

THE EFFECT OF NATURAL VOLATILE OIL PLANTS ON *APIS MELLIFERA* HONEYBEE AND ON THE *VARROA JACOBSONI* IN THE BEE COLONIES

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Introduction

Varroasis is a dangerous pest of the honeybee, caused by the ectoparasitic mite *Varroa jacobsoni* Oud. It spreads epidemically, destroying thousands of bee colonies and reducing the honey production to minimum levels. The Varroa disease causes a serious problem in world beekeeping, due to its harmful effect on the host insect (RITTER, 1981; IFANTIDIS, 1983; FOULY, 1988 and FATHY & FOULY, 1993).

Many chemical acaricides, such as Sinecar (STOR et al., 1981), Folbex and Folbex-Forte (RITTER, 1981) have already been used against the Varroa mite. IFANTIDIS (1987) mentioned that the acaricides effective against the Varroa mites are available, but their application within the hives tends to contaminate the beeswax and the honey. Moreover, chemotherapeutical acaricides lead to problems with residues in honey, in beeswax and in other bee products (MORITZ and MOUTS, 1990).

Therefore, some natural plant products, such as spraying with diluted tobacco solutions and with a dilution of Beibu (*Stemona Japonica* extract), (FAN and LI, 1988) have been used to

control the parasitic mite. CERVANICA and ASPIRAS (1987) used the ground leaves of *Premna japonica*. FATHY and FOULY (1993) used the essential oil of *Eucalyptus rostrata* against the Varroa mite and their data showed very promising results in Egypt. Also, EMARA et al. (1994) were concerned with processing and evaluating certain plant extracts for the control of Varroa mites.

Plant extracts and non-chemical methods are very used - lately - for the control of pests, in order to minimize the chemical pesticides which are considered to be harmful for humans and for the environment as well.

Therefore, the present study deals with the application of some volatile oils of lemongrass, thymus, rosemary, fennel, marjoram and sage on honeybees to control *V. jacobsoni* in the Dakahlia Governorate.

Materials and Methods

The present study was carried out in the apiary of Baramon 1, Mansoura region, while the laboratory experiments were carried out in the Faculty of Agriculture, at the Mansoura University, in 1995.

I. Preparation of Plant Extracts:

Six volatile oil plants *Rosmarinus officinalis* (rosemary); *Thymus vulgaris* (thymus); *Marjorana hortensis* (marjoram); *Foeniculum vulgare* (fennel); *Cymbopogon flexuosus* (lemongrass); *Salvia officinalis* (sag) were first identified by the taxonomists at the Agricultural Research Center, in Sabahia, Alexandria and at the Faculty of Science at the Alexandria University.

A special steam distillation method was used to extract the volatile oils from dry leaves, according to GUENTHER (1949) and the percentages of extracted oil were calculated.

II. Laboratory Assessment:

A series of successive experiments, using 6 concentrations mixed with 1 ppm Tween 20 (as an emulsifier), in addition to the control (1 ppm Tween 20), were carried out to study the plant extracts side-effects on the worker bees in the laboratory. The concentrations were 0.5; 1; 10; 100; 1,000 and 10,000 ppm. Each experiment was replicated three times (25 worker bees each time) and kept in glass containers (2 l capacity). The aqueous spray solutions (15 ml) of the tested substances were sprayed by using a manual atomizer. The number of dead workers and the mean mortality percentages were counted and calculated at 24 and at 48 hours after the treatment. To correct the mortality percentages of the worker bees, ABOTT'S formula (1925) was used.

From these experiments, the safe concentrations of each oil were detected, as follows:

- 0.5, 1 and 10 ppm for lemongrass and thymus.

- 1, 10 and 100 ppm for rosemary, marjoram, sag and fennel.

Moreover, 1 ppm of Tween 20 was used as control.

III. Apiary Experiments:

Fifty - seven Varroa - infested bee colonies were chosen. They were identical in strength (9 combs each), covered with worker bees and each colony was headed by a prolific nearly 1-year old queen.

These colonies were subsequently divided into six groups, of 9 colonies each, according to the safe concentrations which were obtained in the laboratory. The first four groups were sprayed with 1, 10 and 100 ppm of the emulsion oils of rosemary, marjoram, sag and fennel.

