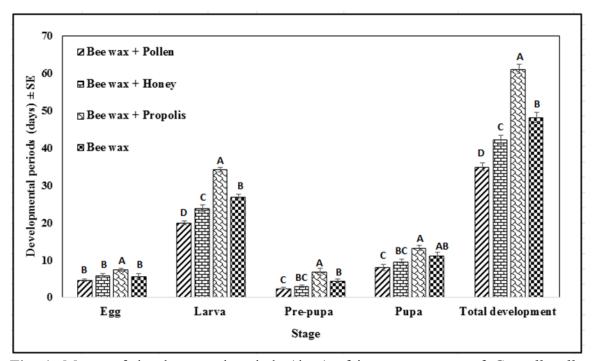


Fig. 1: Means of developmental periods (days) of immature stages of *G. mellonella*, which were fed on certain honey bee products.

Columns, in the same stage, which were headed by the same letter are not significantly different using the LSD test at P = 0.05.

Effect of Some Honey Bee Products on the Weight of Larvae and Pupae of G. mellonella:

Data in Figure (2) show that the means of the heaviest weight per each larva and pupa were 176.4 ± 3.86 and 158.6 ± 7.61 mg respectively, for larvae that had fed on bee wax mixed with pollen. While the means of weight per each larva and pupa were 157.8 ± 4.12 and 137.2 ± 1.32 mg respectively, for larvae that had fed on bee wax mixed with honey. The means of weight per each larva and pupa were 151.2 ± 2.58 and 116.8 ± 3.67 mg respectively, for larvae that had fed on bee wax only. While the means of the lightest weight per each larva and pupa were 105.2 ± 5.47 and 71.0 ± 2.43 mg respectively, for larvae that had fed on bee wax mixed with propolis.

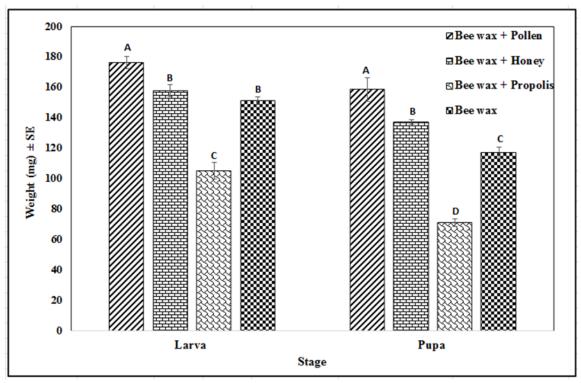


Fig. 2: Means of the weight of larva and pupa of G. mellonella, which were fed on certain honey bee products.

Columns, in the same stage, which were headed by the same letter are not significantly different using the LSD test at P = 0.05.

Effect of Some Honey Bee Products on the Adult Stage of *G. mellonella*: Oviposition Periods:

Data in Figure (3) show that the shortest mean of the pre-oviposition period was 1.4 ± 0.40 days, while the longest mean of the oviposition and post-oviposition periods was 7.0 ± 0.31 and 2.2 ± 0.49 days respectively, for females who had fed on bee wax mixed with pollen during the larval stage. The longest mean of the pre-oviposition period was 3.2 ± 0.37 days, while the shortest mean of the oviposition and post-oviposition periods were 4.4 ± 0.51 and 1.0 ± 0.0 days respectively, for larvae that had fed on bee wax mixed with propolis. The means of the pre-oviposition, oviposition, and post-oviposition periods were 2.6 ± 0.24 , 5.8 ± 0.37 and 1.6 ± 0.24 days respectively, for larvae that had fed on bee wax mixed with honey. The mean of the pre-oviposition, oviposition and post-oviposition periods were 2.8 ± 0.49 , 5.6 ± 0.40 and 1.4 ± 0.40 days respectively, for larvae that had fed on bee wax only.

Adult Longevity:

Data in Figure (4) show that the means of the longest adult longevity of males and females were 19.2 ± 0.37 and 16.4 ± 0.66 days respectively, for adults fed on bee wax mixed with pollen during the larval stage. While the means of the shortest adult longevity of males and females were 9.8 ± 0.49 and 8.4 ± 0.75 days respectively, for larvae that had fed on bee wax mixed with propolis. The means of the adult longevity of males and females were 15.4 ± 0.68 and 12.8 ± 0.58 days respectively, for larvae that had fed on bee wax mixed with honey. While the means of the adult longevity of males and females were 13.0 ± 0.44 and 11.4 ± 0.68 days respectively, for larvae that had fed on bee wax only.

