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Evaluation of Some Essential Oils Against Wax Moth Larvae (Lepedoptera: *Galleria mellonlla* L.) and Adult Honeybee Workers (Hymenoptera: *Apismellifera* L.)

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Abstract: This study was conducted to investigate the effects of five essential oils (garlic, *Allium sativum*; camphor, *Cinnamomum camphor*; menthol, *Mentha sp.*; cinnamon, *Cinnamomum Erum*; and clove, *Dianthus sp.*) on wax moth larvae and adult honeybee workers under laboratory conditions. Cinnamon, garlic, menthol, clove, and camphor oils were found to be highly effective against wax moth larvae, with 100 percent mortality achieved after 48 and 96 hours for cinnamon and garlic oils respectively, and after 120 hours for clove and mint oils. Camphor oil provided 96.60% accumulative mortality after 120 hrs. Moreover, all the essential oils were highly safe for adult honeybee workers under laboratory conditions. Accumulative corrected mortality rates after 72 hr were 15.96, 9.02, 13.18, 29.16, and 8.88% for treatments with cinnamon, clove, camphor, mint, and garlic oils respectively.

1 Introduction

Larvae of *Galleria mellonella* have one of the best effects on honeybee colonies and honeybee frames (Ritter and Akratanakul 2006). Honeybee colonies and its old combs are subjected to attack by wax moth larvae feed on wax skins of pupae, bee pollen (Jindra and Sehnal 1989). *Galleria mellonella* is more popular in honeybee and cause greater problems (Pirk et al 2016). The larvae of *G. mellonella* in fest nearly all colonies of the honeybees, *Apiesmellifera* L., which are important insects and are well known for their significance as pollinators and honey products. High gross losses of managed honeybee colonies have been reported in various parts of the world, (Higes et al 2008, Neumann and Carreck 2010). Consequently, dark comb (where the brood is reared) is preferred by the moth and consequently, it suffers the most injury (Ellis et al 2013). Egg, larval, and pupal duration of the greater wax moth (GWM) take approximately 5 days, 6–7 weeks, and 2 weeks at 29–32°C, respectively. A GWM larva progresses through 8–9 stages (molts) over the course of its development at 33.8°C (Charriere and Imdorf 1999). Tested materials against the wax moth show varied efficacy. Using miacid, methyl salicylate, clove, and basil oils to protect stored wax combs (Ayman and Atef 2007). Volatile plant oils were used as safe agents for humans and bees and reaching lesser wax motherless contaminants. The spraying of water extract of clove, Egyptian propolis cinnamon, mint and Chinese propolis (4%) could protect new combs from larvae of wax moth infection. The cloveex tract reduced the life cycle of wax moths compared with control combs. Moreover, the cinnamon extracts inhibited egg hatchability (29.1%) compared with control (86.3%) (Sanad and Mohanny 2015). Both *Origanum Majorana* and *Cymbopogon Proximus* oils were used to evaluate their action as alternative control agents against *G. mellonella*. The results showed that the first oil is more toxic on *G. mellonella* than the second oil (Hussein et al 2014). Fourth instar larvae of the *G. mellonella* treated with *Menthapiperita*, *Pelargonium graveolens L.* and *Ocimumbasilicum L.* treatments decreased pupation and percentage of adult emergence and extended the larval–pupal periods. In terms of mortality, *O. basilicum* is the most active, followed by *M. piperita* and finally, *P. graveolens* (Elbarky et al 2015). The oils of the five plants (neem, cedar, clove, peppermint, and karang) and one extract (neem seed kernel) were tested at various concentrations and exhibited varying degrees of activity against the larvae of *G. mellonella*. Larvae mortality was highest with neem oil 5% (65.33% at 7 DAT) whereas lowest with kerrang oil (2.33% at DAT) (Bisht et al 2017). The plant oils of freed best alternatives to the insecticides for controlling wax moths (Ncibi et al 2021). Four natural products were examined for GWM control, namely, tobacco extract (*Nicotianatabacum*), eucalyptus oil (*Eucalyptus spp.*), malagueta pepper (*Capsicum frutescens*) and neem oil (*Azadirachtaindica*). The different product concentrations was safe for the bees and effectively controlled the moth where eucalyptus oils and neem at low concentrations caused mortality of wax moth, and they are safe for colony population growth (Telles et al 2020).

The current study aims to evaluate the effect of five essential oils (garlic, *Allium sativum*; camphor, *Cinnamomum camphor*; menthol, *Mentha sp.*; cinnamon, *Cinnamomum verum*; and clove, *Dianthus sp.*) on wax moth larvae and adult honeybee workers under laboratory conditions. Results indicated that cinnamon, garlic**,** menthol, clove, and camphor oils were highly effective against wax moth larvae and extremely safe for adult honeybee workers.

2 Material and Methods

2.1 Rearing of wax moth larvae

This study was conducted in the apiary of the Plant Protection Institute at Quantar, Qaluobia Governorate. The larvae of greater wax moth were collected from naturally infested honeybee hives. Wood boxes of 40 x 30 x 30cm were used for rearing under laboratory conditions with 25 ± 5°C and 70 ± 5% relative humidity. Collected larvae were introduced into 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, then after the emergence of moths that laid eggs, hatched into larvae. For toxicological tests, fourth instar larvae were used (Elkhiat 2012).