The other two groups were sprayed with 0.5, 1 and 10 ppm of thymus and lemongrass, while the seventh group, represented by three colonies, was sprayed with 1 ppm Tween 20 and used as a control group.

All colonies were sprayed between the combs, with the volatile oils during the day (150 ml/colony), by using a manual atomizer on 29/3, 5/4, 19/4 and 26/4/1995.

To determine the mortality percentage of the Varroa mites, the bottom of each colony was covered with a sheet of thick white paper which was pre-

viously coated with vaseline, just before each application. Six days after spraying, the paper sheets were removed and the dead mites fallen on the hive floor were visually counted. At the same time, another sheet of paper replaced the former one just before the next application.

The same technique was repeated before each application. Also, the number of mites parasitizing approximately 50 worker bees, in addition to the individuals inhabiting 30 worker brood cells was determined according to FATHY and FOULY (1993). Moreover, the efficacy of the tested substances was determined, by analysing the reduction in the Varroa percentages population, using the equation of GIORDANI and LE-PORATI (1989).

Results and Discussion

The data listed in Table 1 show that 250 g of dried rosemary leaves gave the greatest amount of volatile oil, followed by thymus, fennel, marjoram and lemongrass, while the sag ranked last.

The average amounts of volatile oils were 41.4, 36.5, 31.3, 25.0, 18.5 and 17.8 ml respectively/250 g of the previously mentioned plants.

Table 1
Percentage of Oils Extracted from 250 g (dry weight) of Different Volatile Oil Plants

Plant species	Extracted oil (ml)	Extracted oil (%)
Lemongrass	18.5	7.4
Thymus	36.5	14.6
Rosemary	41.1	16.5
Marjoram	25.0	10.0
Sag	17.8	7.1
Fennel	31.3	12.5

To detect the toxicity level of the above-mentioned natural bioactive substances upon the worker bee *Apis mellifera* and its relation with the concentration of each substance, a bioassay was carried out under laboratory conditions. The toxic effect of the six natural volatile oils on the worker bees was recorded after 24 and 48 hours of exposure. The mortality percentage was also recorded (Table 2). The results indicated that the rosemary oil has a less strong and constant effect (average mortality = 0.65%) on worker bees in comparison to other oils, at 1 and 10 ppm, while at 100 ppm, it had a stronger effect (1.40%). Both the marjoram and the fennel had a constant effect (1.25%) at all the concentrations (1, 10 and 100 ppm).

Thymus was more toxic for worker bees (1.25%) than the lemongrass (0.65%) at 0.5 ppm, while both oils had an equal effect (1.40%) at 10 ppm. (Table 2).

From the previous results, it may be concluded that the lower concentrations of 0.5, 1 and 10 ppm of both lemongrass and thymus; and the higher ones (1, 10 and 100 ppm.) of rosemary, marjoram, sag and fennel may be used in acaricidal activities, because of their safety for honeybees.

As regards the bioactivity of the substances tested as acaricides, the data listed in Table 3 show that all the tested volatile oils had a variable effect on the parasitic mite. The sag, the lemongrass and the fennel were not as effective as other oils. The rosemary, which gave the greatest amount of ex-

tracted oil, showed the highest level of efficacy. Concentrations of 1, 10 and 100 ppm of rosemary oil caused an average reduction of 18.9%, 28.7% and

39.2% respectively in the population of Varroa mites parasitizing the live worker bees and inhabiting the brood cells (Fig. 1, 2 and 3).

