

تقدير كفاءة بعض المستخلصات النباتية المطبقة على شمع نحل العسل فى مكافحة البيولوجية لفرشة الشمع الكبرى

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الملخص العربى

أجريت هذه الدراسة بمنحل و معامل قسم الحشرات الاقتصادية . كلية الزراعة جامعة المنوفية لدراسة تأثير بعض المستخلصات النباتية علي بعض الصفات البيولوجية وكذلك سميتها على دودة الشمع الكبرى. أظهر التحليل الاحصائي للنتائج وجود فروق معنوية في متوسط وزن اليرقات و العذارى و الفراشات بين المعاملات بالمستخلصات النباتية ونظيرتها فى الكنترول. وكانت النسبة المئوية لموت اليرقات ٣٣.٣ و ٣٦.٦ و ٥٠ % في المعاملات بتركيزات ٠.٢% و ٠.٣% و ٠.٤% من مستخلص نبات النيم علي التوالي مقارنة بالكنترول صفر % . وكانت النسبة المئوية لموت العذارى ١٠٠% وذلك في التركيزات الثلاثة مقارنة ب ١٠% في الكنترول . سجلت المعاملات بالتركيزات ٠.٢% و ٠.٣% و ٠.٤% من مستخلص نبات الزرييح نسب مئوية لموت اليرقات ٢٣.٣ و ٣٦.٦ و ٥٠ % علي التوالي مقارنة بالكنترول الذى لم يحدث به موت ، وكانت النسبة المئوية لموت العذارى ١٠٠ % وذلك في التركيزات الثلاثة مقارنة ب ١٦.٦% في الكنترول . سجلت المعاملات بالتركيزات ٠.٢% و ٠.٣% و ٠.٤% من مستخلص نبات الكافور نسب مئوية لموت اليرقات قدرت ب ٢٦.٣ و ٣٣.٣ و ٤٦.٦ % علي التوالي مقارنة بالكنترول الذى لم تسجل فيه اى نسب موت ، وكانت النسبة المئوية لموت العذارى ٩٣.٣ و ٩٦.٦ و ١٠٠ % وذلك في التركيزات الثلاثة السابق ذكرها ، اما فى الكنترول فقد تم تسجيل نسبة موت ١٣.٣ % من العذارى .

ASSESSING THE EFFICACY OF SOME PLANT EXTRACTS APPLIED TO HONEYBEE WAX IN THE BIOLOGICAL CONTROL OF *GALLERIA MELLONELLA* (Lepidoptera: Pyralidae)

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ABSTRACT: Experiments in this study were conducted in the apiary and laboratory of Economic Entomology Dept., Faculty of Agriculture, Minoufiya University to study the toxicological effects of three plant extracts in controlling *Galleria mellonella*. The statistical analysis of the obtained results indicated that there were significant differences in the weights of larvae and pupae as well as the emerged adults between sprayed treatments and control treatment. Results indicated that the mortality percentages of wax moth larvae were 33.3, 36.6 and 50 % for the 0.2, 0.3 and 0.4 % neem concentrations, respectively, compared with control 0.0 % . Mortality percentages of pupae were 100 % at the three neem concentrations, while it was only 10 % at control treatment. The emergency percentages of adult moths were 90 % at control treatment, while no emerged adults were registered at all neem concentrations. The mortality percentages of wax moth larvae were 23.3, 36.6 and 50 % for the 0.2, 0.3 and 0.4 % of *Chenopodium* concentrations, respectively, compared with control , which was 0.0 % . Mortality percentages of pupae were 100 % at the three *Chenopodium* concentrations, while it was only 16.6 % at control treatment. The emergency percentages of adult moths were 83.3 % at control treatment, while no emerged adults were registered at all *Chenopodium* concentrations. The mortalities of wax moth larvae were 26.3, 33.3 and 46.6 % for the 0.2, 0.3 and 0.4 % of Camphor concentrations, respectively, compared with control, which was 0.0 % . Mortality of pupae were 93.3, 96.6 and 100 % at the three of Camphor concentrations, respectively, while it was only 13.3 % at control treatment. The emergency percentages of adult moths were 86.6 % at control treatment, while it were 6.6, 3.3, and 0.0 % at 0.2, 0.3, and 0.4 % of Camphor concentrations, respectively.

Key words: Honeybee wax, plant extracts, biological control, the greater wax moth, *Galleria mellonella*.

