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Potential of Some Volatile Oils and Chemicals against Lesser Wax Moth, Acheroia gresilla F. (Lepidoptera: Pyralidae)

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Abstract

The oils of six plants viz.; clove; *Eugenia aromatic*., basil; *Ocimum basillicum L.*, thyme; *Thymus vulgaris L.*, blue gum; *Eucalyptus spp.*, spearmint; *Minth viridis L.*, Lemongrass oil; *Cymbopogon citrates Hort.* And six aromatic fragments namely; methyl salicylate, eugenol, menthol, thymol, camphor and naphthalene were tested against *Acheroia gresilla* F. Paradicholorbenzene, acetic and formic acids were used as check and the LC_{50s} were determined against the last larval instar. Results revealed that methyl salicylate, formic acid, clove and basil oils were highly effective.

Keywords: Plant volatile oils; Methyl salicylate; Formic acid; Clove and *Acherioia gresilla*

Introduction

The honeybee is known since many years ago as an important factor for increasing the yield of various crops and produces different products such as propels pollen, royal jelly, bee wax and honey. The honeybees infected by some pests such as the lesser wax moth which is a highly destructive insect that attacks and destroy beeswax combs especially those in storage and can cause substantial losses to combs, hive materials all over the world [1,2]. During its development it builds silk-lined tunnels in the honey comb and feeds on honey, pollen, wax, faces and cocoons of the bee larvae. This leads to destruction of honeycombs and subsequent deterioration of the weakened colonies [3]. In the last three decades the control measures that are available for combs and other equipment not occupied by bees are physical methods involving exposure to heat [4] and store combs in full light. Biological methods involve limited use of microbial insecticides *Bacillus thuringiensis* [5] and the use of juvenile hormone [6]. Actually the most used methods involve the use of different chemicals, such as ethylene oxide and Para dichlorobenzene, carbon dioxide and methyl bromide [7] to kill the different stages of the moth. In fact, an effective and harmless method of control of this pest has not been developed. Recent studies showed that many plant extracts have an effect on insect and mites [8-10]. In this work, we attempted to control LWM with certain volatile plant oils which seem to be safer and less contaminant to bees and humans. Such treatment methods with non-harmful products could contribute considerably to control this pest and reduce the risks of beehive products contamination and may give a possible solution for this apicultural problem.

Material and Methods

Experimental wax moth: To obtain pure culture of wax moth, newly emerged wax moth females and males were collected from naturally-infested wax combs from the apiary of Fac. Agric., Fayoum Univ. and transferred to clean glass jars. After mating females were left to deposit eggs and a black ribbon cloth was hanged inside each cage to serve as oviposition site. The eggs were incubated in a warm at 30ºC, relative humidity of ca 70% and 24 hours darkness until emergence of lesser wax moth. The larval stages were provided as needed with a cultural media (clean beewax) which cut and put in clean small cups for rearing larvae.

Tested plants

Six plants belonging to different families commonly used in Egypt for medical and/or health purposes were used in this experiment. These plants were: clove; *Eugenia aromatica* L., basil; *Ocimum basilicum* L., thyme; *Thymus vulgaris* L., blue gum; *Eucalyptus* spp., spearmint; *Mintha viridis* L., and lemongrass oil; *Cymbopogon citratus* Hort. The oils of these plants were extracted by water-distillation and locally obtained according to [11]. Also six materials contains volatile fragments were tested these materials were: eugenol, methyl salicylate, menthol, thymol, camphor and naphthalene obtained from Horticulture Department, Faculty of Agriculture, Fayoum University, Egypt. On the other hand, three classic chemical substances were tested, these substances were acetic acid, formic acid and paradichlorobenzene were obtained from Chemistry Department, Faculty of Science, Fayoum University, Egypt.

Testing the effect of the different substances

Fifteen substances were tested against the last larval instar, pupa and adult stages to make the investigations. The larvae were collected from the cages and transferred to Petri dishes 10 cm diam., only five larvae or five 5 pupae of LWM per each dish,. In case of adults (5 moths), chimney glasses were used. Clean beeswax cube (approx. 1x1cm wax comb) was added to each dish or chimney glass. Card boards (1x1x0.2 cm) carrying 0.01 ml or 0.1 ml or 0.2 ml (for liquids) and 0.01 g or 0.1 g or 0.2 g (for solids) of fifteen tested materials was added to dishes or glasses. Five replicates of each treatment were applied, and control ones had carded boards free. Daily inspection was carried out for study of some biological aspects.