Table 2

Influence of the Six Natural Volatile Oils on the Mortality Percentage of Worker Bees under Laboratory Conditions

Plant	Conc. (ppm.)	No. of dead bees after 24 hr.	Mort. (%)	No. of dead bees after 48 hr.	Mort. (%)	Average mort. (%)
Lemongrass	0.5	2	2.5	1	0	0.65
	1	4	3.8	1	0	1.40
	10	4	3.8	1	0	1.40
Thymus	0.5	3	2.5	1	1.3	1.25
	1	3	2.5	2	1.3	1.40
	10	3	2.5	2	1.3	1.40
Rosemary	1	1	0	2	0	0.65
	10	2	1.3	1	0	0.65
	100	4	3.8	1	0	1.40
Marjoram	1	3	2.5	1	0	1.25
	10	3	2.5	1	0	1.25
	100	3	2.5	1	0	1.25
Sag	1	3	2.5	1	0	1.25
	10	3	2.5	2	1.3	1.40
	100	3	2.5	1	0	1.25
Fennel	1	3	2.5	1	0	1.25
	10	3	2.5	1	0	1.25
	100	3	2.5	1	0	1.25
Control	-	1	-	1	0	-

Table 3

Percentages of Reduction in the Varroa Population Occurring in the Bee Hives after Successive Treatments with Different Concentrations of Volatile Oils

Plant species	Conc. (ppm)	1 st treatment	2 nd treatment	3 rd treatment	4 th treatment	5 th treatment	Mean reduction (%)
Lemongrass	0.5	8.8	11.7	27.2	32.8	49.4	25.9
	1	14.3	26.7	34.2	39.2	53.8	33.6
	10	26.7	34.2	47.8	53.5	63.8	45.2
Thymus	0.5	18.0	22.0	36.6	41.2	61.4	35.8
	1	21.5	31.8	40.2	54.2	67.7	44.9
	10	26.4	38.8	53.7	65.1	79.6	52.7
Rosemary	1	18.9	23.5	38.5	50.6	63.4	38.9
	10	28.7	40.3	56.5	70.5	81.8	55.6
	100	39.2	49.6	67.7	83.2	87.5	65.4
Marjoram	1	9.7	19.6	28.2	34.3	59.2	30.2
	10	26.9	34.3	42.7	52.6	66.7	44.6
	100	37.4	41.8	52.4	66.4	77.7	55.1
Sag	1	6.2	8.7	14.4	29.0	49.2	21.5
	10	12.9	18.9	26.3	32.0	56.6	29.3
	100	14.3	28.8	37.6	42.9	63.1	37.3
Fennel	1	6.9	11.3	23.9	32.7	48.2	24.6
	10	23.0	32.8	41.2	45.5	57.8	40.1
	100	36.2	38.9	48.3	58.9	68.6	50.2

