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Control of Varroa Mite by Essential Oils and Formic Acid with Their Effects on Grooming Behaviour of Honey Bee Colonies

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ABSTRACT

Four essential oils in two concentrations (50 and 100%) of thyme, *Thymus vulgaris*; Cinnamon, *Cinnamomum zeylanicum*; Lemon grass, *Cymbopagon flexuosus*; Anise, *Pimpinella anisum* and formic acid (65%) were used against varroa mite, *Varroa destructor* infesting honey bee colonies. Results show that, formic acid and the highest concentration (100%) of tested essential oils caused effective control of varroan mite, whereas the reduction percentage of Formic acid, Lemon grass, Cinnamon, Thyme and Anise oils recorded more than 96.6% after the end of treatments on both worker brood and adult workers. Concerning to the grooming behaviour of honey bee colonies against varroa mite in treated and untreated colonies, results indicated that, Thyme, Anise, Cinnamon, Lemon grass oils resulted in 44.8, 41.6, 40 and 38.1% of damaged varroa, respectively, while the Formic acid caused 37.9%. The highest concentration of tested oils resulted in high amount of sealed worker brood particularly in Anise and Lemon grass oils, while, the Cinnamon oil and Formic acid recorded the lowest one. Tested oils and Formic acid caused high number of wax foundation and honey yield in the treated colonies compared with control colonies.
KEY WORDS: Essential oils, honey bee, varroa mite, Formic acid, grooming behaviour.

INTRODUCTION

The ectoparasitic mite *Varroa destructor* (Anderson and Trueman 2000) is considered a severe pest for honey bees causing serious losses to the beekeepers (de Jong *et al*., 1982 and Baker and Peng, 1995). The mite can be found on adult bees, on the brood, and in hive debris. In a heavy infestation, pupae may not develop into adult bees. The adults that do emerge may weigh less than healthy bees (Shimanuki and Knox, 1991). Advanced infestations cause considerable damage to honey bee populations (Baker and Peng, 1995) resulting in a reduction of the quantity and quality of honey production and pollination (Spivak, 1997). Control treatments of the mite are based on the use of acaricides, produced in especialised laboratories as well as substances of natural origin (Calderone and Spivak, 1995a; Sammataro *et al.*, 1998). The repeated use of synthetics varroacides results in the development of resistance in the varroa mite to these products. Varroa population resistant to amitraz was found in the end of the 20th century (Elzen *et al*., 1999) and mite resistant has been reported for almost all chemical substances used (Pohorecka and Bober, 2007; Spreafico *et al*., 2001; Thompson *et al*., 2002). There is current concern about contamination of bee products with synthetic substances against the varroa (Howis, and Nowakowski, 2009).

It is now crucial that beekeepers have access to new control tactics that do not harm bees or contaminate hive products. Ethereal plant oils, also known as essential oils, and other volatile compounds have been used to control bee mites with some success (Ariana *et al*., 2002; Calderone and Spivak, 1995b; Colin *et al*., 1997; El-Shemy, 1997; Imdorf *et al*., 1995; Ruffinengo *et al*., 2007; Sammataro *et al*., 2004). Recently, two commercial essential oil products (Apiguard® and ApiLife Var®) for *Varroa destructor* control have become available in the USA. Both products kill phoretic mites on adult bees through the slow release (Imdorf *et al*., 1999) via sublimation during routine hygienic and trophallaxis bee behaviour, of thymol and other essential oil vapours from crystals embedded in a polyacrylic acid gel matrix. Thymol has been approved by the Environmental Protection Agency as an acaricide against *V. destructor*, and no adverse effects on humans have been found (Whittington *et al*., 2000).

The present investigation aimed to determine the effective control of varroa mite by using some essential oils and Formic acid with their effects on grooming behaviour of honey bee colonies.

MATERIALS AND METHODS

This work was conducted during the period from 24th December 2010 to 5th of March 2011 in private apiary at Ahnasia province, Beni Sauef Governorate, Egypt. Thirty honey bee *Apis mellifera* L. colonies of

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Carniolian hybrid naturally infested by varroa mite were used for this study. Three colonies were used for each concentration of essential oils and Formic acid and the three untreated colonies were left as control.

Four essential oils of thyme; *Thymus vulgaris*, Cinnamon; *Cinnamomum zeylanicum*, Lemon grass; *Cymbopagon flexuosus* and Anise; *Pimpinella anisum* L. were used at concentration of 50 and 100% for each tested oil. While, the Formic acid as a chemical acaricide was applied at concentration of 65%. Five ml of each tested essential oils and Formic acid was applied, which enough to saturate one stripe of cotton (1x2cm) and held in one comb between the brood nest of each tested colony. Each tested colony was treated four times at 12 days intervals from the beginning of experiment.