INTRODUCTION

The greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) is the economic pests attacking the bees wax. The former pest attacks wax combs whether in bee hives or in store. It caused economic losses for beekeeping industry (Ibrahim, 1980). Within 10-15 days, *G. mellonella* larvae could completely damage the combs in bee hives leaving them as frames covered with heavy layer of silk threads and feces

(Beck, 1960, and Abou Bakr and El-Shemy, 1991). Recently, Shashidhar *et al* (2007) stated that the greater wax moth, *Galleria mellonella*, during its larval development, caused heavy damage by consuming as high as 937.5 and 974.41 mg of crude wax and comb of honey bees, respectively. Sixth instar larva consumed the highest amount of wax (345.83) and comb (295.67 mg). A single larva of *G. mellonella* per comb of *Apis cerana* and *A. mellifera* caused a

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damage of 103.67 and 91.67 cm² after 35 days of release, respectively. Three larvae per comb of *A. cerana* and 10 larvae per comb of *A. mellifera* caused a damage of 340.0 and 818.33 cm² which was equivalent to 1.13 and 1.02 frame comb area of *A. cerana* and *A. mellifera*, respectively. Ellis and Hayes (2009) stated that *Galleria mellonella* and *Achroia grisella* larval feeding can cause significant damage in active honey bee *Apis mellifera* colonies and stored equipment. This damage may lead to significant material and financial losses. Traditional control methods use toxic chemicals that may leave residues in wax and honey and are potentially hazardous to bees and humans. Evaluation of the use of a product that is not harmful to bees or humans are very necessary.

From these points of view, the aim of this article is to assess the efficacy of three plant extracts in the control of the greater wax moth, *Galleria mellonella* as a trial to avoid the bad effects of using chemical pesticides on bees, environment, and humans.

MATERIALS AND METHODS

Experiments in this study were conducted in the apiary and laboratory of Economic Entomology & Agricultural Zoology Dept., Faculty of Agriculture, Minoufiya University to study the toxicological effects of three plant extracts in controlling *G. mellonella* with special references to some biological parameters.

1- Rearing of wax moth:

Second instar larvae of the greater wax moth were collected from naturally infested honey bee hives. Wood boxes 40 x 30 x 30cm were used in the rearing process which was potted in the laboratory under 25 ± 5 °C and 70 ± 5 % Relative Humidity. Collected larvae were putted in the boxes with infested wax combs and left to feed and grow. Boxes were covered with polyethylene plastic. Wax combs were added as needed until pupation process, then after emergency of moths which laid eggs, hatched to larvae. Fourth instar larvae were used in the toxicological tests.

2- Collection and preparation of the Plant Extracts

Three plants were chosen, i.e., Neem (*Azadirachta indica*, Meliaceae), Camphor (*Cinnamomum camphora*, Lauraceae), and Chenopodium (*Chenopodium hybridum*, Chenopodiaceae). Leaves of collected plants were naturally air dried under laboratory conditions at room temperature (25-30 °C) then dried in an oven at 45°C for 48 hrs, and ground to powder with an electric blender.

3- Preparation of plant extracts

Plant leaf samples were exposed to the extraction process, where each plant sample (100 g) was extracted using acetone solvent. Dry ground leaves were putted in flasks (1/2 liter) filled with acetone solvent and left for 24 h at room temperature. The flasks were plugged and shaken in an electric shaker, and then the suspensions were filtered through Whitman no.1 filter paper. The solvents were evaporated from the filtrate by leaving it in the room temperature to obtain the crude extracts. Ten grams of each crude was dissolved in 100 ml acetone (Roman pavela *et al* 2009) to obtain a 10% stock solution (w/v), which was stored under refrigeration until needed. This stock solution was serially diluted with the solvent as required for the bioassay tests

4- Toxicity of liquid formulations of tested plants against the greater wax moth, *Galleria mellonella* under laboratory conditions:

The purpose of this experiment was to study the effect of the three plant extracts against different stages of wax moth (larva, pupa, adults) when applied as liquid formulations of acetone extraction sprayed on the pieces of wax at Petri dishes 10cm under laboratory conditions where 10 fourth instar larvae of the greater wax moth was potted in each dish which contain 20 g of small pieces of pure wax, then, 5 ml of each concentration was added to the dish above the pieces of wax and closed with other dish to prevent worms from escaping. Treatments

were replicated three times. Different biological aspects were recorded until the emergency of moths.