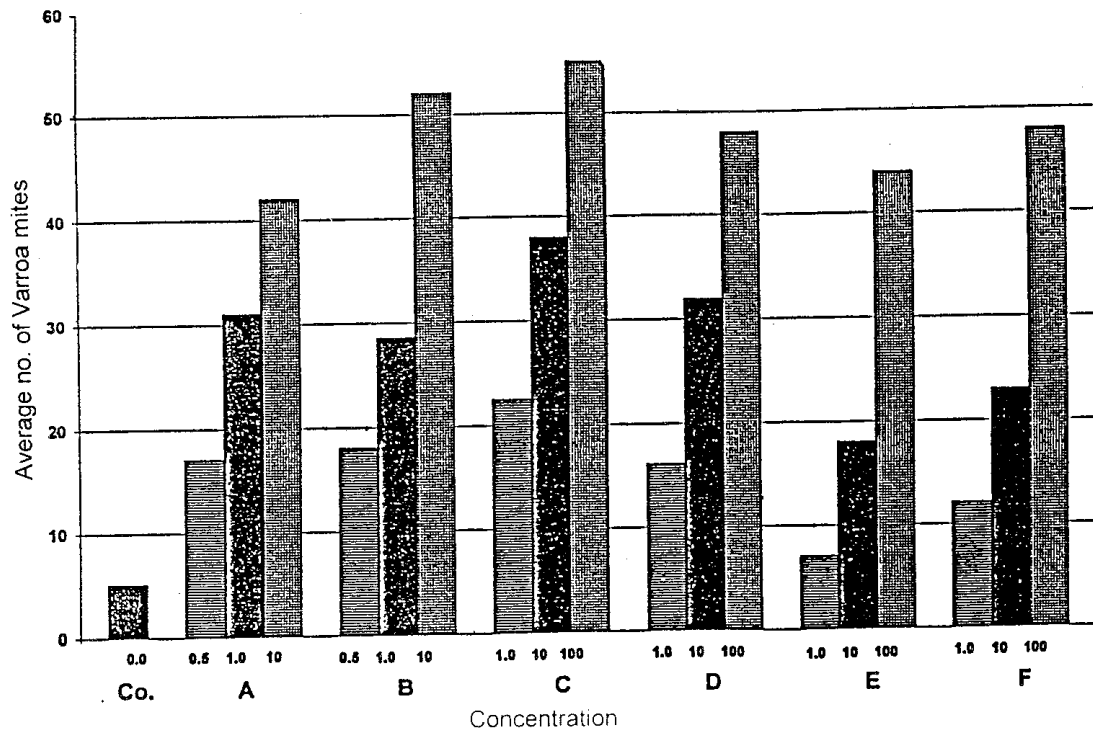


Fig. 1 - Average Number of Varroa Mites Fallen on the Hive Floor after Successive Treatments with Different Volatile Oils, 1995

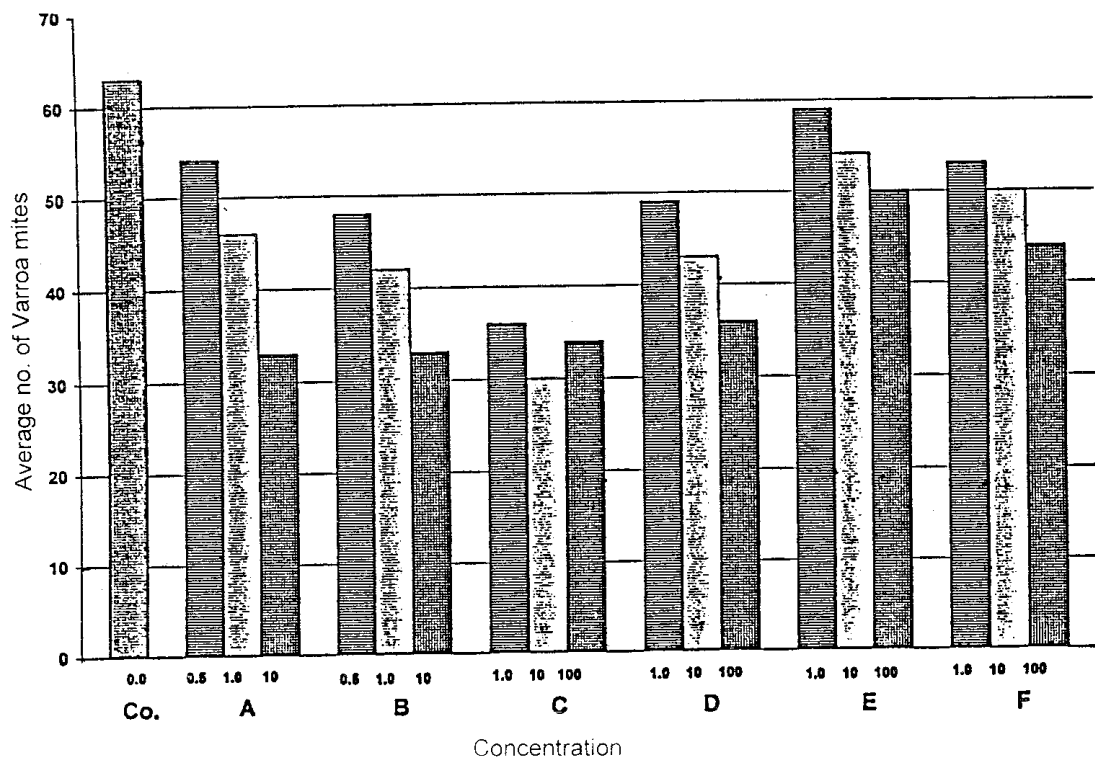


Fig. 2 - Average Number of Varroa Mites Living on Worker Bees after Successive Treatments with Different Volatile Oils

Co.: Control, A: Lemongrass, B: Thymus, C: Rosemary, D: Marjoram, E: Sage, F: Fennel

