

Evaluation of Some Volatile Plant Oils and Mavrik Against *Varroa destructor* in Honey Bee Colonies

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Abstract: Different concentrations (25%, 50% and 100%) of *Citrus aurantium* L (Sour Orange), *Cymbopogon flexuosus* (Lemon grass) and Citronella volatile oils and Mavrik were used against varroa mites in honey bee colonies. The percentage of infestation by varroa mites on worker brood and adult workers, number of dead varroa fallen on the sheet and amount of brood rearing were determined in the tested colonies. The highest concentrations (100%) of tested volatile oils caused a highly effective in controlling varroa mites. 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), *Cymbopogon flexuosus* (Lemon grass) and after the third week of treatment with Citronella oils. Mavrik strips caused highest reduction of varroa in the worker brood cells. The highest concentrations of tested volatile oils caused highly amount of sealed worker brood particularly in Citronella oil as well as in Mavrik strips

Key words: Honey bee colonies, varroa mite, volatile plant oils and mavrik

INTRODUCTION

The varroa mite, *Varroa destructor* was described in its original host *Apis cerana* in tropical Asia^[29]. Its developmental cycle and reproduction inside the capped cells, where it feeds on the haemolymph of the pupae^[11]. Mated females emerge from the cell when the adult host emerges^[30]. Parasitism can result in a loss of up to 25% of adult weight, severe deformations of the wing and reduced longevity of worker and drone honey bees^[8,9,22]. Colonies infested with *Varroa destructor* have significantly reduced worker bee populations and eventually die if left without controlling^[18,1,32].

Varroa mite populations were successfully controlled with the Pyrethroid pesticide Fluvalinate (Mavrik) that could be applied in low doses on plywood strips to the brood nests of bee colonies to bring about a high degree of control of varroa mites^[26]. Also, 10% of Fluvalinate impregnated in plastic strip (10x1 inch) named commercially Apistan was used for controlling varroa mites^[20]. In recent years resistance to this compound has been demonstrated by many authors^[28,6,12,15,27]. Furthermore, chemicals used to control varroa such as Fluvalinate can contaminate honey, wax and pollen^[35,3]. Recently, many authors have been published reporting that some volatile plant oils for controlling varroa mites such as Thyme, Sage, Eucalyptus, Neem and Grapefruit oils^[5,33,14,36,10]. Citronella oil^[23,24] and lemongrass oil^[16] were used for controlling varroa mites. These botanical oils tend to have low mammalian toxicity, little

environmental effect and wide public acceptance^[4]. The present investigations aimed to evaluate the following three volatile plant oils; *Citrus aurantium* L (Sour Orange), *Cymbopogon flexuosus* (Lemon grass), Citronella oil and Mavrik for controlling varroa mite in infested honey bee colonies.

MATERIALS AND METHODS

This work was carried out in the apiary yard at Kaha location, Qualubey governorate during the period from 28th December 2004 to 15th of March 2005.

Experimental procedure:

Tested honey bee colonies: Thirty-six honey bee colonies of Carniolian hybrid *Apis mellifera* L. naturally heavily infested by varroa mites were used for this study. Three colonies were used for each concentration of tested natural plant oil and Mavrik (spraying and stripes) and the three untreated colonies were left as control.

Tested chemicals and concentrations:

1. Three natural plant oils of *Citrus aurantium* L (Sour Orange), *Cymbopogon flexuosus* (Lemon grass) and Citronella oils were used at concentration of 25%, 50% and 100% for each tested oil. Each tested colony was treated one time by using one cm³ of each oil, which added in Petri dish (3m inside diameter) and inserted below the brood combs.

2. Mavrik acaricide 20% (20 mL / Litter of water) was used in two forms, the first was applied as spraying directly on the honey bee combs including mature and immature stages of bees, while the second was used a stripe of cotton (2 x 20 cm) which impregnated for 24 hr. in Mavrik and held in one comb between the brood nest of each tested colony.

Determination of varroa infestation percentages: The percentage of varroa infestation in the tested colonies during the experimental period, was determined in sealed brood (pupae with pigment eyes), in worker brood cells, 50 individuals for each. In adult workers the percentage of infestation was determined in approximately 100 living worker bees taking directly from the combs^[7]. The percentage of infestation was determined two times at 3 days intervals before applying the treatments, while during the treatments they were determined at 7, 14, 21, 28 days after treatments. The reduction percentages of varroa infestation were calculated from the collected data according to the equation of Henderson and Tilton^[19].