The percentage of varroa infestation in the tested honey bee colonies during the experimental period, were determined in worker brood cells and adult workers. In worker brood, 50 sealed worker cells were randomly selected from the brood nest and opened (pupae with pigment eyes), the pre-pupae or pupae inside it were carefully examined and adult female mites on it were counted. In adult workers, the infestation percentage was determined in approximately 100 living worker bees taking directly from the combs of each tested colony (De Jong, 1988). The infestation percentage in worker brood cells and adult bees were determined two times at 3 days intervals before applying the treatments, while during the treatments they were determined at 12, 24, 36 and 48 days post treatment. The reduction percentages of varroa infestation were calculated from the collected data according to the equation of Henderson and Tilton (1955).

Dead varroa mite and bees fallen on the bottom board, covered with sheet of white paper were collected and counted in all tested colonies (treated and control) at 12 days before treatments and 12, 24, 36 and 48 days post treatment after Abd El-Wahab and Ebada (2006).

To detect the grooming behaviour in the treated and untreated colonies; the collected varroa mite on the bottom broad were carefully examined under dissecting microscope at 10X magnification. The injury mite legs or bodies, that caused by grooming behaviour of bees were recorded and calculated at 12, 24, 36 and 48 days post treatment according to Boecking and Spivak (1999) and Flottum (1997).

The following biological activities were measured for each tested colony during the experimental period, *i.e.*, the number of combs covered with bees and activity of wax foundation in square inches were determined at 12 days intervals according to Nour (1992). While, area of sealed worker brood was measured in square inches at 12 days intervals according to the technique of Fresnay (1962). At the end of experimental honey yield was measured as Kg /colony in all tested honey bee colonies.

Analysis of variance (ANOVA) was carried out by using F-test and at least significant difference (L.S.D.) was used to compare between the different treatments (Fisher, 1950 and Snedecor and Chochran, 1972).

RESULTS AND DISCUSSION

The effect of experimented essential oils and Formic acid (65%) against infestation with varroa mites is shown in Table (1). Data indicated that the percentage of varroa mite infestation on sealed brood and adult workers was clearly reduced after the end of treatments (fourth treatment) in all experimented essential oils and Formic acid. After the second and following treatments a significant differences were showed in the mean percentage of infested worker brood and adult workers between the treated colonies with all tested materials and control ones. In the colonies treated with essential oils and Formic acid the percentage of infestation with varroa mites on the worker brood and adult workers reduced gradually from the first to the fourth week (end of the treatment) after the treatments. Formic acid 65% and the highest concentrations (100%) of tested essential oils caused a highly effective in controlling varroa mites, whereas the reduction percentages of Formic acid, Lemon grass, Cinnamon, Thyme and Anise oils recorded (99.3&100%), (99.1&98.5%), (98.8&98%), (98.3&97.8) and (96.6&96.9%) after the fourth treatment on the worker brood and adult workers, respectively.

From the foregoing results it could be concluded that the experimented essential oils were more effective against varroa mite infestation and could provide an attractive tool in an integrated pest management program for varroa control in honey bee colonies.

The obtained data are agreement with the finding of Eguaras *et al*. (1996) and Allam *et al*. (2003), they found that formic acid killed 91.7% of the varroa mites and honey bee colonies became more active with large number of worker brood area were detected. Abd El-wahab and Ebada (2006) observed that, the mean percentage of varroa infestation on the worker brood and adult workers reduced to 100% after the fourth week of treatment with *Citrus aurantium* L. (Sour Orange) and *Cymbopagon flexuosus* (Lemon grass). According to the investigations of Hamaad *et al*. (2008) they found that the Thyme oils spray resulted 65.9% of varroa mite mortality and it has potential for varroa mite control under Egyptian conditions. Also, Daher-Hjaij and Alburaki (2006) observed high mite mortality when beehives were fumigated with extracts of seeds of Anise *Pimpinella anisum*. According to studies of Noel and Amrine (1996) they summarized that during the late fall and early winter the varroa mite is very susceptible to control by essential oils due to the formation of a cluster and to the lack of brood. By treating hives with the essential oils, the varroa mites will have no place to hide and all can be killed.

Table (1): The reduction percentages of varroa mite infestation on worker brood and adult workers in honeybee colonies treated with different concentrations of tested essential oils and formic acid.

