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Control of Varroa Mite (Varroa Destructor) on Honey Bees by Sycamore Leaves (Ficus Sycomorus)

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Abstract: The effect of sycamore leaves (*Ficus Sycamorus*) against varroa mites in honey bee colonies was studied. The percentage of infestation by varroa mites on worker brood, adult workers and number of dead varroa fallen on the sheet were determined in the tested colonies. The percentage of varroa infestation on the worker brood and adult worker reduced to 94.8 and96.18% after the fourth week of exposed to the smoke of ten grams of sycamore leaves and reduced to 96.38 and 98.09% after fourth weeks of treatment with oxalic acid. Treatment with the extract of methylene chloride of sycamore leaves caused the highest number of dead varroa fallen on the sheet in comparison with the other tested extracts and control. Sycamore leaves constituents were identified by GC/MS and these components arrived to 30 compounds.

Key words: sycamore leaves, varroa mites.

INTRODUCTION

Honey bee colonies are subject to infestation by insects, mites and diseases. The ectoparasitic mite Varroa destructor (Anderson and Trueman),[5]. is considered one of the most serious pests of beehives, causing tremendous damage to honey bees (*Apis mellifera* L) and great economic loss to the beekeeping industry[6,19,2]. 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[11,10,16]. Colonies infested with Varroa destructor have significantly reduced worker bee populations and eventually die if left without controlling [14,1,25]. The development of infested brood is also affected because emerged bees have a low weight and shorter life span. This has a negative effect on honey production and other beehive products[31]. Efforts to control varroosis have been focused on the use of control synthetic miticides.Som of these have a high degree on the mite, however, these miticides have important disadvantages: they may promote the mites to develop resistance against their active ingredient, they are toxic to bees and humans and may leave chemical residues in honey which is a product for human consumption[18,22,13,28,23]. In the short term, mite control may be achieved by using natural miticides, which have low Toxicity and low environmental impact, because no residues are left in honey or because these breakdown or volatilized rapidly [7,20]. Few natural products have shown effectiveness against varroa, formic acid, oxalic acids and thymol essential oil are among them[15,29,12]. Phyto therapy was used in varrosis control using the following plants: garlic (Allium sativum). Tobacco (Nicotina tabacum), walnut (Juglans regia), tomato (Lycopersicon esculentum). Worm wood (Artemisia absinthium). Pine (Pinus sylvestris), tansey (Tanacetum vulgare) and other. The 50 up to 80% efficiency using these plants was attained when their extracts were applied 2-3 times a week[4,17]. The experimental reported here were carried out to investigate the role of sycamore leaves (Ficus sycamorus) as control agent against varroa mite depending on the reduction in percent in and the number of fallen mites.

MATERIALS AND METHODS

Experimental Honeybee Colonies: The field part of the present investigations was carried out in an apiary located at Tallen, Menia El-Kamh center, Sharkia governorate during spring season of 2007. Two experiments were carried out. Thirty colonies of hybrid Italian honeybees, (*Apis mellifera*) L. naturally heavily infested by varroa mites were used for this study. Three colonies were used for each treatment and the three untreated colonies were left as control.

Control Agents Treatments: Each experimental colony was exposed to the smoke of five and ten grams of sycamore dry leaves (*Ficus sycamorus*) as carried out by [27]. The dry leaves were placed in a lighted smoker with burlap and fifteen puffs were blown inside the hive through the entrance. The entrance was blocked for fifteen minutes and the colony was completely closed from all sides. Oxalic acid 3% was sprayed directly - on bee one time at the rate of 3-4 ml per comb side. The treatments were repeated every seven days with four applications during each period (28-days).A sample containing 10 gm of sycamore dry leaves was extracted with 100 ml of each methylene chloride, diethyl ether, petroleum ether,

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acetone, ethanol for 24 hrs. The extracts were filtered then concentrated to 5 ml on rotary evaporator. Each extract was used once as a strip of cotton (2x20 cm) which impregnated for 15 minute and held in one comb between the brood rest of each tested colony.

Evaluating the Efficiency of Control Agents: The percentage of varroa infestation in the tested colonies during the experimental period, were determined in worker brood cells and adult bees.

For sampling mites on worker brood cells, two frames with recently sealed brood (pupae were not older than the purple to dark-purple eye stage) were selected from each colony and examined in the laboratory. The frames were returned to the colonies immediately after being examined. Three sample units of 50 sealed worker cells each were randomly selected from the two brood frames using a numbered grid. Each sealed cell was uncapped, the pre-pupa or pupa inside it was carefully examined and adult female mites on it were counted. The walls of the cells and removed caps were also examined as the mite frequently hides there. In adult workers, the percentage of infestation was determined in approximately 100 living worker bee taking directly from each colony. To have a representative sample, between 4 to 6 frames were chosen both from the brood chamber and the honey super. Samples were taken during the day[9,8]. Percentage of infestation 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 7,14,21 and 28 days after treatments, according to the procedure adopted by[26].

