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POTENTIAL EFFICACY OF CERTAIN PLANT VOLATILE OILS AND CHEMICALS AGAINST GREATER WAX MOTH, *Galleria mellonella* L. (LEPIDOPTERA: PYRALIDAE)

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INTRODUCTION

Wax moths are serious pests of beeswax worldwide. Greater wax moth (GWM), *Galleria mellonella* L., and lesser wax moth, *Acheroia gresilla* L., are known to be harmful to deposited and stored beeswax. GWM causes the greatest damage in apiaries which lead to financial losses every year, beside damaging wax combs by larval feeding, and destroying frames and wooden parts in the hive. Adult wax moths and larvae can also transfer pathogens of serious bee diseases, *e.g.* foulbrood. However, in colonies infested with this disease, feces of wax moth contain large amounts of spores of the causative bacteria, *Paenibacillus larvae* **(Charrière and Imdorf, 1997)**.

Possibilities for controlling wax moth including some manipulations in the hive and other treatments to stored combs *i.e.* technical, physical, biological and chemical methods *e.g.* sulphur fumigation, acetic and formic acids evaporation and applying paradichlorobenzene are practiced **(Calderone, 2000)**. Although the use of these chemicals is somewhat easy and effective, some precautions of safety and contamination of bee products are considered. Also, some of these materials seemed to be ineffective against eggs of the wax moth **(Fraser, 1997)**.

In this work, we attempted to control GWM with certain volatile plant oils which seem to be more safe and less contaminant to bees and humans. Also, these materials are cheap, available to beekeepers, and could be used to control other hive infestations *e.g. Varroa* and acarine mites *.etc*.

MATERIAL AND METHODS

1.Culturing of GWM

Naturally-infested wax combs with GWM were obtained from the apiary of Fac. Agric., Fayoum Univ. and were taken to a rearing chamber in the same Faculty.

To ascertain pure culture, infested wax cubes (feeding medium) were cut and transferred to clean 10 kg-glass jars. Emerged moths were taken to new jars fortified with uninfected waxes and left to copulate and lay eggs. Emerged larvae were monitored to obtain the desired instars for the following assay. Rearing and treatments were conducted at room conditions (20±5 ^{o}C temp. and 65±5% R.H.) during June, 2006 to March, 2007.

2.Tested materials

2.1. Volatile oils: six plant volatile oils were selected. These were: clove; *Eugenia aromatica* L., basil; *Ocimum basilicum* L., thyme; *Thymus vulgaris* L., blue gum; *Eucalyptus* spp., pepperrmint; *Mintha viridis* L., and lemongrass oil; *Cymbopogon citratus* Hort. The oils were extracted by water-distillation and locally obtained.

2.2.Volatile fragments: six materials were tested. These materials were: eugenol (Win Lab., UK), methyl salicylate, menthol, thymol, camphor and naphthalene obtained from El-Gomhoria Chem. Co., Egypt.

2.3. Chemicals: three chemicals: 80% acetic acid, 85% formic acid and paradichlorobenzene were tested.

3. Assay

3.1.Preliminary experiments: each 5 larvae of the 5th instar or 5 pupae of GWM were transferred to 10 cm diam. Petri-dishes,. 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 0.01g or 0.1g or 0.2g (for solids) of 15 tested materials were added to dishes or glasses. Five replicates of each treatment were applied, and control ones had card boards free. Daily inspection was carried out for some biological aspects.

3.2. Determination of LC_{50s}: to determine LC_{50s} for the tested materials, the procedure of **Barakat *et al.* (2006)** with some modification was carried out as following:

The 5th larval instar individuals of GWM were selected. Gradual concentrations of each tested material (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, then left to evaporate for about 3 -5 minutes. Larvae (20 / dish) were placed in treated dishes. Four replicates were applied and control ones had acetone only. After 24 hrs., % mortalities were calculated and corrected by the formula given by **Abbott (1925)**.The LC_{50} and LC_{90} values were estimated from the toxicity lines of the tested materials according to **Finney (1971)**. The Probit program designed by Windows® was also applied.

4. Latent effects

The tested materials, except the last three ones (table, 1) were dissolved in 1 ml acetone, while the control had acetone only. After dryness, larvae of 5th instar were transferred to treated dishes (20 larvae / dish). After 1 hr., the same larvae were transferred to untreated clean dishes where 4 replicates were used. After 24 h, survived larvae in each treatment were transferred to clean untreated dishes (20 larvae / dish) and provided with beeswax comb (about 1 x 1 x 1 cm cube). Such dishes (4 replicates / treatment) were daily inspected to record subsequent biological aspects *e.g.* mortalities of larvae and pupae, pupation, emergence, oviposition and egg hatching.