5-Statistical analysis

The obtained data was statistically analyzed using analysis of variance (ANOVA) at 5% probability. The measurements were separated using Duncan's Multiple Range Test (DMRT) through Costat software program (version 3.03). Mortality percentages were counted according to Abbott formula.

RESULTS AND DISCUSSION

Effect of three concentrations of liquid formulation of acetone extract of plants on the greater wax moth :

1- Neem experiments :

Data in Tables (1, 2,) show the effect of spraying wax pieces by three concentrations 0.2, 0.3, 0.4 % of liquid formulation of Neem acetone extract on some biological aspects of the greater wax moth under laboratory conditions of 25 ± 5 °C and 70 ± 5 % relative humidity .

Data presented in Tables 1 and 2 show the effect of feeding larvae of *Galleria mellonella* on sprayed wax with three concentrations of a liquid formulation of neem extract, i.e. weights of 30 larvae, 30 pupae, number emerged adults, adult duration, in addition to the mortality percentages of larvae and pupae and emerged adults.

Results in Table (1) indicated that weights of 30 larvae was 0.96, 1.12 and 1.19 g at neem concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 1.69g. The larval duration was 19.6 day for the 0.4 % concentration , 18.3 day for 0.3 % concentration and 17 day at 0.2 % concentration , compared with only 11 day at control. Weights of 30 pupa was 0.12, 0.14 and 0.15 g at neem concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 0.19 g. The pupal duration was 60 days for all tested , compared to 30 days at control. No emerged adults were registered at all neem concentrations , while there was 27 moths emerged from 30 pupae at control treatment.

The statistical analysis of the data Table (1) indicated that there were significant differences in the weights of larvae and pupae as well as the emerged adults between sprayed treatments and control treatment.

As for the mortality percentage Table (2) results indicated that the mortality percentages of wax moth larvae were 33.3, 36.6 and 50 % for the 0.2, 0.3 and 0.4 % neem concentrations, respectively, compared with control , which was 0.0 % . Mortality percentages of pupae were 100 % at the three neem concentrations, while it was only 10 % at control treatment. The emergency percentages of adult moths was 90 % at control treatment , while no emerged adults were registered at all neem concentrations.

Table (1): Effect of spraying wax pieces by three concentrations of liquid formulation of Neem acetone extract on some biological aspects of the greater wax moth under laboratory conditions of 25 ± 5 °C and 70 ± 5 % relative humidity

Neem Con. %	weights 30 larva (g)	larval period (days)	weights 30 pupa (g)	pupal period (days)	number emerged adults	Adult period (days)
0.2	0.96 b	17.0 a	0.12 d	60 a	00.0 b	0
0.3	1.12 b	18.3 a	0.14 c	60 a	00.0 b	0

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0.4	1.19 b	19.6 a	0.15 b	60 a	00.0 b	0
Control	1.69 a	11.0 b	0.19 a	30 b	27.0 a	10
LSD 5%	0.35	4.3	0.02	1.8	2.9	-

* Values in each column followed by different letter are significantly different at 5% level.

Table (2): Mortality percentages of wax moth larvae , pupae , and emerged adults as influenced by three concentrations of liquid formulation of Neem acetone extract

% Neem con.	% Mortality of larva	% Mortality pupae	% Emerged adult
0.2	33.3	100.0	00.0
0.3	36.6	100.0	00.0
0.4	50.0	100.0	00.0
control	0.0	10.0	90.0

2 Chenopodium experiments:

Data in Tables (3, 4) show the effect of spraying wax pieces by three concentrations 0.2, 0.3, 0.4 % of liquid formulation of Chenopodium acetone extract on some biological aspects of the greater wax moth under laboratory conditions of 25 ± 5 °C and 70 ± 5 % relative humidity .

Data presented in Tables 3 and 4 show the effect of feeding larvae of *Galleria mellonella* on sprayed wax with three concentrations of a liquid formulation of Chenopodium extract, i.e. weights of 30 larvae, 30 pupae, number emerged adults, adult duration, in addition to the mortality percentages of larvae and pupae and emerged adults.

