

BIOCIDAL EFFECT OF CERTAIN BOTANICAL EXTRACTS AGAINST VARROA MITE, *Varroa destructor* IN HABITING THE EGYPTIAN APIARIES

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ABSTRACT

Monitoring the bio-effect of certain medicinal plant extracts and certain botanical oils with different forms (direct oils, tablets and capsules) against Varroa mite was made to find out the more efficient compounds for controlling the ectoparasite mite in the Egyptian apiaries. Comparison was worked at with the results given after using Mavrik as a recommended acaricide for this particular mite. The highest reduction value in mite infestation was obtained after using thyme and worm wood extracts as well as marjoram, cedar wood and clove oils as botanical oils.

Also so, clove and marjoram oils used as tablets or capsules gave the efficient control measurement for Varroa mite in the experimental colonies.

Keywords : Honey bee, Varroa mite, Biocide, Botanical extracts.

INTRODUCTION

The haemophagous Varroa mite, *Varroa destructor* has become the most dangerous destructive pest of honeybee colonies, not only in Egypt but also in many world countries. This mite was recorded for the first time in Egypt in 1983 (Wienands and Madel 1988).

Migratory beekeeping, importation of colonies, packages of bees and queens are considered to be the possible ways between countries and locations of Varroa spread (Griffiths and Bowman, 1981). The spread of Varroa within colonies is due to swarming, robbing and foraging. Using traditional acaricides for controlling this pest develop the resistance in the biological systems of the mite against these compounds. Therefore, it is important to find out natural compounds which control this mite efficiently without any human hazards. Such materials are more safe to human beings and bees, cheap, available and easy to use.

The aim of the present work is to evaluate the efficacy of some plant extracts and botanical oils, either directly or in form of tablets and capsules against Varroa mite in the experimental colonies.

MATERIAL AND METHODS

Field experiments were conducted in the apiary of Beekeeping Research Department, ARC, Ministry of Agriculture during 2003 and 2004 years to evaluate the following natural compounds of botanical origin against *Varroa destructor* infested the experimental colonies.

First hybrid Carniolan honeybee colonies infested with Varroa mite with relatively similar strength (8 combs covered from both sides with adult workers each) were selected. Each colony headed by a prolific queen of nearly the same age as those of the other colonies. The experimental colonies (27 in 2003 and 21 in 2004) were divided into 9 and 7 groups, respectively, each contained 3 colonies (replicates).

- a- Aqueous plant extracts of thyme 50 % (*Thymus vulgaris*), Ginger 25 % (*Zingiber officinalis*) and worm wood 50 % (*Artemisia herba*) were prepared, adding 500 gm of powdery dry plant to 5 liters of distilled water, heating at 100°C for 6 hours, the mixtures was filtered after 24 hrs and one liter of extract was taking for control experiments. 25 ml of the extract were sprayed on the combs of each tested colony and repeated 4 times every month (once / week).
- b- Volatile oils of marjoram (*Marjorana hortensis*), clove (*Dianthus barbatus*), cedar wood (*Ziziphus spina*) and mint (*Mentha viridis*), which were brought from private sector shops and used directly by soaking 2 ml of each oil in cotton piece, put in each tested hive and repeated at 4 times every month (once / week).
- c- Marjoram oil, clove oil and ginger powder mixed (cotton seed oil) in form of tablets using of 4 tablets/colony, 2 times at 15 day intervals.

The tablets preparation was as follows :

The two volatile oils were prepared as tablets by 20 ml of distilled water to 80 gm of the mixture of talc powder and grain flour (as carrier). These compounds were mixed till they became as paste form. Tablets were made by filling a plastic frame (2 cm in diameter and 0.4 cm in height) with the prepared paste and left in electric oven at 105°C for dryness.

Oil was then added drop by drop on both sides of the tablet using micro-pipette. It was found that one ml of oil soaked in one tablets was considered as suitable amount.

To obtain ginger tablet cotton seed oil was used as sticking agent. Ginger powder was mixed with the cotton seed oil at the rate of 85 gm of ginger powder to 15 gm of cotton seed oil to obtain a homogenized paste. The paste was prepared as tablets using fore mentioned previous. Marjoram oil and clove oil in form of capsules by using 6 capsules/colony, 3 times at 10 day intervals.