5. Field application

The tested materials which showed high efficacy against GWM in the laboratory 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).

RESULTS AND DISCUSSION

I. Activity of tested materials against GWM larvae

From the preliminary experiments, it was noticed that the tested materials exhibited variable efficacies against GWM. 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) and Figure (1). The estimated LC_{50} values were obviously different being: 0.48, 3.17, 5.75, 6.57, 10.26, 11.40, 23.92, 48.10 and 54.12 (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.

II. Latent effects of tested materials against GWM

The LC_{50s} of most active materials were applied to the 5^{th} larval instar of GWM. After 24 h, survived larvae were monitored for latent effects on some biological aspects compared to control. The obtained data in Table (2) showed different effects on the tested parameters as the following:

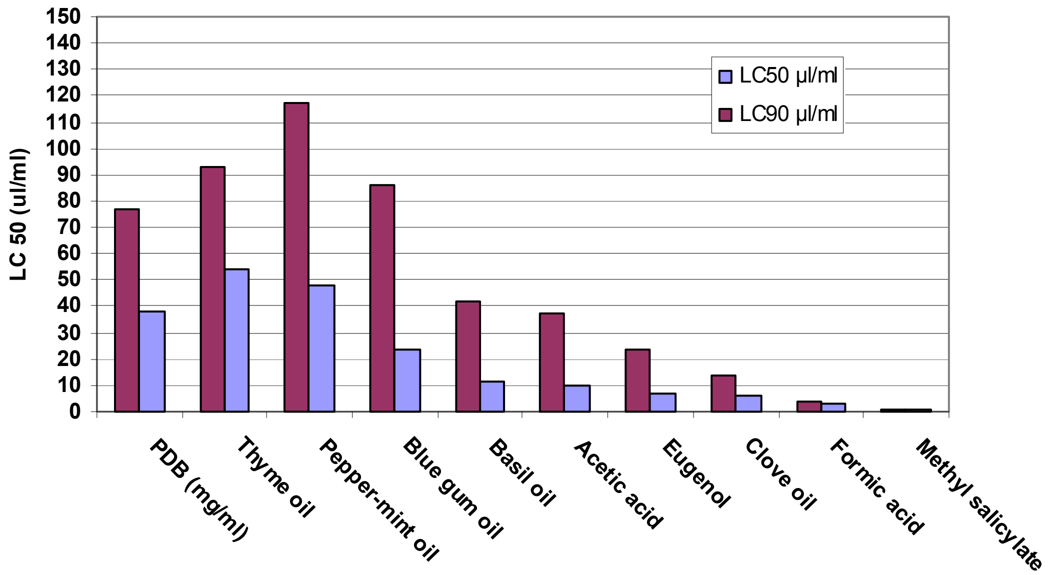
Table (I)

The LC_{50s} and LC_{90s} values (μl/ml) for most active tested volatiles and chemicals against the 5th larval instar of GWM.

Materials / Parameters	Methyl salicylate	Formic acid	Clove oil	Eugenol	Acetic acid	Basil oil	Blue gum oil	Pepper-mint oil	Thyme oil
LC_{50}	0.48	3.17	5.75	6.57	10.26	11.40	23.92	48.10	54.12
LC_{90}	0.88	3.76	13.39	23.85	37.37	41.91	86.19	116.93	93.08
Slope	4.98	17.23	3.49	2.29	2.28	2.27	2.30	3.32	5.43
±SE	0.91	3.09	0.63	0.40	0.39	0.40	0.34	0.84	2.10

Figure (I)

The LC_{50} values (μl/ml) for most active tested volatiles and chemicals against the 5th larval instar of GWM.



1. Larval and pupal mortalities

Cumulative larval mortality was observed for those larvae treated with LD_{50}. The highest mortality was recorded for eugenol (29.0%) followed by formic acid (17.6%), clove (14.0%), methyl salicylate (10.0%) and basil oil (7.0%). No mortality was found in acetic acid or control treatments with significant differences with the other treatments. Also eugenol and clove differed significantly than the other ones.

The highest pupal mortality was recorded for methyl salicylate (65.0%) followed by eugenol (57.0%) and clove (50.0%). However, acids or basil had median mortalities compared to control which had the lowest mortality (1.0%) with significant differences.