Results in Table (3) indicated that weights of 30 larvae was 1.03, 1.04 and 1.08 g at neem concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 1.68 g. The larval duration was 19.0 day for the 0.4 % concentration, 16.3 day for 0.3 % concentration and 15 day at 0.2 % concentration , compared with only 11 day at control. Weights of 30 pupa was 0.18, 0.20 and 0.21 g at Chenopodium concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 0.22 g. The pupal duration was 60 days for

all tested , compared to 30 days at control. No emerged adults were registered at all neem concentrations , while there was 25 moths emerged from 30 pupae at control treatment.

The statistical analysis of the data Table (3) indicated that there were significant differences in the weights of larvae and pupae as well as the emerged adults between sprayed treatments and control treatment.

As for the mortality percentage Table (4) results indicated that the mortality percentages of wax moth larvae were 23.3, 36.6 and 50 % for the 0.2, 0.3 and 0.4 % of Chenopodium concentrations, respectively, compared with control , which was 0.0 % . Mortality percentages of pupae were 100 % at the three Chenopodium concentrations, while it was only 16.6 % at control treatment. The emergency percentages of adult moths was 83.3 % at control treatment , while no emerged adults were registered at all Chenopodium concentrations.

3- Camphor experiments:

Data in Tables (5, 6) show the effect of spraying wax pieces by three concentrations 0.2, 0.3, 0.4 % of liquid formulation of Camphor acetone extract on

some biological aspects of the greater wax moth under laboratory conditions of $25 \pm 5^\circ\text{C}$ and $70 \pm 5\%$ relative humidity .

Data presented in Tables 5 and 6 show the effect of feeding larvae of *Galleria mellonella* on sprayed wax with three concentrations of a liquid formulation of Camphor extract, i.e. weights of 30 larvae, 30 pupae, number emerged adults, adult duration, in addition to the mortality percentages of larvae and pupae and emerged adults.

Results in Table (5) indicated that weights of 30 larvae was 1.2, 1.23 and 1.24g at Camphor concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 1.7 g. The larval duration was 21.6 day for the 0.4 % concentration, 21.3 day for 0.3 % concentration and 19.6 day at 0.2 % concentration , compared with only 11 day at control. Weights of 30 pupa was 0.22, 0.24 and 0.25 g at Camphor concentrations of 0.2, 0.3 and 0.4 %, respectively, compared to control treatment 0.26 g. The pupal duration was 60 days for all tested , compared to 30 days at control. Number of emerged adults were 2.0, 1.0, 0.0 at 0.2, 0.3, 0.4 % of Camphor concentrations respectively , while there was 26 moths emerged from 30 pupae at control treatment.

The statistical analysis of the data Table (5) indicated that there were significant differences in the weights of larvae and pupae as well as the emerged adults between sprayed treatments and control treatment.

As for the mortality percentage, results in Table (6) indicated that the mortality percentages of wax moth larvae were 26.3, 33.3 and 46.6 % for the 0.2, 0.3 and 0.4 % of Camphor concentrations, respectively, compared with control, which was 0.0 %. Mortality percentages of pupae were 93.3, 96.6 and 100 % at the three of Camphor concentrations, respectively, while it was only 13.3 % at control treatment. The emergency percentages of adult moths was 86.6 % at control treatment, while it was 6.6, 3.3, and 0.0 % at 0.2, 0.3, and 0.4% of Camphor concentrations, respectively.

These results are in harmony with those obtained by Beck, 1960 , Ibrahim, 1980 Malczewska *et al.*,(1988), Abou Bakr and El-Shemy, 1991 Gelbic and Nemeč (2001) Swamy *et al.*, (2006) Shashidhar *et al.*, (2007), Ellis and Hayes (2009) , Zaitoun (2007), Izhar *et al.*, (2008) who tested successively different medicinal and aromatic plants against the greater wax moth , *Galleria mellonella*.

Table (3): Effect of spraying wax pieces by three concentrations of liquid formulation of Chenopodium acetone extract on some biological aspects of the greater wax moth under laboratory conditions of $25 \pm 5^\circ\text{C}$ and $70 \pm 5\%$ relative humidity

Chenopodium Con. %	weights 30 larva (g)	larval period (days)	weights 30 pupa (g)	pupal period (days)	number emerged adults	Adult period (days)
0.2	1.03 b	15.0 b	0.18 d	60 a	0.0 b	0.0
0.3	1.04 b	16.3ab	0.20 c	60 a	0.0 b	0.0
0.4	1.08 b	19.0 a	0.21 b	60 a	0.0 b	0.0
Control	1.68 a	11.0 c	0.22 a	30 b	25.0 a	10.0
LSD 5%	0.45	3.60	0.03	1.8	2.9	-

* Values in each column followed by different letter are significantly different at 5% level.