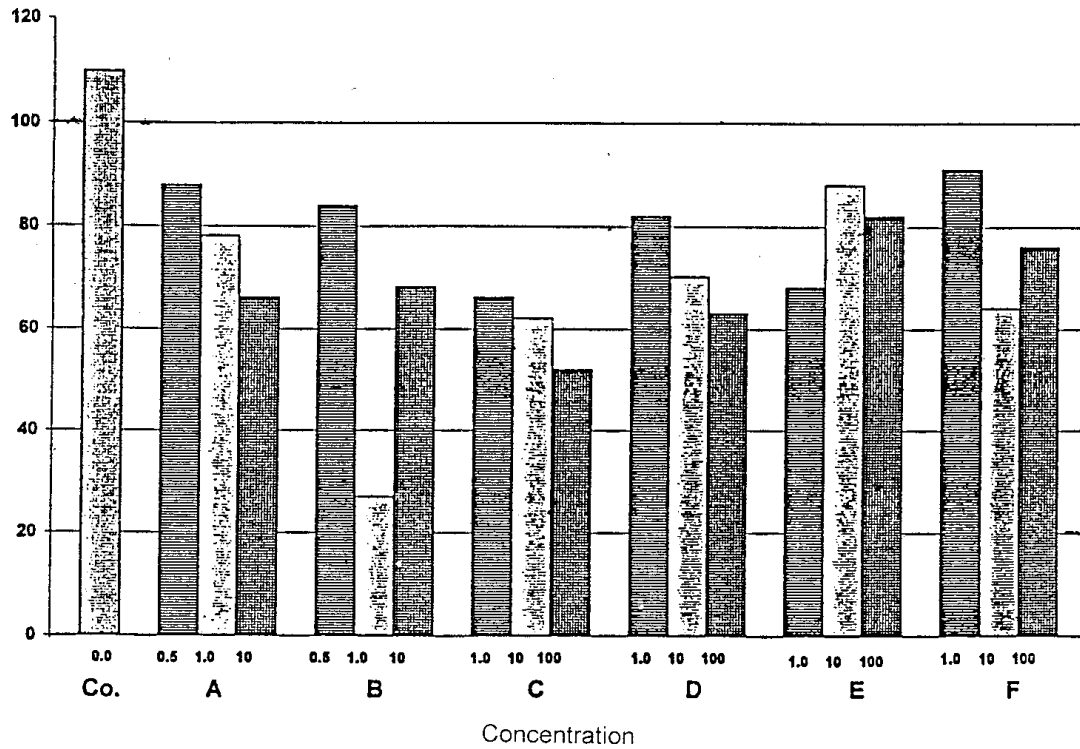


Fig. 3 - Average Number of Varroa Mites within the brood cells after successive treatments with different volatile oils

