J. Plant Prot. and Path., Mansoura Univ., Vol. 5 (6): 687 - 695 , 2014

EVALUATION OF SOME NATURAL OILS FOR CONTROLLING VARROA MITE *(Varroa destructor)* INFESTING HONEYBEE COLONIES IN SOME EGYPTIAN GOVERNORATES

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ABSTRACT

The study was conducted in four governorates namely Dakahlia, Behera, Gharbia and Alexandria, in order to study the effect of some natural substances on varroa mites, which affects honeybee colonies during the period from the first of August to the end of September, 2013. Results showed that the average percentage reduction in the incidence of varroa mite was 79.66%, 77.43%, 77.04% and 79.88% in the governorates of Dakahlia, Behera, Gharbia and Alexandria, respectively (in the brood + on adult bees) when using clove oil and menthol oil with concentration (12.5% ، 25%). While the percentage of reduction of using eucalyptus oil in the same concentrations 65.6%, 73.8%, 70.9% and 66.73% for the previous governorates. From the previous findings it is advised to using one of these oils preferably clove oil for controlling varroa mite.

Keywords: Honeybee, Varroa mite, Essential oils, Clove, Camphor, Menthol, Reduction.

INTRODUCTION

The serious ectoparasitic mite, *Varroa destructor* Anderson & Trueman (2000) (formerly *V. jacobsoni* Oud.), (Acari: Varroidae) has become a serious pest of the honey bee, *Apis mellifera* and *Apis cerana* (Hymenoptera: Apidae) worldwide. The disease caused by the mites is called ‘‘varroatosis’’. Varroa mites damage immature and adult bees by feeding on hemolymph, thus, greatly weakening or killing the bees (Weinberg and Madel, 1985).

Migratory beekeeping, importation of colonies packages of bees and queens are considered to be the possible ways of varroa spread. The spread of varroa with in colonies is due to swarming, robbing and foraging. A significant mite infestation may lead to the death of an entire honey bee colony. The varroa mite is thus considered as the parasite with the most pronounced economic impact on the beekeeping industry (Guzman-Novoa *et al*., 2010). The use of synthetic acaricides has been the major effective method against varroa mites at bee colonies. But the intensive use of these acaricides resulted in the development of resistance and reduction of their efficacy (Milani, 1999). In addition, the remaining of these chemicals has numerous adverse effects on environment (Wallner, 1995; Kochanskg and Wilzer, 2001) and lead to contamination of colony products, especially wax and possibly honey, as well (Wallner, 1999). Therefore, there are urgent needs to search for new pesticides with a new or different mode of action and/or improved effectiveness against varroa mites and safe to honey bees. In this respect, there are considerable interests, nowadays, in the use of

natural products for controlling parasitic bee mites (Jacobson, 1983; Colin, 1990; Liu and Nasr, 1993; Kraus and Berg, 1994; Calderone and Spivak, 1995; Xie *et al*., 1995; Calderone *et al*., 1997; Hagigatian, 2000). The natural climatic conditions and the beekeeping technology in the Arab Republic of Egypt allow for the successful application of the alternative means and methods for fighting against varroatosis. These circumstances allow for development and testing of the effectiveness of new preparations based on acids, essential oils and other natural substances under the conditions of our country and to register them as veterinary medicinal preparations (Gurgulova *et al*., 2004). The application of some plant extracts or essential oils based products against infested apiaries were found able to maintain mite infestation rates below economic injury levels (Calderone *et al*., 1997 and Hagigatian, 2000). In addition, a high insecticidal efficacy has been attributed to several plant extracts (Shaddel-Telli *et al*., 2008) and especially those of meliaceous species such as the highly reputed neem tree, *Azadirachta indica* and including certain members of the genus Swietenia (Mikolajczak and Reed, 1987; Jimenez *et al*., 1997; Omar *et al*., 2007).

The objective of this study was designed to detect the assessment of the miticidal effect of some natural oils (clove, menthol, camphor) against *V. destructor* in field experiments at different governorates.

MATERIAL AND METHODS

The experiment was carried out in four governorates: in apiaries in Manzala, Dakahlia governorate; Mehala, Gharbia governorate; Sabaheia, Alexandria governorate and El-Dalangat, Behera governorate during the period of 1/8/2013 to 28/9/2013 to study the effect of some natural substances for controlling *Varroa destructor* in honeybee colonies.