The capsules preparation was as follows :

Since marjoram oil is soluble in vaseline, capsules were prepared by putting 10 gm vaseline (as carrier and anti-evaporation agent) in 50 cm³ beaker and melted on electric heater, then 10 gm at marjoram oil were added, mixed well and left to freezing point. Rubber stoppers were filled with vaseline-oil mixture using spatule and the mean weight of marjoram oil in each capsule was determined by weighing 10 rubber stoppers before and after filling with mixture. It was found that each capsule weighed 0.2 gm and contained 0.1 gm marjoram oil.

In case of clove oil, this oil was not soluble in vaseline. Therefore, it was mixed with talc powders at the rates of 1 : 1 by weight (clove oil : talc

powder) to obtain a paste. Rubber stoppers were filled with this paste using spatule. It was found that each capsule weighed 0.3 gm and contained 0.15 gm oil. Using of capsules rate 6/colony 3 times at 10-day intervals.

d- Mavrik (fluvalinate 10 %, w : w) as recommended traditional acaricide was used to compare the data obtained after using it with those obtained after using the natural materials. Mavrik strips, were used at 2 strips/colony/month.

Each hive floor was covered with a plastic sheet coated with thin layer of vaseline to capture the fallen Varroa mite individuals. The mites were counted, removed at the end of each treatment coated with vaseline and the plastic sheet was reentered the hive.

The procedure was repeated 1, 2, 3, 7, 14, 21 and 28 days after application. The rates of Varroa infestation in sealed worker brood cells and on adult bees were determined at the beginning and the end of the experiments using the following procedures :

25 sealed worker brood cells from each colony were opened, the number of infested cells with Varroa mite was recorded and the percentage of infestation was calculated.

Reduction in infestation with Varroa mite was calculated according the formulae of Henderson and Tilton (1955).

RESULTS AND DISCUSSION

1- Mean number of fallen Varroa mite, in honeybee colonies treated with natural extracts and oils :

Data presented in Table (1) indicate that treating Varroa mites infesting first hybrid honeybee colonies with the tested natural substances caused significantly higher number of fallen Varroa mites as compared to that of untreated colonies, i.e., a mean total of fallen Varroa (131.67 individuals) was recorded after using ginger extract, followed by mint oil (96.30 individuals), Cedar wood oil (94.97 mites), clove oil (90.64 individuals), marjoram oil (87.31 mites), thyme extract (63.01 mites) and worm wood extract (55.34 mites). The least number of fallen Varroa was recorded in untreated colony (22.98 mites).

It is important to notice that using the recommended traditional acaricide for controlling Varroa mite caused very high number of fallen Varroa (774.31 mites) on the floor of the experimental hive. The number of dead fallen mites were increased gradually in case of using natural materials, this might be due to slow action against Varroa mite (Imdorf, 1995 ; El-Abbodi and Nazer, 2003 and Rice *et al.*, 2004). In contrast, chemical compounds, i.e. Mavrik was effective and the number of dead fallen mite were sharply increased after 24 hr from application.

Table 1. The changes in the population density of *Varroa* survivors fall on the hive floor in 1st hybrid Carniolan honey bee colonies treated with certain natural extracts and selected oils during winter of 2003.

Tested groups	Types of Plants	No. of <i>Varroa</i> of fall/colony after the following days from treatments :							
		1	2	3	7	14	21	28	Total
Aqueous extracts	Thyme	3.67± 0.33	8.67± 2.33	5.00± 1.00	7.00± 3.21	7.00± 1.53	12.00± 5.51	19.67± 0.57	63.01
Medicinal plants extracts	Ginger	12.00± 2.08	14.00± 1.55	23.67± 4.26	35.00± 3.46	24.00± 9.29	14.33± 4.81	8.67± 1.45	131.67
"Spray"	Worm wood	7.33± 1.76	2.67± 0.33	8.67± 2.84	8.67± 3.48	11.33± 4.37	9.67± 3.18	7.00± 1.00	55.34
Botanical oils Soaking in cotton	Marjoram	5.66± 1.45	6.00± 1.52	5.00± 2.08	15.66± 1.85	27.33± 14.49	19.33± 10.33	8.33± 2.84	87.31
	Clove	5.66± 0.33	6.66± 0.66	7.66± 0.33	6.33± 0.88	14.00± 2.00	30.33± 3.92	20.00± 4.61	90.64
	Cedar wood	3.66± 0.33	5.33± 0.33	7.66± 1.85	7.00± 1.52	21.00± 5.50	29.66± 6.22	20.66± 5.54	94.97
	Mint	5.66± 0.66	7.66± 1.2	7.33± 1.85	7.66± 0.33	17.33± 1.20	33.00± 6.42	17.66± 2.6	96.30
Acaricide (strips)	Mavrik	248.33± 65.65	238.33± 50.57	140.0± 63.05	69.33± 3.29	52.0± 11.78	16.66± 4.25	9.66± 1.2	774.31
Control (check)	Untreated	3.00± 0.57	2.00± 1.15	2.66± 1.2	4.00± 2.3	1.00± 0.0	5.66± 2.84	3.66± 1.85	22.98
F-value									65.24
L.S.D. at (5%)									3.87