2.2 Tested materials

Five essential oils were examined for this study (cinnamon, garlic, clove, camphor, and menthol oils) as mentioned in **Table 1**. Samples were obtained from the Society Du El Capitan (CAP PHARM) for the extraction of natural oils plants and cosmetics.

Table 1. List of selected plants

English name	Scientific name	Family name
Cinnamon oil	*Cinnamomumverum*	Lauraceae
Garlic oil	*Allium sativum*	Alliaceae
Clove oil	*Dianthus sp.*	Caryophyllaceae
Camphor oil	*Cinnamomum camphor*	Lauraceae
Menthol oil	*Mentha sp.*	Lamiaceae

2.3 Wax moth larvae assay

Preliminary experiments: The assay was performed on fourth instar larvae, 10 of which were used (replicated). Three replicates were used for each essential oil. The larvae were transferred into a 10 cm diameter Petri dish provided with 10 gm wax. Samples of 12 ml of each tested essential oil were added to a piece of cotton. The untreated (control) dishes were left as it. The dead larvae were counted and recorded daily for five days, and the percentages of mortality were evaluated.

Mortality percent of Larval = No. of dead larvae/No. of tested larvae × 100.

2.4 Honeybee workers' assay

For the assay, 50 adult honeybee workers were transferred into special cages. Samples of 12 ml of each essential oil tested in a Petri dish were poured into each cage. The untreated (control) dishes were left as it. The number of honeybee workers and percentages of mortality were evaluated daily for three days. Abbott's formula = (%T–%C/100–%C) × 100 (Abbott WS 1925).

2.5 Statistical analysis

The experiment employed a completely randomized design. Results were analyzed using SAS system (Ying and Liu 2006). The general linear modules were used to test for differences (alpha = 0.05), the least significant of which was applied as a mean separation.

3 Results and Discussion

3.1 Wax moth larvae assay

Table 2 indicates the number and percentages of dead and (%) of mortality of 4th instar wax moth larvae after treatment with essential oils under laboratory conditions. The data showed that all the tested wax moth larvae were dead after 48 and 96 hrs. when treated with cinnamon and garlic oils respectively, and after 120 hrs when treated with clove, camphor, and mint oils. All the tested larvae died after 120 hrs.

3.2 Accumulative mortality (%) of several essential oils on fourth instar wax moth larvae

Table 3 demonstrates the accumulative mortality of several essential oils tested on wax moth larvae. The results indicated that all the tested essential oils were highly effective against the wax moth larvae. The accumulative mortality was 100% for cinnamon and garlic oils after 48 and 72 hrs, respectively, and after 120 hrs., for clove and mint oils. The camphor oil provided 96.60% accumulative mortality after 120 hrs.

3.3 Honeybee worker's assay

Table 4 displays the numbers and percentages of dead adult honeybee workers after treatment with essential oils under laboratory conditions (50 adult honeybee workers/replicates). The results revealed that, after three days, the numbers of dead adult honeybee workers were 29, 19, 25, 48 and 18 when treated with cinnamon, clove, camphor, mint, and garlic oils, respectively and 6 for untreated cheek (control), respectively.

3.4 Accumulative corrected mortality (%) of some essential oils on adult honeybee workers

The data in **Table 5** shows that all the essential oils were highly safe for adult workers of honeybee under laboratory conditions. The accumulative corrected mortalities were 15.96%, 9.02%, 13.18%, 29.16%, and 8.88% after 72 hrs when treated with cinnamon, clove, camphor, mint, and garlic oils, respectively. Using synthetic insecticides is one of the most prevalent strategies to control wax moth infestations, especially for weak bee colonies. However, insecticide residues lead to toxicity in bees and contamination of their products, thereby increasing the risks to human health and the environment. Sanad and Mohanny (2015) sprayed 4% clove and mint extract on new combs and found that the spray with clove extract reduced the wax moth's life cycle to 42.9 days while untreated combs were 72.0 days. In addition, Elbarky et al (2015) found that the essential oils increased the larval and pulpal periods and decreased pupation and percentage of adult emergence. Ncibi et al (2021) investigated the efficacy of natural products that were discovered to be safe for adult honeybees while effectively controlling fourth instar wax moth larvae.