Tested essential oils and formic acid		Mean percentage of varroa infestation on worker brood				Mean percentage of varroa infestation on adult workers			
		Concentration of tested oils				Concentration of tested oils			
		50%	% of reduction	100%	% of reduction	50%	% of reduction	100%	% of reduction
Thyme oil	**Before treatment**	**15**	---	**18.6**	---	**5.6**	---	**5.3**	---
	First treatment	7.3 **ab**	71.9	6.6 **ab**	79.4	4.1 **a**	54.3	2.7 **a**	**67.2**
	Second treatment	3.2 **c**	87.3	2.6 **cd**	91.5	1.3 **d**	84.1	1.4 **d**	**82.1**
	Third treatment	1.9 **e**	93.5	1.5 **f**	95.9	0.6 **g**	95.9	1.0 **f**	**93.4**
	Fourth treatment	1.0 **g**	96.7	0.6 **h**	98.3	0.6 **i**	96.1	0.3 **h**	**97.8**
Cinnamon	**Before treatment**	**15**	---	**15**	---	**3.8**	---	**5.6**	---
	First treatment	6.0 **a**	76.9	3.6 **a**	85.9	1.6 **b**	72.1	4.5 **b**	**48.7**
	Second treatment	2.6 **c**	89.4	1.3 **c**	94.7	2.5 **de**	55.5	1.4 **d**	**83.2**
	Third treatment	2.2 **e**	92.5	3.0 **f**	90.3	2.1 **g**	80.5	1.1 **f**	**93.2**
	Fourth treatment	0.6 **g**	97.8	0.3 **h**	98.8	1.1 **i**	90.1	0.3 **h**	**98**
Lemon grass oil	**Before treatment**	**20**	---	**18.6**	---	**4.6**	---	**7.3**	---
	First treatment	6.0 **a**	82.7	3.6 **a**	88.7	2.8 **ab**	61.7	5.4 **bc**	**52.8**
	Second treatment	2.6 **c**	92.1	1.6 **c**	97.4	3.1 **e**	55.9	2.6 **d**	**75.3**
	Third treatment	2.4 **e**	94	1.6 **f**	95.5	2.9 **g**	78.5	0.8 **f**	**95.9**
	Fourth treatment	0.6 **g**	98.4	0.3 **h**	99.1	0.9 **i**	93.2	0.3 **h**	**98.5**
Anise oil	**Before treatment**	**10.3**	---	**14.3**	---	**2.9**	---	**3.6**	---
	First treatment	9.6 **ab**	46.1	10.6 **b**	57.2	2.5 **ab**	43.8	2.3 **a**	**59.4**
	Second treatment	4.6 **c**	73.2	2.7 **cd**	88.6	1.1 **d**	72.7	1.4 **d**	**73.5**
	Third treatment	3.86 **e**	81.3	1.5 **f**	94.7	1.1 **g**	86.6	0.6 **f**	**93.1**
	Fourth treatment	2.3 **g**	89.1	1.0 **h**	96.6	0.66 **i**	92.2	0.3 **h**	**96.9**
Formic acid (65%)		**Formic acid 65%**		---	---	**Formic acid 65%**		---	---
	Before treatment	**20**	---	**20**	---	**4.3**	---	**4.3**	---
	First treatment	10 **b**	71.2	10 **b**	71.2	1.3 **b**	80.4	1.3 **a**	**80.4**
	Second treatment	5.3 **c**	84.2	5.3 **d**	84.2	1.2 **d**	80.6	1.2 **d**	**80.6**
	Third treatment	2.1 **e**	94.8	2.1 **f**	94.8	0.9 **g**	92.7	0.9 **f**	**92.7**
	Fourth treatment	0.3 **g**	99.1	0.3 **h**	99.3	0.0 **i**	100	0.0 **h**	**100**
Control	**Before treatment**	**6.3**	---	**6.3**	---	**4.3**	---	**4.3**	---
	First treatment	11.0 **b**	---	11**b**	---	6.8 **c**	---	6.8 **ac**	---
	Second treatment	10.6 **d**	---	10.6 **e**	---	6.3 **f**	---	6.3 **e**	---
	Third treatment	12.6 **f**	---	12.6 **g**	---	12.3 **h**	---	12.3 **g**	---
	Fourth treatment	13.0 **h**	---	13 **i**	---	12.6 **j**	---	12.6 **i**	---
L.S.D.	First treatment	4.0	---	6.6	---	1.8	---	1.8	---
	Second treatment	4.2	---	3.7	---	1.3	---	1.4	---
	Third treatment	2.4	---	4.1	---	2.6	---	2.5	---
	Fourth treatment	**2.5**	---	**1.4**	---	**1.8**	---	**1.4**	---

Mean followed with the same letter(s) in a single treatment are not significantly different at 5% level of probability.