Dead varroa mites fallen on the bottom board, covered with sheet of white paper with a layer of sticky substances (Vaseline, oil) were collected and counted in all tested colonies (Treatments and Control) at 7,14,21 and 28 days after the treatments. At each count, the old sheets were taken out for counting and replaced with new Vaseline, oil.

Gas Chromatographic / Mass Spectrometry (Gc/ms) Analysis: A sample containing 10 gm of dry sycamore leaves was placed in a glass thimble equipped with a fritted disk, after which the thimble was placed in the soxhlet extractor. The round bottom extraction flask was filled with 200 ml of methyline chloride, and extraction at the boiling point was allowed to proceed for 5 hours. The extraction flask was then fitted on a rotary evaporator. The extractor was then rinsed into the concentrator with on additional 25 ml of solvent. The concentrator was kept in a water both until the solvent volume was less than 1 ml[21]. Also, 100 gm powder of dry sycamor leaves burned and the fumigants were collected under condensation in liquid form. The composition of methylene – chloride extract and liquid form in sycamore leaves were analyzed by gas chromatography / Mass spectrometry (GC/MS). The identification of components was based on comparison of their mass spectra with computer search of their 70 ev mass spectro with those stored in the library of the GC/MS dado system, as well as by retention indices. The components was analyzed by GC/MS, using GC mode 1 trace GC 2000 produced by thermo and mass spectrometer model: SSQ 7000 with DB-5 (5% phenyle) methyl poly siloxane column (internal diameter, 25 mm). the temperature was programmed from 50°C (3min) to 300°C at 3°c/min and the final temperature was held for 47 min, injector and detector flow of 1ml/min. the injector and detector temperatures 220°C, oven temperature programmed from 40 (3min), 50-300°c (3°C/min), carrier gas helium at a flow of 1 mi/min., operating at 70 ev. Analysis of variance (ANOVA) was carried out for the obtained data according to the method of[30].

RESULTS AND DISCUSSION

The effective of sycamore leaves (*Ficus sycamorus*) for controlling varroa mites were illustrated in table (1 and 2). In the colonies treated with 5 gm and 10 gm of sycamore leaves, the percentages of infestation with varroa mites on the worker brood and adult worker reduced gradually from the first to the fourth week (end of the treatment) after the treatment. The percentage of varroa infestation on the worker brood and adult worker reduced to94.8 and 96.18% after the fourth week of treatment with 10 gm of sycamore leaves and reduced to 96.38 and 98.09% after fourth week of treatment with oxalic acid (Table 1). It is observed that, the efficiency of smoke with sycamore leaves against varroa mite was noticed during 6-days. Recording the greatest number of captured mites, so, the treatments with 5 and 10 gm sycamore leaves were repeated every 7 days for 4 week. This finding may be due to the higher numbers of adult mites attached to the bees bodies beside the higher efficiency of the freshly installed control agent.

According to the obtained data in Table (1) the highest total number of dead varroa mite were counted in the tested colonies after the first 1-3 weeks after treatment, there after, the numbers of captured mites decreased sharply. This reduction in the number of fallen mites could be attributed to either the reduced rate of infestation or the reduced efficiency of the control agent tested[27]. After the first week of treatment the mean number of dead varroa fallen on the sheet of treated colonies with 10gm sycamore leaves (smoke) recorded 160 individuals. While reached 72 at the end of treatment (fourth week) compared with 37.88 individuals of varroa in control.

Statistical analysis indicated that there ware highly significant differences between the tested sycamore leaves (5 and10 gm), oxalic acid and control in the mean percentage of varroa infestation on worker brood and adult worker. Also, on the varroa fallen on the sheet during the experimental period (Table 1)

Table 1: The reduction percentages of varroa mites on brood and adult workers as well as the number of dead varroa fallen on the sheet in honeybee colonies as affected by sycamore dry leaves and oxalic acid.