Toxicity tests

A series of concentrations for each material (eight concentrations) to determine LC_{50s} for these materials, the procedure of Barakat et al. [12] was carried out as following:

The last larval instar individuals of LWM were selected for this investigation. The concentrations of tested materials (1 µl, 2 µl, 4 µl, 8 µl, 16 µl, 32 µl 64 µl and 128 µl) were dissolved in 1ml acetone in 10-cm Petri dishes, and dispersed by gentle hand shaking, and then left to evaporate for about 3-5 minutes. Larvae (20/dish) were placed in treated dishes. Three replicates were applied and control ones had acetone only. After 24 hrs. % mortalities were calculated and corrected by the formula given by Abbott [13].The LC_{50} and LC_{90} values were estimated from the toxicity lines of the tested materials according to Finney [14].

Retarding effects

The tested materials, (nine materials were chosen) (Table 1) were dissolved in 1 ml acetone, while the control had acetone only. After dryness, larvae of last instar were transferred to treated dishes (20 larvae/dish). After 1 hr., the same larvae were transferred to untreated clean dishes where three replicates were used. After 24 h, survived larvae in each treatment were taken to clean untreated dishes (20 larvae/dish) and provided with clean beewax. Such dishes (3 replicates/treatment) were daily inspected to record subsequent biological aspects such as mortalities of larvae and pupae, pupation, emergence, oviposition and egg hatching.

Field application

The tested materials which appeared high efficacy against LWM were selected for field application. Renewable source of material in glass bottles were applied with different concentrations (0.25 ml, 0.50 ml, 1.0 ml and 2.0 ml/comb/week).

Statistical analysis

Collected data were subjected to analysis of variance (ANOVA), and means were compared using Fisher,s least significant differences [15].

Results and Discussion

Effect of tested materials against LWM

Volatile oils of six plants belonging to different families, fragments of six aromatic materials and three chemicals substances were used in this experiment. From the preliminary results it was noticed that the tested materials exhibited variable efficiencies against LWM. The least effective materials including lemongrass oil, camphor, menthol, naphthalene and thymol, which gave lower larval mortality rates even when applied at high concentrations were excluded. Although PDB showed noticeable effect on treated larvae (slow paralysis followed by death) it had high LC_{50} value (38 mg/ml) compared to other tested materials.

On the other hand, potential activities of active materials were summarized in Table 1. The estimated LC_{50} values were obviously different being: 0.29, 2.55, 3.45, 4.34, 7.62, 9.88, 19.55, 34.67 and 43.67 (µl/ml) for methyl salicylate, formic acid, clove oil, eugenol, acetic acid, basil oil, blue gum oil, spearmint oil and thyme oil, respectively. From the obtained data, the tested materials could be screened according to LC_{50} values in the following two categories:

a) Most effective materials which exhibited high mortality rates with low LC_{50} values including; methyl salicylate, formic acid, clove oil, eugenol, acetic acid, and basil oil.

b) Moderate effective materials which showed moderate mortality rates with moderate LC_{50} values including; blue gum, spearmint, and thyme oils.

T- material LCS	Methyl salicylate	Formic acid	Clove oil	Eugenol	Acetic acid	Basil oil	Blue gum	Spearmint- oil	Thyme oil
LC50	0.2 9	2.55	3.45	4.34	7.62	9.88	19.55	34.67	43.67
LC90	0.6 5	1.95	11.75	21.98	29.56	32.32	79.34	90.65	82.24
Slope ± SE	**3.45 ± 0.54**	13.35 ± 2.88	2.56 ± 0.43	1.98 ± 0. 25	1.95 ± 0.23	1.76 ± 0.33	1.89 ± 0.22	2.32 ± 0.76	4.56 ± 2.10

Table 1: Toxicity of different materials against the last larval instar of LWM

Retarding effects of more effective materials against LWM

The LC_{50s} of most active materials were applied to the last larval instar of LWM. After 24 hour, survived larvae were monitored for retarding effects on some biological aspects in comparison with control. The obtained data in Table 2 showed different effects for the tested parameters as the following:

Mortalities of larval and pupal stages: Cumulative larval mortality was observed for those larvae treated with LC_{50}. The highest mortality was recorded for eugenol (34.0%) followed by formic acid (19.8%), clove (17.5%), methyl salicylate (13.0%) and basil oil (11.23%). Minimum mortality was found in acetic acid (4.10) and no mortality with control treatments and significant differences with the other treatments. Also eugenol and clove differed significantly with the other ones. The highest pupal mortality was recorded for methyl salicylate (69.87%) followed by eugenol (65.0%) and clove (56.0%). However, acetic acid or basil had median mortalities compared to control which had the lowest mortality (2.0%) with significant differences.