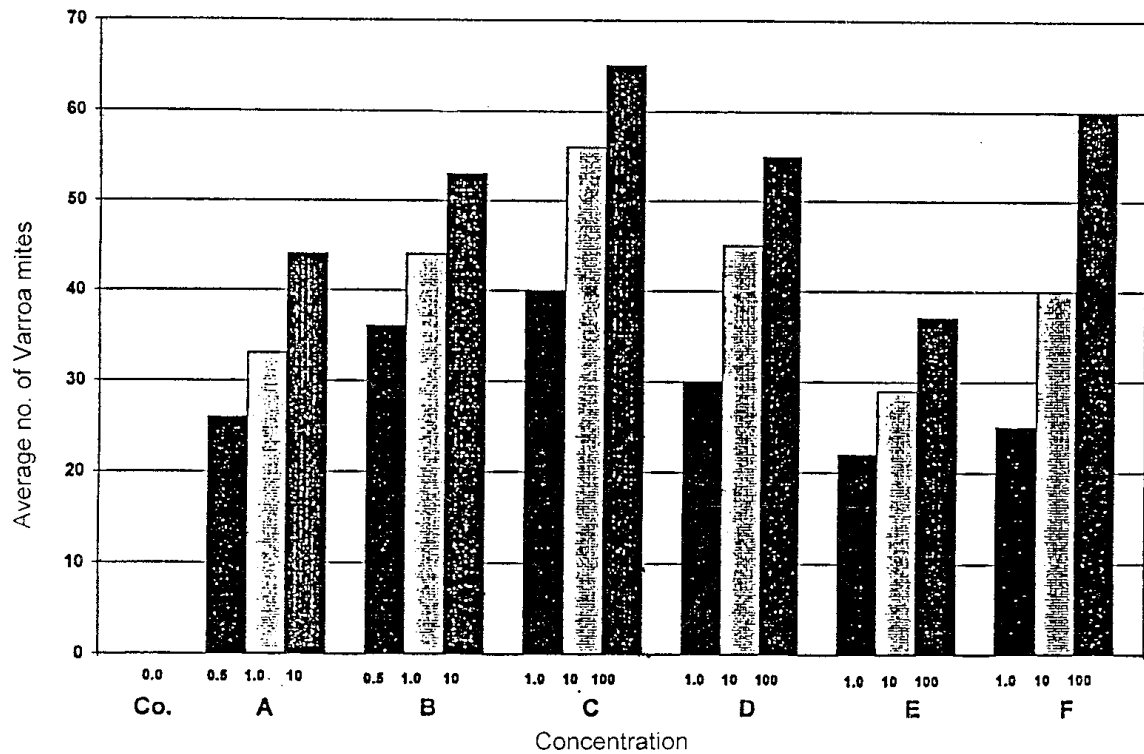


Fig. 4 - Mean Reduction (%) in Varroa Population Occurring in Bee Hives after Successive Treatments with Different Concentrations of Volatile Oils

Co.: Control; A: Lemongrass; B: Thymus; C: Rosemary; D: Marjoram; E: Sage; F: Fennel

Also, these values linearly increased when the treatment with all the tested substances was repeated. This is in agreement with the findings of WACHENDORFER et al. (1985) and FATHY and FOULY (1993), who repeated the treatment with formic acid and with camphor oil to increase the efficacy of the Varroa control.

The data in Table 3 also show that the mean percentages of reduction after five treatments averaged 25.9%, 33.6% and 45.2%; and 35.8%, 44.9% and 52.7% respectively for the mite populations occurring in colonies treated with 0.5, 1 and 10 ppm of lemongrass and thymus, respectively (Fig. 4).

On the other hand, in the bee hives sprayed with 1, 10 and 100 ppm of oils extracted from fennel and marjoram, the mean reduction percentages were 24.6%, 40.1% and 50.2%; and 30.2%, 44.6% and 55.1%, respectively.

The highest level of toxicity was generally observed when the bee hives were sprayed with rosemary. The Varroa population was reduced by 38.9%, 55% and 65.4% for the concentrations of 1, 10 and 100 ppm, respectively. In all the cases, the sage oil proved to be the last substance from the point of view of its effectiveness. Thus, the reductions in the mite population were of only 21.5%, 29.3% and 37.3%, respectively, when the bee colonies were sprayed with the same concentrations of this substance (Fig. 4).

The previous results proved that some oils extracted from different volatile oil plants, such as rosemary, thymus, marjoram and fennel, may be used as acaricides for the control of the

Varroa mite, while other substances, such as lemongrass and sage, were ineffective. Similar results were obtained by EMARA et al. (1994), who found that the worm seed plant, (*Artemisia china* L.), at concentrations of 0.3 x 10² and 3 x 10², was not as effective as *Hctate gum*, as *Pipper sp.* and as *Eucalyptus globulus* L.

Therefore, it may be concluded that there are some volatile oil plants which are considered to be a promising source of natural acaricides against varroasis.

Also, to obtain a high level of mortality of the Varroa population, high concentrations of volatile oils and the repetition of the treatments are necessary. It is also recommended to use rosemary because of its high level of effectiveness and of the great amount of oil that it gives.

Also, the effect of natural substances, such as essential or volatile oils, even if the treatment is repeated, cannot completely eliminate the Varroa invasion in colonies containing capped brood cells in countries like Egypt, where the sealed brood cells are present almost all around the year. The control of the Varroa mite by using natural products is more recommendable than the use of other chemical acaricides in order to avoid harmful effects.

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