Treatments	Mean percentage of varroa infatuation				Mean No. of dead varroa fallen on the sheet
	Worker brood	% reduction	Adul worker	% reduction	
Sycamore dry leaves (5g)					
Before treatment	30.7	-	40.0	-	68.0
First week	20	34.79	13.3	66.68	119.0
Second week	15	51.09	6.7	83.33	94.7
Third week	11	64.13	4.0	90.00	80.0
Fourth week	7.3	76.12	2.3	94.18	55.7
Mean	15.80b		13.27b		76.80b
Sycamore dry leaves (10g)					
Before treatment	37.0	-	39.3	-	74.0
First week	6.3	82.98	4.7	88.45	176.0
Second week	5.7	84.68	3.0	92.37	150.7
Third week	2.7	92.78	2.7	93.24	141.3
Fourth week	1.90	94.80	1.50	96.18	72.0
Mean	10.72bc		10.24b		118.80a
Oxalic acid (3%)					
Before treatment	27.7	-	47.0	-	73.0
First week	5.0	81.93	3.0	93.62	151.7
Second week	2.5	90.97	2.31	95.08	129.0
Third week	1.4	94.94	1.1	97.65	54.7
Fourth week	1.0	96.38	0.9	98.09	34.7
Mean	7.52c		10.86b		90.50ab
control					
Before treatment	31	-	35	-	32.0
First week	25	-	41.7	-	28.7
Second week	29	-	35.3	-	33.3
Third week	29.3	-	41.0	-	32.7
Fourth week	34.7	-	39.7	-	29.3
Mean	29.80a		38.53a		35.20c
Total	15.68		17.54		80.15
Significantly	**		**		**

According to the obtained results in Table (2) the highest total number of dead varroa mites were counted in the tested colonies after the first and second week of treatment with leave extracts especially methylene chloride, diethyl ether and petroleum ether extract then decreased after third week. Treatment with sycamore methylene chloride extract caused the highest number of dead varroa fallen on the sheet in comparison with the other tested extracts and control. Increasing the mean number of fallen varroa mites on the sheet in tested honey be colonies treated with sycamore methylene chloride extract or sycamore dry leaves (smoke) may be due to the activity of active material in plant leaves (Fig.1) or the activate of the defense behavior mechanisms of honey be workers by these components against varroa mites[24]. found that the honey bee colonies which fed on neem extract showed highest mean number of varroa mites fallen on the sheet[3]. suggested that Different concentrations (25%, 50%and 100%) of Citrus aurantiumL (Sour Orange), Cymbopagon flexuosus (Lemon grass) and Citronella volatile oil caused changes in the haemolymph of honey be worker and therefore increased the number of varroa mites fallen on the sheet. There were highly significant differences between in dead varroa mites fallen on the sheet between the treatment with extracts and control colonies during the experimental period(Table 2).

Data in Table (2) show that, the honey bee colonies treated with sycamore ethanol, acetone and petroleum ether extracts caused deleterious effects on honey bee worker especially ethanol extract prevent laying the eggs for two weeks.

Table 2: Comparison of sycamore leaf extracts on the number of dead varroa mites fallen and dead honey bee for three weeks post treatment.

Treatment	Total No. of fallen mites				Total No. of dead honeybee		
	First week	Second week	Third week	Mean	First week	Second week	Third week
Control	37.28	48.00	69.28	51.56c	-	-	-
Methylene chloride extract	506.72	965.28	112.00	498.00a	-	-	-
Diethyl ether extract	133.28	186.72	80.00	133.33bc	7.67	2.33	0.00
Petroleum ether extract	526.00	240.00	181.28	225.78b	7.00	7.0	0.00
Acetone extract	213.28	208.00	133.28	213.33b	20.33	3.33	0.00
Ethanol extract	128.00	90.00	-	109.33bc	76.67	5.33	-
Significantly				**			

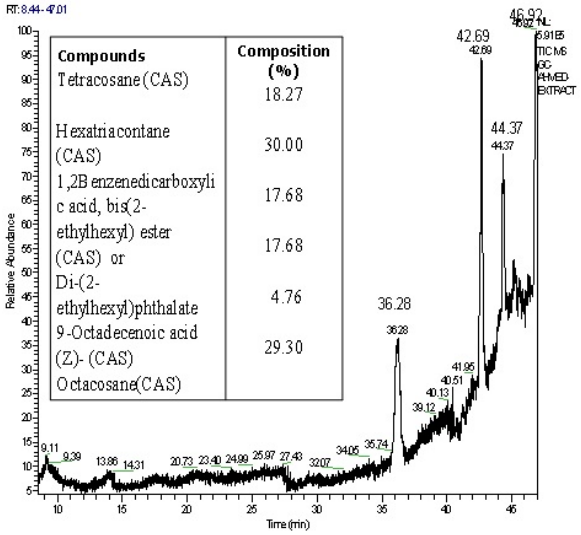

Fig. 1: Composition of the five fumigants of *sycomore leaves (Ficus sycomrus)*.