2- Efficiency of the natural extracts and oils against *Varroa* mite :

Table (2) show that thyme extract caused reduction of infestation being 81.29 % and 89.31 % for brood cells and adult, respectively. The mean reduction of infestation reached 85.3 % for both brood and adult. Ginger extract caused reduction of infestation being 75.01 % and 79.82 % for brood cells and adult, respectively. The mean reduction of infestation reached 79.82 % for both brood and adult, respectively.

Worm wood extract caused reduction of infestation being 87.6 % and 83.79 % for brood cells and adult, respectively. The mean reduction of infestation reached 85.7 % for both brood cells and adult.

It could be concluded that, the mean of reduction of infestation with *Varroa* mite could be arranged descendingly as follow : worm wood (85.7 %), thyme (85.3 %) and ginger plant extracts (79.82 %), respectively.

On the other hand, when using botanical oils soaking in the cotton, the marjoram oil caused reduction of infestation being 81.27 % and 83.45 % for brood cells and adult, respectively. The mean reduction of infestation reached 82.36 %. Clove oil caused reduction of infestation being 70.9 % and 83.37 % for brood cells and adult, respectively. The mean reduction of infestation reached 77.14 %. Cedar wood oil caused reduction of infestation being 66.8 % and 85.35 % for brood and adult, respectively. The mean reduction of infestation reached 76.08 % for brood and adult. Mint oil caused

reduction of infestation being 73.8 % and 74.9 % for brood and adult, respectively. The mean reduction of infestation reached 74.35 %.

It could be concluded that, the mean reduction of infestation when using botanical oils soaking in the cotton against *Varroa* mite could be grouped descendingly as follows : Marjoram oil, clove oil, cedar wood oil and mint oil, respectively. These results agree with of the findings of Fries *et al* (1997) and Lodesani (2004) who found that essential oils were found to be very effective against the mite, but without any appreciable side effect on bees.

Meanwhile, when using Mavrik strips as recommended by traditional acaricides, the reduction of infestation being 81.29 % and 94.65 % for brood and adult, respectively. The mean reduction of infestation for both brood and adult was 87.97 %. The highest reduction in mite infestation was obtained after using thyme and worm wood extracts as well as marjoram, cedar wood and clove as botanical oils. These results similar with those of Mahmoud, (2005) since she found that the highest reduction of infestation against *Varroa* mite were thymol, Mavrik, eucalyptus and peppermint, respectively.

Table 2. Mean percentages infestation of *Varroa destructor* in 1st hybrid Carniolan honeybee colonies (brood and adult) treated with certain natural extracts and selected oils during winter of 2003.

Tested groups	Types of plants	Infestation % on brood cells		Reduction %	Infestation % on adult bees		Reduction %	Mean of reduction (%)
		Before treatment	28 days after treat.		Before treatment	28 days after treat.		
Aqueous extracts Medicinal plants extracts "Spray"	Thyme	10.67± 3.53	4.00± 4.00	81.29	4.57± 0.57	1.60± 0.93	89.31	85.30
	Ginger	5.33± 1.33	2.67± 1.15	75.01	5.63± 0.82	2.83± 0.59	84.62	79.82
	Worm wood	10.67± 1.67	2.67± 2.67	87.60	3.57± 0.30	1.89± 0.96	83.79	85.70
Botanical oils (soaking in cotton)	Marjoram	5.33± 1.33	2.00± 1.15	81.27	4.99± 0.65	2.70± 1.41	83.45	82.36
	Clove	17.66± 5.78	10.3± 3.81	70.90	10.43± 1.73	5.67± 0.44	83.37	77.14
	Cedar wood	18.00± 2.60	12.00± 0.58	66.80	11.83± 0.88	5.67± 0.88	85.35	76.08
	Mint	9.50± 0.76	5.00± 0.57	73.80	6.50± 1.17	7.00± 0.57	74.90	74.35
Acaricide (strips)	Mavrik	10.67± 1.33	4.00± 2.30	81.29	10.6± 1.55	1.86± 1.01	94.65	87.97
Control (check)	Untreated	5.33±1. 33	10.67± 1.30	-	3.67±0. 43	11.98± 2.02	-	-
F-value				0.93			19.47	
L.S.D. at 5 %				n.s.			2.35	

n.s. : non-significant

3- Mean numbers of fallen Varroa mite in honeybee colonies treated with preparation of natural oil compounds :