Table (4): Mortality percentages of wax moth larvae and pupae , and emerged adults as influenced by three concentrations of liquid formulation of Chenopodium acetone extract

% Chenopodium	% Mortality of	% Mortality of	%Emerged adult
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con.	larva	pupae	
0.2	23.3	100.0	00.0
0.3	36.6	100.0	00.0
0.4	50.0	100.0	00.0
control	00.0	16.6	83.3

Table (5): Effect of spraying wax pieces by three concentrations of liquid formulation of Camphor acetone extract on some biological aspects of the greater wax moth under laboratory conditions of 25 ± 5 °C and 70 ± 5 % relative humidity

Camphor con. %	weights 30 larva (g)	larval period (days)	weights 30 pupa (g)	pupal period (days)	number emerged adults	Adult period (days)
0.2	1.20 b	19.6 a	0.22 b	60 a	2.0 b	5.0
0.3	1.23 b	21.3 a	0.24 ab	60 a	1.0 bc	5.0
0.4	1.24 b	21.6 a	0.25 a	60 a	0.0 c	0.0
Control	1.70 a	11.0 b	0.26 a	30 b	26 a	10.0
LSD 5%	0.39	4.5	0.02	1.8	1.6	-

Values in each column followed by different letter(s) are significantly different at 5% level.

Table (6): Mortality percentages of wax moth larvae and pupae , and emerged adults as influenced by three concentrations of liquid formulation of Camphor acetone extract

% Camphor con.	% Mortality of larva	% Mortality of pupae	%Emerged adult
0.2	26.6	93.3	6.6
0.3	33.3	96.6	3.3
0.4	46.6	100.0	00.0
control	0.0	13.3	86.6

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تقدير كفاءة بعض المستخلصات النباتية المطبقة على شمع نحل العسل في مكافحة البيولوجية لفرشة الشمع الكبرى

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الملخص العربى

أجريت هذه الدراسة بمنحل و معامل قسم الحشرات الاقتصادية . كلية الزراعة جامعة المنوفية لدراسة تاثير بعض المستخلصات النباتية علي بعض الصفات البيولوجية وكذلك سميتها على دودة الشمع الكبرى. أظهر التحليل الاحصائي للناتج وجود فروق معنوية في متوسط وزن اليرقات و العذارى و الفراشات بين المعاملات بالمستخلصات النباتية ونظيرتها فى الكنترول. وكانت النسبة المئوية لموت اليرقات ٣٣.٣ و ٣٦.٦ و ٥٠ ٪ في المعاملات بتركيزات ٠.٢٪ و ٠.٣٪ و ٠.٤٪ من مستخلص نبات النيم علي التوالي مقارنة بالكنترول صفر ٪ . وكانت النسبة المئوية لموت العذارى ١٠٠٪ وذلك في التركيزات الثلاثة مقارنة ب ١٠٪ في الكنترول . سجلت المعاملات بالتركيزات ٠.٢٪ و ٠.٣٪ و ٠.٤٪ من مستخلص نبات الزريخ نسب مئوية لموت اليرقات ٢٣.٣ و ٣٦.٦ و ٥٠ ٪ علي التوالي مقارنة بالكنترول الذى لم يحدث به موت ، وكانت النسبة المئوية لموت العذارى ١٠٠ ٪ وذلك في التركيزات الثلاثة مقارنة ب ١٦.٦٪ في الكنترول .

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سجلت المعاملات بالتركيزات ٠.٢٪ و ٠.٣٪ و ٠.٤٪ من مستخلص نبات الكافور نسب مئوية لموت اليرقات قدرت ب ٢٦.٣ و ٣٣.٣ و ٤٦.٦ ٪ علي التوالي مقارنة بالكنترول الذى لم تسجل فيه اى نسب موت ، وكانت النسبة المئوية لموت العذارى ٩٣.٣ و ٩٦.٦ و ١٠٠ ٪ وذلك في التركيزات الثلاثة السابق ذكرها ، اما في الكنترول فقد تم تسجيل نسبة موت ١٣.٣ ٪ من العذارى .