Results obtained in Table (2) indicate that the highest number of dead varroa fallen on the sheet was recorded after the end of the first treatment and markedly decreased by the following treatments until the end of treatments particularly in concentrations 100% of the almost experimented essential oils and 65% of Formic acid. While, in the control colonies the fallen varroa clearly increased. Thyme oil recorded the highest number of varroa fallen on the sheet followed by Formic acid, Lemon grass, Anise and Cinnamon, represented by 125, 79, 76, 72 and 65 individuals, respectively.

Concerning to the grooming behaviour of honey bee colonies against varroa mites in treated and untreated colonies, the data in Table (2) indicate that the concentration of 100% of the experimented essential oils showed the highest percentage of damaged varroa in comparison with untreated colonies (control). Whereas, Thyme, Anise, Cinnamon, Lemon grass oils resulted 44.8, 41.6, 40 and 38.1% of damaged varroa after the end of treatments, respectively, while the Formic acid caused 37.9% of damaged varroa.

Increasing the mean number of varroa mite fallen on the sheet in tested honey bee colonies treated with essential oils may be due to the activation of the defense behaviour mechanisms of honey bee workers by these plant oils against varroa mite. Zakaria and Allam (2007) recorded that the mean number of cutting varroa bodies by bee workers was 18.6% with formic acid treatment, while in colonies treated with black cumin oil was 14.1% in comparison with control which recorded 4.3% of varroa fallen on the sheet. Salem *et al*. (1998) found that the honey bee colonies which fed on Neem extract showed the highest mean number of varroa mite fallen on the sheet. They suggested that these extracts caused changes in the haemolymph of honey bee workers and therefore increased the number of varroa mite fallen on the sheet. Abd El-Wahab (2001) stated that some defense

behaviour mechanisms against varroa mite were detected in some races and hybrids of honey bees. These mechanisms resulted in increasing the number of fallen varroa mite on the bottom board of bee hive.

Table (2): The effect of different concentrations of tested essential oils and formic acid on the number of dead and damaged varroa mites fallen on the sheet

Tested essential oils and formic acid		Concentration of tested oils							
		50%				100%			
		No. of fallen varroa	No. of varroa damage	% of varroa damage	No. of dead bees	No.of fallen varroa	No. of varroa damage	% of varroa damage	No. of dead bees
Thyme oil	**Before treatment**	**26**	**8**	**30.7**	**0.0**	**30**	**9**	**30**	0.0
	First treatment	33	16	48.4	0.0	39	16	41.2	0.0
	Second treatment	37	11	29.7	0.0	35	19	54.3	0.0
	Third treatment	26	10	38.5	0.0	29	15	51.7	0.0
	Fourth treatment	16	6	37.5	0.0	22	6	27.2	1
	Total	**112**	**43**	**38.3**	**0.0**	**125**	**56**	**44.8**	**1**
Cinnamon	**Before treatment**	**11**	**2**	**18.1**	**0.0**	**8**	**2**	**25**	0.0
	First treatment	18	6	33.3	0.0	10	5	26.3	0.0
	Second treatment	11	3	27.2	0.0	14	6	42.8	0.0
	Third treatment	6	2	33.3	2	17	5	29.4	1
	Fourth treatment	9	2	22.2	0.	24	10	41.6	0.0
	Total	**44**	**13**	**29.5**	**2**	**65**	**26**	**40**	**1**
Lemon grass oil	**Before treatment**	**12**	**2**	**16.6**	**2**	**11**	**3**	**27.2**	**0.0**
	First treatment	19	4	21.05	2	22	10	45.4	0.0
	Second treatment	9	2	22.2	0.0	10	3	30	1
	Third treatment	8	2	25	0.0	30	10	33.3	0.0
	Fourth treatment	12	4	33.3	2	14	6	42.8	0.0
	Total	**48**	**12**	**25**	**4**	**76**	**29**	**38.1**	**1**
Anise oil	**Before treatment**	**18**	**5**	**27.7**	**0.0**	**10**	**3**	**30**	**0.0**
	First treatment	20	6	30	2	18	8	44.4	0.0
	Second treatment	15	5	33.3	0.0	17	9	52.9	0.0
	Third treatment	12	3	25	0.0	26	9	34.6	1
	Fourth treatment	10	2	20	0.0	11	4	36.3	0.0
	Total	**57**	**16**	**28.7**	**2**	**72**	**30**	**41.6**	**1**
Formic acid (65%)		**Formic acid 65%**				**Formic acid 65%**			
	Before treatment	**10**	**2**	**20**	**2**	**10**	**2**	**20**	1
	First treatment	18	6	33.3	2	18	6	33.3	1
	Second treatment	26	13	50	0.0	26	13	50	0.0
	Third treatment	21	6	28.5	2	21	6	28.5	1
	Fourth treatment	14	5	35.7	1	14	5	35.7	1
	Total	**79**	**30**	**37.9**	5	**79**	**30**	**37.9**	**3**
Control	**Before treatment**	**20**	**4**	**20**	**2**	**20**	**4**	**20**	**2**
	First treatment	22	4	18	2	22	4	18	2
	Second treatment	14	3	21.4	2	14	3	21.4	2
	Third treatment	16	4	25	1	16	4	25	1
	Fourth treatment	26	5	19.2	1	26	5	19.2	1
	Total	**78**	**19**	**20.9**	6	**78**	**19**	**20.9**	**6**