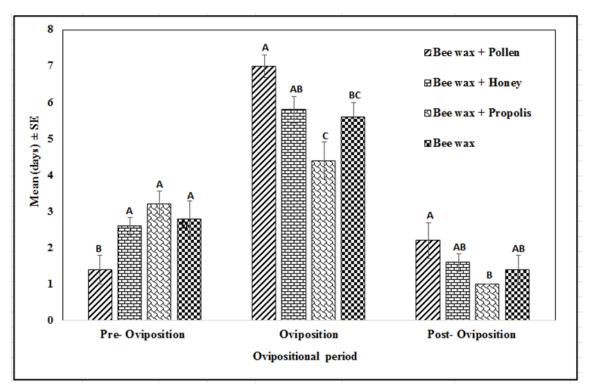


Fig. 3: Means of ovipositional periods (days) of adult females of *G. mellonella*, which were fed on certain honey bee products.

Columns, in the same ovipositional period, which were headed by the same letter are not significantly different using the LSD test at P = 0.05.

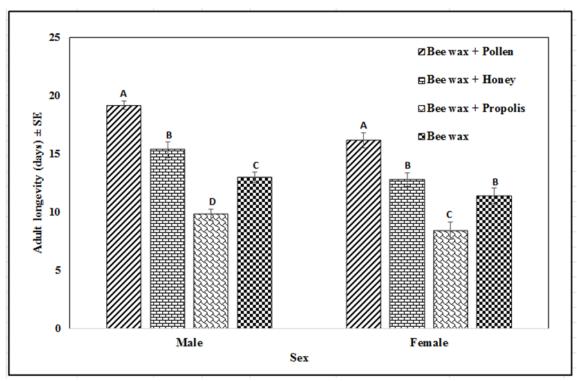


Fig. 4: Means of the longevity of the adult (days) of *G. mellonella*, which were fed on certain honey bee products.

Columns, in the same sex, which were headed by the same letter are not significantly different using the LSD test at P = 0.05.

Effect of Some Plant Oils on Larval Mortality Percentage of G. mellonella:

Data in Figure (5) show the effect of concentrations 1.5 or 3% of the clove, garlic and rosemary oils on the 4th larval instar of *G. mellonella* after 3, 5 and 7 days of treatment. Results show that the mean of percentage of larval mortality for clove oil were 26.66 ± 3.33 , 40.0 ± 2.89 and $50.0 \pm 2.89\%$; for garlic oil were 21.66 ± 4.41 , 30.0 ± 0.0 and $36.66 \pm 1.66\%$ and for rosemary oil were 13.33 ± 1.66 , 21.66 ± 1.66 and $28.33 \pm 3.33\%$ at 1.5 % concentration. Increasing the concentration to 3% increase the percentage means of larval mortality for clove oil to be 38.33 ± 3.33 , 55 ± 2.89 and $68.33 \pm 3.33\%$, for garlic oil to be 28.33 ± 1.66 , 36.66 ± 6.67 and $51.66 \pm 1.66\%$ and for rosemary oil to be 20.0 ± 2.89 , 25.0 ± 2.89 and $38.66 \pm 4.41\%$ after 3, 5 and 7 days of treatment, respectively. Generally, the percentage of larval mortality increased vertically by increasing the concentration and horizontally by the time of exposure.

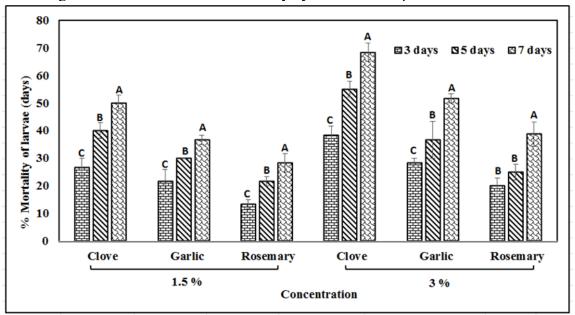


Fig. 5: Means of percentage larval mortality of *G. mellonella* on bee wax treated with different concentrations of plant oils.

Columns, in the same plant oil, which were headed by the same letter are not significantly different using the LSD test at P = 0.05.