1. Dates and places of treatments application: as shown in table (1)

Apiary	Governorate	District	Date	
			from	to
1	Dakahlia	Manzala	25/8/2013	26/9/2013
2	Gharbia	Mehala	16/8/2013	17/9/2013
3	Alexandria	Sabaheia	1/8/2013	1/9/2013
4	Behera	El-Dalangat	29/8/2013	29/9/2013

The strength colony contains at least nine frames covering with bees (1^{st} hybrid Carniolan). These colonies were headed with equal queen ages. Twenty one honey bee carniolan colonies were selected. The colonies have been divided into 7 groups (each of 3 colonies). Colonies in each group were in high infested with Varroa mite.

2. Preparation of the experimental colonies:

Twenty one honeybee colonies (in each governorate, 18 for treated and 3 colonies as untreated) first hybrid Carniolan were infested with Varroa mite, the experimental colonies were divided into seven groups each composed of three colonies.

3. Preparing the natural substances used:

Such groups were treated as follows:

Group (1): Menthol oil 12.5% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline, 1:1) containing 12.5% Clove and put in aluminum foil.

Group (2): Menthol oil 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline) containing 25% Clove and put in aluminum foil.

Group (3): Camphor oil 12.5% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline) containing 25% Camphor and put in aluminum foil.

Group (4): Camphor oil 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline) containing 25% Camphor and put in aluminum foil.

Group (5): Clove oil 12.5% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline) containing 12.5% Clove (12.5 g.) and put in aluminum foil.

Group (6): Clove oil 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 20g of (talc powder+ Vaseline) containing 25% Clove and put in aluminum foil.

Group (7): Untreated colonies (Control).

The Clove and Camphor were obtained from El Gomhouria Co; whereas the Menthol was obtained from El Nasr Pharmaceutical chemicals Co. Abou Zabal. The plates were placed on the top of board facing the brood chamber after open cover the plate to allow the bees to enter the plate and remove the product. The treatment period was four weeks from August 2013 till September, 2013. The honeybee colonies were fed on sugar syrup (sugar 1:1 water) once/week. The bottom board of the hive was covered with a plastic sheet coated with raw Vaseline to capture the fallen mites. Died Varroa mites were counted and removed at the end of each treatment. One plate was placed for each colony and the treatment was repeated every 10 days for 3 times for each concentration.

4. Determination of Varroa infestation:

4.1. On adult workers:

The percent infestations of Varroa mite on workers before and after treatments were determined according to Korneili (1988). Accordingly, samples of hundred bees/colony were collected randomly in vial partially filled with water containing few drops of detergent. The samples were shaken and the bees were washed in a strainer, individual mites that fell, off from worker bees were found at the bottom of the white container (Ritter, 1981). All worker bees and mite were counted for each sample, where the number of mites/100 workers was calculated. The fallen varroa on the plastic sheet was counted periodically every 72 hours, one week, two weeks and three weeks from the beginning of the treatment till the end.

4.2. In brood cells:

The infestation percent of varroa mites in brood cells was determined by using twenty five worker cells (square inch) randomly that were opened

and the Varroa mite occurring with these cells were counted. Reduction percentage in mite infestation was calculated according to **Henderson and Tilton (1955)** equation:

% Reduction of infestation = 1 – {(Ta x Cb) / (Tb x Ca)} x 100

Where: T=% infestation of treated mites and C=% infestation of untreated mites (a=after; b=before treatment).

RESULTS AND DISCUSSION

1. Efficiency of some natural oils against Varroa mite in different governorates:

1. In Dakahlia governorate:

In Dakahlia governorate, from table (2) it could be concluded that menthol (12.5%) on the shape plates as pest form caused reduction of infestation being 64.74% and 77.69% for brood cells and adult, respectively. The mean reduction of infestation reached 71.22% for both brood and adult. In addition, Camphor (12.5%) caused reduction of infestation being 63.97% and 79.51% for brood cells and adult, respectively. The mean reduction of infestation reached 75.33% for both brood and adult. Meanwhile, Clove (12.5%) caused reduction of infestation being 67.26% and 75.35% for brood cells and adult, respectively. The mean reduction of infestation reached 71.31% for both brood and adult. Clove oil 12.5% gave the highest reduction percentage with 79.88% and menthol 12.5% was the least with 61.08%, respectively.