Results in Table (2) show that the experimented essential oils had insignificant adverse effect on the worker bees in comparison with control colonies. Through the experimental period tested oils caused very low toxicity to the worker bees (1 died bee/each oil), as well as Formic acid (3 died bees) in the treated colonies compared with control colonies (6 died bees). These results indicated that the treated colonies with tested products keep the social life of honey bee away from any harmful effect (Whittington *et al.*, 2000).

The present findings are in agreement with Dimetry *et al.* (2005) who found that the Neem-Azal-T/S and Citrullus extracts caused low toxicity to the worker bees compared with the control colonies, while, the Vapcomic (a commercial formulation of Abamectin 1.8%) caused a highest number of dead bees.

Concerning the data in Table (3), the honey bee colonies treated with essential oils and Formic acid recorded remarkable increase of the sealed worker brood, the number of combs covered with bees, wax foundation and honey yield in comparison with control colonies during the experimental period. The highest concentration (100%) of the experimental essential oils resulted highly area of sealed worker brood particularly in Anise oil (110.6 inch^2) and Lemon grass oil (99.9 inch^2), while, the Cinnamon oil recorded the lowest value (90 inch^2). In the colonies treated with Formic acid the mean area of worker brood was 90 inch^2. Tested oils and Formic acid caused a highly number of wax foundation and honey yield (Kg) in the treated colonies compared with control colonies; however, the Cinnamon oil produced the lowest values especially in wax foundation. Increasing in the mean area of sealed worker brood and the other biological activities in colonies treated with

100% of tested oils and Formic acid resulted from reducing the infestation of varroa mite by theses tested components.

Table (3): The effect of tested essential oils and formic acid 65% on some biological activities in treated honey bee colonies.

Concentration (%) of tested essential oils and formic acid		Biological activities before treatments		Biological activities after the end of treatments			
		Mean brood area (inch²)/colony	Mean no. of combs covered with bees/colony	Mean brood area (inch²)/colony	Mean no. of combs covered with bees/colony	Mean no. of wax foundation/colony	Honey yield (Kg)/colony
Thyme oil	50	99.0	4.6	199.8	8.66	2.66	4.3
	100	109.9	5.3	229.9	9.0	3.0	4.3
Cinnamon	50	99.0	4.3	199.6	8.33	2.0	4.0
	100	90.0	4.6	210.0	8.33	2.0	4.3
Lemon grass oil	50	90.0	4.6	218.6	8.33	2.66	4.0
	100	99.9	4.6	229.9	8.66	2.66	4.3
Anise oil	50	95.3	5.3	217.6	8.33	2.0	4.0
	100	110.6	5.0	221.3	8.66	2.66	4.3
Formic acid (65%)	65	90.0	6.0	249.6	9.33	3.0	4.3
Control	---	98.0	4.6	39.6	6.0	0.3	3.0

Generally, the majority of honey bee activities increased gradually after the treatments as a result of curing the colonies from infestation by using the experimental essential oils. In all cases control of varroa mite using naturally plant products are more recommended than other chemical acaricides to keep the social life of honey bee away from any harmful effect (Dimetry *et al.*, 2005, El- Nabarawy *et al*., 2001and Whittington *et al*., 2000).

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