Dead varroa mites fallen on the bottom board, covered with sheet of white paper were collected and counted in all tested colonies (treatments & control) at 7, 14, 21, and 28 days after the treatments.

Brood rearing activity: The amount of sealed worker brood in the tested colonies was measured in square inches at 12 days intervals according to the technique of Fresnoy^[17].

Analysis of variance (ANOVA) was carried out for the obtained data according to the method of Waller and Duncan^[34].

RESULTS AND DISCUSSIONS

The effective of some volatile oils for controlling varroa mites were illustrated in Table (1&2). In the colonies treated with tested volatile oils 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 treatment. The highest concentrations (100%) of tested volatile oils caused a highly effective in controlling varroa mites, whereas 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, *Cymbopogon flexuosus* (Lemon grass) and after the third week of treatment with Citronella oils.

Statistical analysis indicated that there were significant differences between the tested volatile oils in different concentrations (25%, 50% and 100%) and

control in the mean percentage of varroa infestation on worker brood and adult workers particularly at the second week, third week and fourth week after treatments.

Whittington *et al*^[36] and Dimetry *et al*^[10] found that Nem oil spray killed 90-94% of varroa mites but thymol oil spray reduced varroa mites to 79% in honey bee colonies. Fathy and Fouly^[16] recorded that 10 ppm of lemon grass oil caused 44.9% of the reduction percentage of varroa mites infested bee colonies. Citronella oil is more effective repellent to varroa mites and reduced the varroa population in treated colonies to the lowest value^[23,24].

According to the obtained data in Table (3) the highest total number of dead varroa mites were counted in the tested colonies after the first and second week of treatment particularly in 100% of all tested volatile oils in comparison with control colonies. Treatment with Citronella oil (100%) caused the highest number of dead varroa fallen on the sheet in comparison with the other tested volatile oils and control.

Increasing the mean number of fallen varroa mites on the sheet in tested honey bee colonies treated with Citronella oil (100%) may be due to the activate of the defense behaviour mechanisms of honey bee workers by these plant volatile oils against varroa mites. Salem *et al*^[31] found that the honey bee colonies which fed on Neem extract showed highest mean number of varroa mites 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 mites fallen on the sheet. Abd El-Wahab^[2] stated that some defense behaviour mechanisms against varroa mites were detected in some races and hybrids of honey bees. These mechanisms resulted in increasing the number of fallen varroa mites on the bottom board of bee hive.

Prior to the treatment with Mavrik as acaricide against varroa mites, data in Table (4) show that after the first week of treated colonies with Mavrik the level of varroa infestation reduced on the worker brood (34.11% & 54.51%) and adult workers (50.53% & 73.18%) for spraying and strips applications respectively. At the end of treatment (Fourth week) Mavrik strips recorded the highest reduction percentage of varroa infested worker brood (94.24%) in comparison with Mavrik spraying (91.32%), while no significant differences were recorded between them in reduction varroa mites infested adult bee workers (96.28% & 97.54% respectively).

Statistical analysis indicated significant differences in reduction the level of varroa infestation on worker brood and adult workers between the treated colonies with Mavrik (spraying or strips) and control colonies during the experimental period (Table 4).

Table 1: The reduction percentages of varroa mite infestation on worker brood in honey bee colonies treated with different concentrations of the tested volatile plant oils.

Treatments	No. of doses	Mean percentage of varroa infestation on worker brood					
		Concentration of tested oils					
		25 %	% Reduction	50 %	% Reduction	100 %	% Reduction
Sour Orange	Before treatment	29.5	----	36.4	----	32.2	----
	First week	18.9 a	41.46	15.8 a	60.34	14.6 b	58.57
	Second week	11.3 b	67.78	8.4 b	80.59	6.5 b	83.02
	Third week	8.6 b	77.64	5.4 b	88.62	0.8 b	98.09
	Fourth week	6.5 b	86.06	4.6 b	92.07	0.0	100
Lemon grass	Before treatment	24	----	17.3	----	21.8	----
	First week	13.2 bc	49.75	10.5 b	44.55	8.3 c	65.21
	Second week	10.1 c	64.61	7.6 c	63.05	5.2 c	79.94
	Third week	5.2 b	83.38	2.8 c	87.58	1.6 c	94.37
	Fourth week	4.8 c	87.35	2.2 c	91.95	0.0	100
Citronella oil	Before treatment	19.8	----	22.4	----	26.5	----
	First week	10.6 b	51.09	11.2 c	54.32	6.4 d	77.93
	Second week	7.9 d	66.44	5.6 d	78.97	5.9 d	81.27
	Third week	5.8 b	77.53	3.2 c	89.04	0.0	100
	Fourth week	4.1 d	86.90	2.6 c	92.65	0.0	100
Control	Before treatment	14.8	----	14.8	----	14.8	----
	First week	16.2 ac	----	16.2 a	----	16.2 a	----
	Second week	17.6 a	----	17.6 a	----	17.6 a	----
	Third week	19.3 a	----	19.3 a	----	19.3 a	----
	Fourth week	23.4 a	----	23.4 a	----	23.4	----
L.S.D.	First week	3.55	----	0.670	----	0.72	----
	Second week	1.091	----	0.505	----	0.40	----
	Third week	5.30	----	0.607	----	0.60	----
	Fourth week	0.696	----	0.60	----	0.51	----