Preparation of natural oil compound were prepared as tablets (marjoram, clove and ginger) and capsules (marjoram and clove) to study the efficiency of these preparations as acaricides for controlling Varroa mite in the experimental honeybee colonies. The data given in Table (3) show that the highest total number of Varroa fallen on the floor of a hive (109.99 mites) was recorded after using tablets of marjoram oil followed by tablets of ginger oil (102.63 mites). An intermediate total number of Varroa (58.65, 47.64 mites/colony) after using tablets of clove oil and capsules of clove oil, respectively were obtained. The lowest total number of Varroa fallen on the floor of a hive was obtained in untreated colony (11.98 mites) or after using capsules of marjoram (15.31 mites). The data clearly show that tablets were found to be more efficient than capsules for controlling Varroa mite in infested honeybee colonies. In this case, the total number of fallen Varroa after using tablets of marjoram oil was 109.99 mites, compared to same oil which was prepared as capsules (15.31 mites/colony). However, clove oil didn't apparently affected with oil preparations, i.e. 58.65 and 47.64 mites/colony were obtained after using clove oil as tablets and capsules, respectively.

The obtained results in the present work are in agreement with those recorded by Abou El-Enain *et al* ,(1999) ; Abou-Lila *et al* (2001) and Al-Abbodi and Nazer (2003).

Table 3. The changes in the population density of Varroa survivors fall on the hive floor in 1st hybrid Carniolan honey bee colonies treated with certain preparations of natural selected oil compounds during winter of 2004.

Tested groups	Types of plants	No. of Varroa of fall/colony after the following days from treatments :							
		1	2	3	7	14	21	28	Total
Tablets	Marjoram	8.00± 1.00	8.00± 1.00	9.33± 0.88	16.33± 6.11	27.33± 8.95	26.00± 3.60	15.00± 6.92	109.99
	Clove	4.33± 1.33	6.00± 1.00	7.00± 1.52	6.33± 0.88	14.0± 1.52	14.33± 2.90	6.66± .02	58.65
	Ginger	9.33± 7.88	7.66± 2.18	9.66± 0.33	12.33± 1.45	18.66± 7.31	19.33± 6.56	25.66± 14.81	102.63
Capsules	Marjoram	1.00± 1.00	2.33± 0.33	3.33± 0.33	0.66± 0.66	1.33± 0.66	2.33± 0.88	4.33± 1.20	15.31
	Clove	3.33± 1.76	5.33± 1.20	7.33± 2.33	7.66± 2.72	9.33± 3.17	10.00± 3.21	4.66± 2.33	47.64
Acaricide (strips)	Mavrik	125.0± 18.92	123.3± 13.01	100.6± 8.08	85.00± 7.63	48.3± 8.81	28.3± 7.26	6.30± 1.85	516.80
Control (check)	Untreated	3.33± 0.66	1.00± 1.00	2.33± 0.33	1.66± 1.20	1.00± 0.57	0.66± 0.33	2.00± 1.00	11.98
F-value									6.45**
L.S.D. at (5%)									6.54

4. Efficiency of some preparation of natural compounds against *Varroa* mite survivors :

Table (4) show that, when using natural compound as tablets, the marjoram caused reduction of infestation being 76.8 % and 91.76 % for brood and adult, respectively. The mean reduction of infestation reached 84.28 % for both brood and adult. Clove caused reduction of infestation being 82.6 % and 91.11 % for brood and adult, respectively. The mean reduction of infestation was 86.86 % for both brood and adult. Ginger caused reduction 84.6 % and 76.2 % for both brood and adult, respectively. The mean reduction of infestation reached 80.4 %.

Table 4. Mean percentages infestation of *Varroa destructor* in 1st hybrid Carniolan honeybee colonies (brood and adult) treated with certain preparations of natural selected oil compounds during winter of 2004.