DISCUSSION

Results show that the female produced from the larvae which had fed on bee wax mixed with pollen gave the lowest mean of eggs, larval, pre-pupal, pupal and development periods which were 4.4 ± 0.24 , 20 ± 0.54 , 2.4 ± 0.23 , 8.0 ± 0.77 and 35.0 ± 1.05 days respectively. The shortest mean of pre-oviposition was 1.4 ± 0.40 days and the longest mean of oviposition and post-oviposition period were 7.0 ± 0.31 and 2.2 ± 0.49 days respectively. The longest mean of males and females longevity was 19.2 ± 0.37 and 16.4 ± 0.66 days, respectively. Results reported in the literature are in parallel with the results of the present work. For instance, Eischen and Dietz (1990) reported that larval duration of wax moth larvae fed on a diet contained 5% pollen or combination with honey and bee wax at 32° C, 40% R.H., became shorter by 2-5 days. Liu *et al.* (1997) recorded a 6.8 days reduction in larval duration of larvae fed on Chinese honey bee combs as compared to those fed on natural feeding. Hanumanthaswamy (2008) studied the biology of *G. mellonella* on the combs of *A. cerana*. Results show that the egg, larval,

pre-pupal, and pupal periods were 8.6, 49.3, 2.1 and 8.6 days, respectively. The preoviposition, oviposition and post oviposition periods were 1.1, 4.6 and 1.2 days, respectively. The males and females lived for 16.4 and 6.9 days, respectively. Opoosun and Odebiyi (2009) mentioned that the egg, larval and pupal periods of wax moth last for 9.5, 22.7 and 25.4 days, respectively. The oviposition period was 5.8 days. The longevity of adult females and males were 10.6 and 11.1 days, respectively. Jun (2010) reported that the larval stage duration recorded 38.60 days. The shorter duration period of the greater wax moth larvae could be attributed to the existence of honey bee wax in their diets. Hanumanthaswamy et al. (2013) mentioned that at 35 °C, with a decrease in R.H. from 94.5 to 70 %, there was a corresponding increase in the duration of eggs, larval, prepupal and pupal periods, which were from 8.2 to 9.3, 33.60 to 45.95, 3.0 to 3.70 and 7.25 to 8.25 days, respectively. While the pre-oviposition and post-oviposition periods were from 1.10 to 1.30 and 1.0 to 1.1 days, respectively. Also, there was a corresponding increase in the longevity of males and females from 8.9 to 6.8 and 4.9 to 4.6 days, respectively. Mohamed et al. (2014) studied the feeding of newly hatched larvae of G. mellonella on wax combs viz., empty old wax comb aged > 3 years (OW), old wax comb with pollen (OWP), bee-collected pollen (BP), empty new wax comb aged < 2 years (NW) and new wax comb with pollen (NWP). Results show that the effect of all five natural diets on the development of G. mellonella varied significantly. They found that the diet NW to be the poorest larval diet inducing prolonged immature stage duration, prolonging the entire life-cycle duration, retarding oviposition and shortening adult longevity. In contrast, feeding larvae on diets of OW, OWP, NWP and BP positively affected the development and vitality of all life stages. Hosamani et al. (2017) studied the effect of different honey bee species combs on some biological parameters of G. mellonella. Results show that the egg, larval, pre-pupal and pupal periods range from 8.60 to 8.70, 49.30 to 58.60, 2.10 to 2.40 and 9.65 days, on different species of Apis cerana F., Apis dorsata F., Apis mellifera L., and Apis floreae F., respectively. Similarly, the male and female longevity ranges from 16.40 to 16.00 and 6.90 to 6.50 days respectively on different species combs. Pre-oviposition, oviposition and post-oviposition period ranges from 1.10 to 1.20, 4.60 to 3.90 and 1.20 to 1.40 days, respectively. El-Gohary et al. (2018) studied the effect of feeding on some biological aspects of eggs, larvae, pupae, and adults of G. mellonella. Results revealed that diet D3 (4 parts scratched bee wax and 1-part bee collected clover pollen) proved to be the best followed by natural (control) feeding. The superiority of diet D3 was represented by shortened larval duration, increased larval and pupal weight (186 and 197 mg/mature). Vijayakumar et al. (2019) mentioned that the larval and pupal periods were from 35 to 45 and 14 to 16 days and the longevity of adult males and females were 16.50 and 6.88 days on diet composed of honey, respectively. This variation might be due to a change in the quality of food consumed in their larval stage.