2. Pupal period

Treatments showed declined pupal periods of treated larvae reaching 9.2 day for clove; 16.0 day for acetic or control and ranged between 12.8 – 17.2 day in other treatments.

3. Adult longivity

For males, the highest longivity 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 differnces were found between clove or formic and the other ones.

For females, longivity ranged between 7.2 day for formic to 40.8 day for methyl salicylate, while the control had 35.0 day.

4. Oviposition and hatching

Egg laid / female was affected by those treatments reaching its minimum rate (46.2 egg/female) for formic and was 238.2 egg/female for clove, while the maximum one was recorded for the control being 528 egg/female with significant differences with the other treatments. Methyl salicylate and clove exhibited obvious decrease in egg hatching compared to the other treatments.

Table (II)

Latent effects of tested volatile oils and chemicals on some biological aspects of *G. mellonella*.

Parameters	% Mortality *		Pupal period (day)	Longivity (day)		Oviposition	
Treatments	Larvae	Pupae		Male	Female	Egg/female	% Hatchability
Methyl salicylate	10.0 ±2.73	65.0 ±4.46	13.6 ±0.68	24.6 ±2.24	40.8 ±2.57	226.6 ±22.69	46.0 ±5.09
Formic acid	17.6 ±1.12	7.2 ±1.16	12.8 ±0.37	4.0 ±0.99	7.2 ±1.49	46.2 ±2.48	93.0 ±3.74
Clove oil	14.0 ±1.87	50.0 ±2.73	9.2 ±0.58	5.6 ±0.87	8.6 ±0.51	238.2 ±40.45	46.0 ±3.99
Eugenol	29.0 ±1.89	57.0 ±1.38	13.6 ±0.51	26.6 ±3.17	40.0 ±1.97	61.4 ±8.19	91.8 ±3.88
Acetic acid	0.0 ±0.00	17.0 ±2.54	16.2 ±0.58	22.0 ±3.74	34.6 ±0.51	96.0 ±1.70	85.6 ±2.97
Basil oil	7.0 ±1.22	13.0 ±1.22	17.2 ±0.97	30.6 ±0.60	37.4 ±2.18	123.0 ±13.40	94.8 ±1.71
Control	0.0 ±0.00	1.0 ±0.99	16.0 ±0.55	26.4 ±2.22	35.0 ±2.53	528.0 ±54.99	98.6 ±0.98
L.S.D. $_{5\%}$	**3.86**	**14.39**	**1.29**	**6.96**	**5.78**	**81.76**	**10.82**

N.B. * 1- Cumulative mortality. 2- Mean ± SE.

III. Field application

The tested materials which showed high efficacy (methyl salicylate, clove, basil and formic acid) against GWM 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 stored combs.

In this respect, the use of natural products, especially volatile oils or their components and organic acids is the most preferable approach to control pests and diseases of the honeybee. 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 GWM, while thyme oil was the least one. Many essential oils and their components were registered for controlling bee parasites *e.g. Varroa* mites **(Imdorf and Bogdanov, 1999)**. Recent studies to control severe bee disease *e.g.* foulbroods using plant products are highly considered **(Williams *et al.* 1998)**.

From the obtained results it is clear that the tested materials showed marked effects on GWM 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 (1999)** showed that formic acid was the most effective material on hatchability of GWM 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 the use of methyl salicylate, clove oil, formic acid and basil oil with 1 ml / comb / week to protect stored wax combs from infestation with wax moths.

SUMMARY

The possible efficacy of some volatile plant oils and chemicals against the greater wax moth (GWM), *Galleria mellonella* L., was investigated. The oils of six plants were tested namely; clove; *Eugenia aromatica* L., basil; *Ocimum basilicum* L., thyme; *Thymus vulgaris* L., blue gum; *Eucalyptus* spp., spearmint; *Mintha viridis* L. and lemongrass oil; *Cymbopogon citrates* Hort. Also, six aromatic fragments namely; methyl salicylate, eugenol,, menthol, thymol, camphor and naphthalene were tested. To evaluate the relative efficacies of these materials as alternative control agents against GWM, using paradichlorobenzene, acetic and formic acids were compared and the LC_{50s} were determined against the 5^{th} larval instar. Latent effects of those active materials were studied on some biological aspects of GWM and its developing stages. The obtained results showed that efficacies of the tested materials were different. Thus, highly effective ones (methyl salicylate, formic acid, clove and basil oils) were applied to protect wax combs stored ordinarily in the apiary.

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