A,b,c,d for comparison between Sour Orange, Lemon grass, Citronella oil and control at First, Second, Third and Fourth week after treatment.

After the first week of treatment the mean number of dead varroa fallen on the sheet of treated colonies with Mavrik (strips or spraying) increased significantly than the control colonies. At the end of treatment (fourth week) Mavrik spraying recorded the highest total number of

dead varroa (1286.3) in comparison with Mavrik strips (1131.9) Table (4). The obtained results are agreement with Herbert *et al*^[21] and Llorente Martinez *et al*^[25] found that Mavrik solution in slits of wood reduced 99% of varroa mite infestation in bee colonies.

Table 2: The reduction percentages of varroa mite infestation on adult workers in honey bee colonies treated with different concentrations of the tested volatile plant oils.

Treatments	No. of doses	Mean percentage of varroa infestation on adult worker					
		Concentration of tested oils					
		25 %	% Reduction	50 %	% Reduction	100 %	% Reduction
Sour Orange	Before treatment	24	----	21.9	-----	23.3	-----
	First week	16.4 b	35.03	13.3 b	42.26	7.4 b	69.80
	Second week	6.4 b	77.21	5.5 b	78.54	4.9 b	82.03
	Third week	4.2 b	85.68	3.3 b	87.67	0.1 b	99.64
	Fourth week	3.5 b	88.55	2.2 b	92.10	0.0	100
Lemon grass	Before treatment	11.9	----	8.5	-----	12.5	----
	First week	5.7 c	54.46	5.5 c	38.48	4.6 bc	65.01
	Second week	3.6 c	74.15	2.4 c	75.87	1.4 c	90.43
	Third week	3.2 c	77.99	2.5 c	75.93	0.0	100
	Fourth week	2.9 b	80.87	1.5 c	86.14	0.0	100
Citronella oil	Before treatment	10.9	----	13.6	----	17.8	----
	First week	6.9 d	39.81	4.2 d	70.64	3.6 c	80.77
	Second week	3.4 c	73.34	2.6 c	83.66	1.5 c	92.79
	Third week	2.8 d	78.98	1.9 d	88.56	0.0	100
	Fourth week	2.1 b	84.87	1.5 c	91.34	0.0	100
Control	Before treatment	13.5	----	13.5	----	13.5	----
	First week	14.2 a	----	14.2 a	----	14.2 a	----
	Second week	15.8 a	----	15.8 a	----	15.8 a	----
	Third week	16.5 a	----	16.5 a	----	16.5 a	----
	Fourth week	17.2 a	----	17.2 a	----	17.2	----
L.S.D.	Before treatment	----	----	-----	----	----	----
	First week	0.55	----	0.57	-----	2.81	----
	Second week	0.38	----	0.25	----	0.97	----
	Third week	0.39	----	0.29	----	0.16	----
	Fourth week	2.06	----	0.38	-----	----	----

A,b,c,d for comparison between Sour Orange, Lemon grass, Citronella oil and control at First, Second, Third and Fourth week after treatment.

Table 3: The effect of different concentrations of the tested volatile plant oils on the number of dead varroa mites fallen on the sheet every week after treatment.

Treatments	No. of doses	Mean number of died varroa fallen on the sheet every week		
		Concentrations of tested oils		
		25 %	50 %	100 %

Table 3: Continued.

Sour Orange	Before treatment	115	110	128
	First week	205	227	215
	Second week	145	162	200
	Third week	75	105	88
	Fourth week	37	36	41
	Total	462	530	544
Lemon grass	Before treatment	95	110	118
	First week	152	218	462
	Second week	86	175	294
	Third week	43	72	110
	Fourth week	16	54	96
	Total	297	519	962
Citronella oil	Before treatment	105	90	112
	First week	163	272	488
	Second week	68	169	295
	Third week	30	81	133
	Fourth week	44	62	84
	Total	305	584	1000
Control	Before treatment	30	30	30
	First week	36	36	36
	Second week	42	42	42
	Third week	47	47	47
	Fourth week	58	58	58
	Total	183	183	183

Table 4: The reduction percentages of varroa mite infestation on brood and adult workers and mean number of dead varroa fallen on the sheet in honey bee colonies treated with Mavrik.