Tested groups	Types of plants	Infestation % on brood cells		Reduction %	Infestation % on adult bees		Reduction %	Mean of Reduction (%)
		Before treatment	28 days after treat.		Before treatment	28 days after treat.		
Tablets	Marjoram	18.33± 3.33	6.66± 4.40	76.8	9.79± 3.03	0.95± 0.95	91.76	84.28
	Clove	18.33± 3.33	5.00± 2.88	82.6	11.36± 1.38	1.19± 1.19	91.11	86.86
	Gingar	15.00± 5.00	3.60± 1.10	84.6	11.88± 1.11	3.33± 3.33	76.20	80.40
Capsules	Marjoram	16.66± 1.66	3.33± 3.33	87.3	8.49± 1.19	2.70± 1.45	73.10	80.20
	Clove	16.66± 3.33	3.33± 3.33	87.3	11.67± 2.37	1.90± 1.90	86.30	86.60
Acaricide (strips)	Mavrik	17.70± 2.02	5.50± 1.00	80.3	12.3± 1.45	0.92± 0.08	93.80	87.05
Control (check)	Untreated	16.00± 4.93	25.0± 5.77	-	8.71± 1.02	10.25± 1.60	-	-
F-value				0.1			36.17	
L.S.D. at 5 %				n.s.			1.55	

n.s. : non-significant

It could be concluded that, the mean reduction of infestation when using natural compound as tablets against *Varroa* mite could be arranged descendingly as follows : clove, marjoram and ginger, respectively. Meanwhile, when using of natural compound as capsules, the marjoram caused reduction of infestation being 87.3 % and 73.1 % for brood and adult, respectively. The mean reduction of infestation reached 80.2 % for both brood and adult. Clove caused reduction of infestation being 87.3 % and 86.3 % for brood and adult, respectively. The mean reduction of infestation reached 86.6 %.

Meanwhile, using Mavrik strips, as recommended traditional acaricides, the reduction of infestation being 80.3 % and 93.8 % for brood and adult, respectively. The mean reduction of infestation for both brood and adult was 87.05 %.

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التأثير الإبادى لبعض المستخلصات النباتية ضد طفيل الفاروا في المناحل المصرية
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تم إجراء هذا البحث في منحل قسم بحوث النحل بالدقى (محافظة الجيزة) عامى ٢٠٠٣،
٢٠٠٤ لتقييم استعمال المواد الطبيعية في صورة مستخلصات للنباتات الطبية الزعتر، الزنجبيل،
والشيع رشا على النحل المصاب بالفاروا بمعدل ٣ سم³/برواز نحل وكررت ٤ مرات كل أسبوع
مرة. وأستعملت زيوت البردقوش والقرنفل والسيدر و النعناع بمعدل ٢ سم³ على قطعة
قطن/طائفة وكررت ٤ مرات كل أسبوع مرة. وتم عمل مستحضرات من زيوت البردقوش
والقرنفل ومسحوق الزنجبيل في صورة أقراص بمعدل ٤ أقراص/طائفة على دفعتين كل ١٥ يوم
قرصان، وزيوت القرنفل والبردقوش في صورة كبسولات بمعدل ٦ كبسولة/طائفة على ٣ دفعات
كل ١٠ يوم كبسولتين، وأستعمل مبيد أكاروسى "المافريك" بمعدل ٢ شريط/طائفة لمدة شهر
لمقارنته بالمواد الطبيعية السابق ذكرها.

وأوضحت النتائج فعالية المواد الطبيعية بصورة جيدة ضد طفيل الفاروا

- ١- بنسب مئوية في خفض تعداد الطفيل باستعمال الزعتر ٨٥,٣% ، الزنجبيل ٧٩,٨٢% ،
والشيع ٨٥,٧% في صورة مستخلصات نباتية
- ٢- وبلغت النسب المئوية للخفض عند استعمال زيوت البردقوش ٨٢,٣٦% والقرنفل ٧٧,١٤%
والسيدر ٧٦,٠٨% و النعناع ٧٤,٣٥%
- ٣- وبلغت تلك النسب عند استعمال مستحضرات من زيوت البردقوش ٨٤,٢٨% والقرنفل
٨٦,٨٦% ومسحوق الزنجبيل ٨٠,٤% في صورة أقراص
- ٤- و عند استعمال زيوت القرنفل ٨٦,٦% والبردقوش ٨٠,٢% في صورة كبسولات
و تقاربت تلك النسب المئوية للخفض في تعداد الطفيل عند استعمال المافريك. وتوصى
النتائج باستعمال المواد الطبيعية السابقة في صورها المختلفة خاصة الأقراص والكبسولات حيث
لن هذه المواد ليست لها تأثيرات ضارة على النحل ومنتجاته.