Table 2. Numbers of dead and mortality (%) of 4th-instar wax moth larvae after treatment with essential oils under laboratory conditions (10 larvae/replicates)

Date of inspection	Essential oil												L.S.D
	Cinnamon		Clove		Camphor		Mint		Garlic		Control		
	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	Live	Dead	
27/1/2021	15 5.00 (50)	15 5.00 ± 1.15a (50)	24 8.00 (80)	6 2.00 ± 0.57bc (20)	27 9.00 (90)	3 1.00±0.57bc (10)	21 7.00 (70)	9 3.00±1.00ab (30)	21 7.00 (70)	9 3.00 ±1.52ab (30)	30 10.00 (100)	0.00 ± 0.00c	2.90
28/1 /2021	0.00	15 5.00 ± 1.15a (50)	21 7.00 (70)	3 1.00 ± 0.00b (10)	26 8.67 (86.7)	1 0.33± 0.33b (3.3)	9 3.00 (30)	12 4.00 ± 0.57a (40)	6 2.00 (20)	15 5.00 ± 0.57a (50)	30 10.00 (100)	0.00 ± 0.00ba	1.82
29/1/2021	0.00	0.00 ± 0.00a	16 5.33 (53.3)	5 1.66 ± 1.20a (16.6)	24 8.00 (80)	2 0.66 ± 0.33a (3.3)	5 1.66 (16.66)	4 1.33 ± 0.88a (13.33	0 0.00 (0.00)	6 2.00 ± 1.15a (20)	30 10.00 (100)	0.00 ± 0.00a	2.40
30/1/2021	0.00	0.00 ± 0.00b	12 4.00 (40)	4 1.33 ± 0.33a (13.3)	18 6.00 (60)	6 2.00 ± 0.57a (20)	2 0.66 (6.6)	3 1.00±0.57ab (10)	0.00	0.00 ± 000.00b	30 10.00 (100)	0.00 ± 0.00b	1.10
31/1/2021	0.00	0.00 ± 0.00c	0.00	12 4.00 ± 0.57b (40)	0.00	18 6.00 ± 0.57a (60)	0 0.00 (0.00)	2 0.66 ± 0.67c (6.7)	0.00	0.00 ± 0.00c	30 10.00 (100)	0.00 ± 0.00c	1.10
1/2/2021	0.00	0.00 ± 0.00a	0.00	0.00 ± 0.00a	0.00	0.00 ± 0.00a	0.00	0.00 ± 0.00a	0.00	0.00 ± a	30 10.00 (100)	0.00 ± 0.00a	0

values in brackets are mortality (%)

Table 3. Accumulative mortality (%) of some essential oils on 4^{th}-instar wax moth larvae

Treatment	Percentage of corrected mortality after				
	1 day	2 days	3 days	4 days	5 days
Cinnamon oil	50.00	100.00			
Clove oil	20.00	30.00	46.66	59.99	100
Camphor oil	10.00	13.30	16.60	36.60	96.60
Mint oil	30.00	70.00	83.3	93.3	100
Garlic oil	30.00	80.00	100.00		

Where**:** mortality percent of larval= No. of dead larvae/No. of larvae tested × 100

Table 4. Numbers of dead and percentages of adult honeybee workers after treatment with Some essential oils under laboratory conditions (50 honeybee workers/replicates)

Treatment	Repli-cates	No. and percentage of dead honeybee workers after			
		No. of workers	day1	day2	Day3 (total)
Cinnamon oil *(Cinnamomum verum)*	3	150	9 3.00± 1.00a (6)	20 6.66± 1.45ab (13.33)	29 9.66± 1.45ab (19.33)
Clove oil (*Dianthus sp.*)	3	150	7 2.33± 1.33a (4.66)	15 5.00± 2.00ab (10)	19 6.33± 2.33b (12.66)
Camphor oil *(Cinnamomum camphor)*	3	150	12 4.00± 2.00a (8)	21 7.00± 2.08ab (14)	25 8.33± 2.40ab (16.66)
Mint oil *(Mentha sp.)*	3	150	18 6.00± 2.64a (12)	33 11.00± 4.16a (22)	48 16.00± 4.58a (32)
Garlic oil (*Allium sativum)*	3	150	8 2.66± 1.20a (5.33)	15 5.00± 1.52ab (10)	18 6.00± 2.00b (12)
Control (Untreated check)	3	150	4 1.33± 0.66a (2.66)	4 1.33± 0.66b (2.66)	6 2.00± 1.15b (4)
L.S.D			4.97	6.95	7.92

Values between brackets are (%) of mortality of adult workers honeybee

Table 5. Accumulative corrected mortality (%) of some essential oils on adult workers of honeybee %

Treatment	Percentage of mortality after		
	1 day	2 days	3 days
Cannabin oil	3.43	10.9	15.96
Clove oil	2.05	7.54	9.02
Camphor oil	5.48	11.64	13.18
Mint oil	9.59	19.86	29.16
Garlic oil	2.74	7.54	8.88

Abbott's formula = (%T–%C/100–%C) × 100

4 Conclusion

Five essential oils (garlic, *Allium sativum*; camphor, *Cinnamomum camphor*; menthol, *Mentha sp.*; cinnamon, *Cinnamomum verum*; and clove, *Dianthus sp.*). On wax moth larvae and adult honeybee workers under laboratory conditions. The cinnamon, garlic**,** menthol, clove, and camphor oils were highly effective against wax moth larvae where hundred percent mortality was attained after 48 and 96 hours with cinnamon and garlic oils, respectively, and after 120 hrs. For clove and mint oils. Camphor oil provided 96.60% accumulative mortality after 120 hrs. Moreover, all the essential oils were highly safe for adult honeybee workers under laboratory conditions.

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