The results revealed in the current work that the highest mean weight per larva was 176.4 ± 3.86 and 157.8 ± 4.12 mg, also, per pupal were 158.6 ± 7.61 and 137.2 ± 1.32 mg, for those which had fed on bee wax mixed with pollen or honey, respectively. Results of the present work are in general accordance with those of Gross *et al.* (1996) who reported that the addition of 5 g of torula yeast, honeycomb wax, or wheat germ added to the diet or to varying ratios of Gerber's Mixed and Hi-Protein cereals increased the weight of mature *G. mellonella* larvae. Coskun *et al.* (2006) studied the effect of different honeycomb and sucrose amounts on the development of *G. mellonella* larvae. They found after ten days maximum weight is 0.131 g in the diet, which contains 50 g honeycombs. Van Zyl and Malan (2015) reared the wax moth larvae of *G. mellonella* on five different diets to determine which diet resulted in the highest increase in larval

weight. They found that a diet containing 88 g brewer's yeast, 24 g wax powder, 175 ml honey and 175 ml glycerol produced the heaviest larvae, with an average weight of 0.19g per larva.

The results revealed in the current work that the highest mean of the percentage of larvae mortality of G. mellonella for clove oil was $68.33 \pm 3.33\%$, followed by garlic oil, 51.66 ± 1.66 and the lowest mean percentage of larval mortality for rosemary oil was $38.66 \pm 4.41\%$ at concentration 3% after 7 days of treatment, respectively. Obtained results are in accordance with those of Hanumanthaswamy et al. (2006) who observed that the highest reduction in larval population for pongamia oil was 56.42%, followed by neem oil (62.57%) and mahua oil (63.09%), and the lowest larval mortality of G. mellonella for pongamia seed kernel extract was 9.28% and for neem seed, kernel extract was 11.78%. Abdelrahman et al. (2012) studied the efficacy of some plant volatile oils against the greater wax moth. They found that peppermint and ginger are the most toxic oils against the middle and late larval instars of G. mellonella than lemon and camphor. Abou El-Ela (2014) studied the effect of clove (Eugenia aromatic (L.) Baill.), basil (Ocimum basillicum L.), thyme (Thymus vulgaris L.), blue gum (Eucalyptus spp.), spearmint (Minth viridis L.) and Lemongrass oil (Cymbopogon citrates Hort.) against the larvae of Acheroia gresilla F., at different concentrations (0.25, 0.50, 1.0 and 2.0 ml/comb/week). Results revealed that clove and basil oils were highly effective. The larval mortality for clove and basil oils were 17.50 and 11.23%, respectively. Also, clove oil differed significantly from other oils. Elbarky et al. (2015) studied the efficacy of some essential oils viz., peppermint (Mentha piperita L.), geranium (Pelargonium graveolens L.) and basil, O. basilicum, using four concentrations of each oil (0.625, 1.25, 2.50 and 5.0%) against the fourth instar larvae of G. mellonella. They found that basil oil, O. basilicum scored the highest larval mortality (100%), followed by M. piperita (67%), then P. graveolens (50%) at a concentration of 5% after 96 hours of treatment. Bisht et al. (2017) studied the efficacy of some essential oils viz., neem, cedar, clove, piperment, and karanj oil against the 4th larval instar of G. mellonella. They found that the highest percent larval mortality was recorded for clove oil (29.5%) at a concentration of 2% after 7 days of treatment.

Conclusion

Generally, the diet consisting of propolis mixed with beeswax negatively affected some biological parameters for all life stages of *G. mellonella*, while the diet consisting of pollen mixed with beeswax affected them positively. Also, it can be concluded that the tested oils are effective for controlling *G. mellonella*. Clove oil at a concentration of 3% can provide protection for the wax combs stored in the apiary. The obtained results can provide background information for the integrated management of this pest.

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ARABIC SUMMARY

تأثير بعض منتجات نحل العسل والزيوت النباتية على دودة الشمع الكبيرة .(Lepidoptera: Pyralidae)

محمود عبد الحميد محمود ويحيى عبد الفتاح عبد الرحمن قسم وقاية النبات، كلية الزراعة، جامعة الازهر بأسيوط، مصر

کشفت الدراسة الحالیة عن تأثیر بعض منتجات نحل العسل مثل حبوب اللقاح والعسل والبروبولیس وشمع النحل وبعض الزیوت النباتیة علی دودة الشمع الکبیرة (Lepidoptera: Pyralidae) یا النحل وبعض الزیوت النباتیة علی دودة الشمع الکبیرة (الفرات النتائج أن الإناث الناتجة من الیرقات التي تغذت علی شمع النحل الممزوج بالبروبولیس أعطت أعلی متوسط لفترات البیض والیرقات وما قبل العذراء والعذرای والنمو حیث کانت $7.7 \pm 7.7 +$