Treatments	No. of doses	Mean percentage of varroa infestation				Mean No. of dead varroa fallen on the sheet
		Worker brood	% Reduction	Adult worker	% Reduction	
Mavrik (spray)	Before treatment	17.5	----	6.40	----	65
	First week	12.62 c	34.11	3.33 c	50.53	684.2
	Second week	7.00 b	66.36	1.30 c	82.64	371.5
	Third week	4.06 c	82.21	0.60 c	92.32	198.3
	Fourth week	2.4 c	91.32	0.20 b	97.54	32.3
	Total					1286.3

Table 4: Continued.

Mavik (strips)	Before treatment	16.67	-----	7.8	----	48
	First week	8.3 b	54.51	2.2 b	73.18	592.3
	Second week	6.8 b	65.96	1.8 b	80.28	346.4
	Third week	5.6 b	74.23	1.6 b	83.21	165.2
	Fourth week	1.50 b	94.24	0.37 b	96.28	28
	Total					1131.9
Control	Before treatment	14.8	-----	13.5	-----	30
	First week	16.2 a	-----	14.2 a	-----	36
	Second week	17.6 a	-----	15.8 a	-----	42
	Third week	19.3 a	-----	16.5 a	-----	47
	Fourth week	23.4 a	-----	17.2 a	-----	58
	Total					583
L.S.D.	First week	0.37	-----	0.41	-----	----
	Second week	0.50	-----	0.17	-----	----
	Third week	0.77	-----	0.24	-----	----
	Fourth week	0.67	-----	0.40	-----	----

A,b,c,d for comparison between Mavrik strips, Mavrik spraying and control at First, Second, Third and Fourth week after treatments.

Table 5: Amount of sealed worker brood (inch²) in honey bee colonies treated with different concentrations of the tested volatile plant oils and Mavrik.

Time of treatment	Mean amount of sealed worker brood (inch ²)											
	Sour Orange			Lemon grass			Citronella oil			Mavrik		Control
	25%	50%	100%	25%	50%	100%	25%	50%	100%	Spray	Strips	
Before treatment	55.3	58.7	87.4	42.3	54.6	63.6	49.3	60.6	74.3	88.6	114.3	37.6
Second week after treatment	72.1	80.5	110.2	57.4	78.2	85.1	63.3	75.0	95.5	124.0	157.1	46.5
Fourth week after treatment	90	97.40	145.3	73.5	83.0	107.2	88.0	96.9	162.0	218.3	234.4	62.3
Sixth week after treatment	112.3	144.8	187.6	89.2	118.1	153.4	95.5	138.0	214.1	292.5	365.3	81.0
Eight week after treatment	126.8	191.7	230.5	103.0	159.0	184.0	117.0	172.0	270.4	361.2	412.9	92.4
Ten week after treatment	179.7	212.3	290.8	150.2	193.4	238.0	168.6	215.4	324.5	416.7	437.2	133.6
Mean	116.2	145.3	192.9	94.7	126.3	153.5	106.5	139.5	213.3	282.5	321.4	83.5

From the obtained results, it could be concluded that the tested plant volatile oils were more effective against varroa mites infestation particularly in the highest concentrations (100%). Citronella oil (100%) reduced the mean percentage of varroa infestation on worker brood and adult workers to 100% reduction at third week after treatment followed by *Citrus aurantium* and lemon grass

oils. Although, no significant differences between the effects of Mavrik (strips or spray) on varroa mite infestation on adult workers, the Mavrik strips caused highest reduction of varroa inside the worker brood cells.

Concerning the data in Table (5) the honey bee colonies treated with tested volatile oils and Mavrik (spraying or stripes) recorded remarkably increasing of

the mean amount of sealed worker brood (inch²) in comparison with control colonies during the experimental period. The highest concentrations of tested volatile oils resulted highly amount of sealed worker brood particularly in Citronella oil (209.63 inch²) as well as Mavrik strips (316.08 inch²). Increasing in the mean amount of sealed worker brood in colonies treated with 100% of volatile oil and Mavrik strips resulted from reducing the infestation of varroa mites by these tested components. 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^[36,13,10].

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