Name	RT	Area %
1-Methylbutyl nitrite	8.17	1.93
1H-Indene, 1-methylene- (CAS)	13.54	7.22
Dodecane (CAS)	13.79	12.93
methylnaphthalene	16.66	0.37
1-Heptadecanol (CAS)	18.97	1.03
Tetradecane (CAS)	19.20	16.95
5-Cyano-2-pyridinecarboxamide	21.53	1.52
1-Heptadecanol (CAS)	23.89	1.34
pentadecane	24.09	11.93
N-[Bis(methylthio)methylene]-L-phenylalanine methyl ester	25.82	7.65
1-Heptadecanol (CAS)	28.33	0.83
Octadecane (CAS)	28.49	5.92
2-oxo-3-(ethylaminocarbonyl)imidazolo[4,5-b]quinoxaline	29.47	0.74

Discontinue:

7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione	30.72	5.43
trans-1-(phenylthio)-6-oxo-4-oxahept-1-ene	31.63	1.25
4,4,7,7-Tetramethyldeca-1,9-diene	32.38	0.36
butyl 2,4-dimethyl-2-nitro-4-pentenoate	32.51	2.76
Docosane (CAS)	36.18	1.29
Tributyl ester of 3-acetyl-citric acid	36.98	3.08
(3R*,4S*)-3-Ethyl-4-methyltetrahydrofuran-3-ol	39.55	0.61
bis(2-ethylhexyl) phthalate	41.60	3.03
1-Methylbutyl nitrite	42.68	0.36
2-Propenoic acid, 2-methyl-, 2-propenyl ester (CAS)	45.72	0.43
1-Octadecanol (CAS)	46.12	0.77
Decanedioic acid, didecyl ester	47.00	4.45
1-Octadecanol (CAS)	48.86	1.03
(E)-tricos-9-ene	49.67	1.83
cis-3a,8b-dihydro-7-hydroxy-8-acetyl-furo[3,2-b]-benzofuran-2(3H)-one	50.20	1.42
1-Octadecanol (CAS)	51.61	0.44
3-O-(trimethylsilyl)-5,7,3',4'-tetra-O-methylquercetin	52.65	1.11

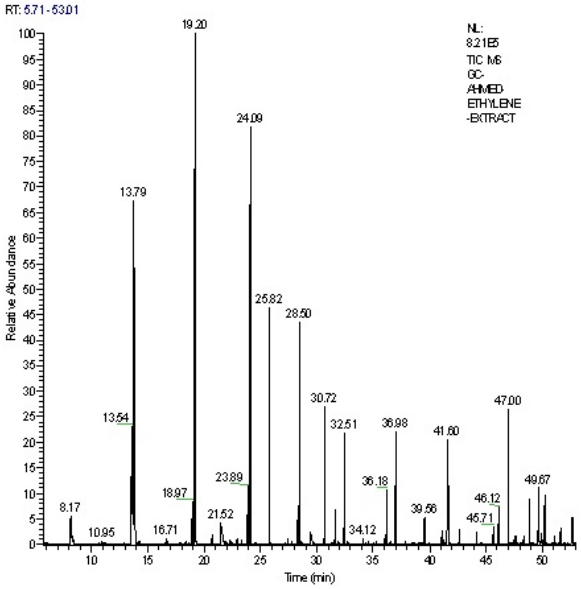

Fig. 2: Composition the extract of methylene chloride of sycamore leaves.

The main chemical components of sycamore leave Fig. (1). Are presented in Fig. (1and2).

The main chemical components of the evaluated sycamore leaves extract are presented in Table 1. the main compounds were Tetradecane, pentadecane, Dodecane, N-[Bis(methylthio)methylene]-L-phenylalanine methyl ester, Octadecane 7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione, Decanedioic acid, didecyl ester, 1H-Indene, 1-methylene- (CAS), Tributyl ester of 3-acetyl-citric acid, bis(2-ethylhexyl) phthalate, butyl 2,4-dimethyl-2-nitro-4-pentenoate (figure 1) On the other hand, the major constituent of the sycamore leaves (In smoke), Hexatriacontan, octacosane, tetracosane ,di- (2-ethyl hexyl) phthalate and 9-octadecenoic acid (figure 2).

Conclusions: From the results obtained from this work, it can be concluded that the percentage of varroa infestation on the worker brood and adult worker reduced to 94.8 and 96.18%after the fourth week of treatment with 10 gm of sycamore leaves and reduced to 96.38 and 98.09% after fourth week of treatment with oxalic acid. Increasing the mean number of fallen varroa mites on the sheet in tested honey be colonies treated with sycamore methylene chloride extract or sycamore dry leaves (smoke) .So, the sycamore leaves could play an important role to use these plants derivates to establish an integrated pest management program to reduce mite populations in *A. mellifera* colonies. Although, this knowledge should be taken into account to control mite based on natural products, further studies need to be done in order to identify some other factors that may be involved in a successful control of Varroosis.

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