Table (2). Effect of some natural oils for reduction infestation of varroa mite in Dakahlia governorate, 2013.

Treatments	% Infestation before treatment		% Infestation after treatment		% Reduction		% Mean Reduction	LSD 5%
	brood	Adult	brood	adult	brood	adult		
menthol 12.5%	13.62	10.27	6.67	4.09	64.74	77.69	71.22	6.32
menthol 25%	12.0	11.04	5.33	4.33	68.02	78.04	73.03	17.04
camphor 12.5%	13.33	10.73	6.67	7.56	63.97	60.54	65.26	11.96
camphor 25%	14.67	12.77	8.00	8.20	60.74	64.04	62.39	13.28
clove 12.5%	14.67	11.88	6.67	5.23	67.26	75.35	71.31	12.79
clove 25%	16.0	13.25	5.33	3.95	76.02	83.3	79.66	19.66
Control	13.33	10.75	18.67	19.18	0.0	0.0	0.0	4.63

1. In Behera governorate:

Table (3). Effect of some natural oils for reduction infestation of varroa mite in Behera governorate, 2013

Treatments	% Infestation before treatment		% Infestation after treatment		% Reduction		% Mean Reduction	LSD 5%
	brood	adult	brood	adult	Brood	adult		
menthol 12.5%	11.66	6.53	6.0	3.16	64.56	69.5	67.03	6.30
menthol 25%	13.33	9.66	5.33	2.83	73.16	81.7	77.43	9.50
camphor 12.5%	17.83	8.5	8.7	2.73	69.4	78.2	73.8	14.12
camphor 25%	16.33	9.1	8.5	2.8	63.63	79.47	71.55	8.38
clove 12.5%	12.83	8.97	6.7	3.73	63.23	73.17	68.2	10.54
clove 25%	14.66	9.63	8.5	3.66	58.13	76.83	67.48	11.27
Control	14.17	6.63	20.33	10.47	0.00	0.00	0.00	3.48

In Behera governorate it was noticed that using 25% menthol oil gave the highest reduction percentage of infestation being 73.16% and 81.7% for brood cells and adult bees, respectively. The mean reduction percentage for both brood and adult was 77.43% in both applications. On contrary, 12.5% menthol oil was the inferior of the reduction percentage of infestation for adult and the mean reduction of infestation giving 69.5% and 67.03%, respectively. On the same hand, 25% clove oil was the inferior of the reduction percentage of infestation for brood cells with 58.13%. It's cleared that clove oil was outstanding in the mean reduction percentage of infestation in Gharbia, Dakahlia and Alexandria governorate being 77.04, 79.66 and 79.88%, respectively.

Meanwhile, 12.5% menthol was the least of the mean reduction infestation for both brood and adult giving 61.08 and 67.93% in both Alexandria and Behera governorate, respectively. In contrast, 25% camphor oil gave the least mean reduction of infestation with 62.39% for both brood and adult in Dakahlia governorate. The same trend was observed for 12.5 Camphor oil being 64.7% in Gharbia governorate. These findings are in are in agreement with Allam, 1999 who recorded 71.80% and 64.3% efficiency of clove sprayed during August-September in two successive season respectively, while smoking eucalyptus, peppermint and clove oil gave variable range (45.5%-92.2%). Many researchers reported gradual mites fall after treating colonies with organic acids or volatile oils and recorded variable ranges (Ghoniemy and Abo-Zaid, 1993 and Ismail *et al*., 2006).

1.In Gharbia governorate:

Table (4). Effect of some natural oils for reduction infestation of varroa mite in Gharbia governorate, 2013.

Treatments	% Infestation before treatment		% Infestation after treatment		% Reduction		% Mean Reduction	LSD 5%
	brood	Adult	brood	adult	brood	adult		
menthol 12.5%	12.0	14.04	5.33	6.13	66.68	65.07	65.88	9.46
menthol 25%	16.0	19.53	6.67	7.31	68.73	70.05	69.39	16.35
camphor 12.5%	14.67	17.63	6.67	8.02	65.80	63.6	64.7	13.70
camphor 25%	13.33	17.53	5.33	6.18	70.01	71.79	70.9	6.37
clove 12.5%	14.67	18.33	6.67	6.91	65.89	69.84	67.87	22.30
clove 25%	17.33	18.93	5.33	5.41	76.93	77.14	77.04	25.11
Control	16.0	17.73	21.33	22.23	0.0	0.0	0.0	9.16