Pupation period: The active materials treatments showed declined pupation periods of treated larvae reaching 12.2 day for clove; 17.29 day for acetic or control and ranged between 12.8-17.2 day in other treatments.

Adult longevity: For males, the highest longevity was observed for basil (30.6 day), while the lowest one (4.0 day) for formic; clove (5.6 day) compared to control (26.4 day). Significant differences were found between clove or formic and the other ones. For females, longevity ranged between 9.2 day for formic to 35.90 day for methyl salicylate, while the control had 32.0 day.

Oviposition and hatching: Eggs laid/female was affected those treatments reaching its minimum rate (55.2 egg/female) for formic acid and maximum rate 221.2 egg/female for clove, while the maximum one was recorded for the control being 339 egg/female with significant differences with the other treatments. Methyl salicylate and clove exhibited obvious decrease in egg hatching compared to the other treatments.

Field application: The tested materials which showed high efficacy (methyl salicylate, clove, basil and formic acid) against LWM were selected for field application. Renewable source of a material in glass bottles (1 ml/comb/week) was found to be the best concentration for protecting comb in this respect, the use of natural products, especially volatile oils or their components and organic is the most preferable approach to control pests and diseases of the honeybee

Parameters	% Mortality *		Pupation (day)	Longivity (in day)		Oviposition	
Active material	Larvae	Pupae	15.60 ± 0.56	Male	Female	Egg/female	% Hatchability
Methyl salicylate	13.0 ± 1.54	69.87 ± 4.46	15.60 ± 0.56	23.65 ± 1.32	35.90 ± 2.44	215.6 ± 22.69	46.0 ± 5.09
Formic acid	19.8 ± 1.32	12.2 ± 0.85	14.67 ± 0.44	6.87 ± 0.89	9.2 ± 1.86	55.2 ± 2.48	93.0 ± 3.74
Clove oil	17.50 ± 1.76	56.0 ± 1.95	12.20 ± 0.54	8.95 ± 0.89	10.6 ± 0.56	221.2 ± 40.45	46.0 ± 3.99
Eugenol	34.0 ± 1.92	65.0 ± 0.94	15.77 ± 0.79	24.54 ± 3.65	36.65 ± 1.43	66.4 ± 8.19	91.8 ± 3.88
Acetic acid	4.10 ± 0.43	23.0 ± 1.66	17.29 ± 0.57	21.87 ± 2.99	32.6 ± 0.95	98.0 ± 1.70	85.6 ± 2.97
Basil oil	11.23 ± 0.98	17.0 ± 0.98	18.98 ± 0.83	27.55 ± 0.89	33.4 ± 2.56	133.0 ± 13.40	94.8 ± 1.71
Control	0.0 ± 0.00	2.0 ± 0.78	17.45 ± 0.60	26.4 ± 2.33	32.0 ± 2.77	339.0 ± 39.99	98.6 ± 0.98
L.S.D. 5%	4.21	13.56	1.33	8.96	4.78	7.63	12.54

Table 2: Retarding effects of tested material soils and chemicals on some biological aspects of Acheroia gresilla. N.B. * 1- Cumulative mortality. 2- Mean ± SE.

Essential oils and their components offer an attractive alternative to synthetic pesticides for the control of honeybee pests, besides they are inexpensive and mostly pose few health risks. Monoterpenes are the main components of essential oils, comprising about 90% of the total. The above mentioned results showed that methyl salicylate, clove, basil and formic acid were the most effective tested materials against LWM, while thyme oil was the least one. Many essential oils and their components were registered for controlling bee parasites *e.g. Varroa* mites. Recent studies to control severe bee disease e.g. foulbroods using plant products are highly considered [16]. From the obtained results it is clear that the tested marked effects compared to untreated ones. Formic acid enforced larvae for earlier pupation compared to other materials. In general, clove and eugenol showed obvious effects on these biological aspects. In this respect Hassan and Aly [17] showed that formic acid was the most effective material on hatchability of LWM eggs. They added that formic acid showed a remarkable action against two serious pests of honey bees; *Varroa* mite and wax moths. In conclusion we recommend by using methyl salicylate, clove oil, formic acid and basil oil with one ml/comb/week to protect stored wax combs from infestation with wax moths.

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