1.4. In Alexandria governorate:

Table (5). Effect of some natural oils for reduction infestation of varroa mite in Alexandria governorate, 2013

Treatments	**% Infestation before treatment**		**% Infestation after treatment**		**% Reduction**		**% Mean Reduction**	**LSD 5%**
	brood	adult	brood	adult	brood	adult		
menthol 12.5%	14.33	7.66	10.0	3.66	45.33	76.83	61.08	14.81
menthol 25%	13.0	11.0	6.0	3.33	66.53	81.63	74.08	20.67
camphor 12.5%	18.0	15.0	11.33	7.0	51.83	72.0	61.92	16.16
camphor 25%	13.33	10.0	6.66	5.66	60.43	73.03	66.73	12.60
clove 12.5%	11.0	13.33	4.0	3.66	72.43	87.33	79.88	12.49
clove 25%	24.66	14.0	12.0	5.0	61.66	82.93	72.29	23.15
Control	18.7	9.33	24.33	20.0	0.00	0.00	0.00	4.98

Essential oils and their components can be effectively used to dispel ticks and mites, both parasitic and free-living (Saad *et al.*, 2006). Imdorf *et al*. (1995) studied the toxic effects of volatile substances on varroa and bees. They found that the air concentration which killed nearly 100% of varroa without noticeable loss of bees was found to be between 5 and 15µg/L for thymol, between 50 and 150 µg/l for camphor and between 20 and 60 µg/l air for menthol. 240 µg/l eucalyptol produced 100% varroa mortality but also 25% bee mortality.

Camphor and menthol also possess the necessary characteristics of an efficient varroacides. Eucalyptol however, is not very suitable for Varroa treatment since its rate of evaporation is difficult to control and only a small difference between its toxicity for Varroa and for bees was observed. This results similarity with Imdorf *et al.* (1995). It is advisable to use Thymol, Menthol, Camphor and mixture of (Thymol, Menthol and Camphor) on the shape plates on strong colony but it was recommended not to use on weak colonies at high temperature greater than 18.5 °C and this results coincide with Alessandra *et al.* (2004).

Results showed that Clove oil was harmless to the honey bees at the concentrations applied. The recent development of resistance to miticides in *V. destructor* populations has caused great concern to the honey bee industry (Elzen *et al.*, 1998; Milani, 1999). The treatments of observation hives with clove oil have the potential to control *V. destructor* in honey bee colonies. Clove oil appeared to be harmless to honey bee workers and brood. Ample research has shown that an IPM-based approach to varroa mite control is more economical than the conventional methods heavily relying on chemical pesticides (Ismail *et al.*, 2006). For example, using any combination of the non-chemical varroa controls mentioned above can lower varroa populations in a colony under economic threshold.

On conclusion, its recommended using clove oil 25% for controlling varroa mite infestation and it could be a useful component of an integrated pest management program (IPM) for the honeybee industry (Elzen *et al*., 1998 and Milani, 1999). As a result there is an urgent need for alternative control strategies that are cost effective, environmentally friendly and with no mammalian toxicity.

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تقييم فعالية بعض الزيوت الطبيعية لمكافحة حلم الفاروا الذى يصيب طوائف نحل العسل فى بعض المحافظات المصرية

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أجرى هذا البحث فى أربع محافظات وهى الدقهلية، البحيرة، الغربية و الإسكندرية، بهدف دراسة تأثير بعض المواد الطبيعية على حلم الفاروا الذى يصيب طوائف نحل العسل وذلك خلال الفترة من الأول من أغسطس إلى نهاية شهر سبتمبر 2013. أوضحت النتائج أن متوسط نسبة الخفض فى الإصابة بحلم الفاروا كانت 79.66%، 77.43%، 77.04% و 79.88% فى محافظات الدقهلية، البحيرة، الغربية و الإسكندرية، على التوالى (فى الحضنة+على النحل البالغ) عند إستخدام زيت القرنفل وزيت المنتول بتركيزى (12.5، 25%). بينما بلغت نسبة الخفض 65.6%، 73.8%، 70.9%، 66.73% بإستخدام زيت الكافور بنفس التركيزات السابقة وبترتيب المحافظات السابقة. ومن النتائج السابقة ينصح بإستخدام أحد هذة الزيوت ويفضل زيت القرنفل فى مكافحة حلم الفاروا.