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EVALUATION OF SOME NATURAL SUBSTANCES FOR CONTROLLING *VARROA DESTRUCTOR* AND THEIR EFFECTS ON INDIVIDUAL ACTIVITY OF HONEYBEE COLONIES.

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ABSTRACT

The present work was carried out at branch Beekeeping Research Department at Qalubia governorate. The aim of investigation evaluate the efficacy of some local natural substances for controlling *Varroa destructor* in honeybee colonies and their comparison with Apiguard 25% Thymol as material import was recommended. The result obtained show that, the mean reduction of infection with Varroa mite could be arranged into the following descending orders as follows: 86.78%, 81.48%, 79.33%, 75.33% and 68.96% when using plates Apiguard, Crystal Thymol, mixture of (Thymol, Menthol and Camphor), and Menthol, respectively. The experimental colonies were treated with (2-plates /colony) one plate at fortnight for (4 weeks). The mean increase of treated honeybee colonies for controlling Varroa mite was 7.96% and 67.69% for both brood rearing and honey production as compared to that of untreated colonies.

Generally, it could be concluded that, in cas of infestation of honeybee colonies with Varroa mite it is advisable to use Apiguard substance, Crystal Thymol and a mixture of (Thymol, Menthol and camphor). At the rate of one plate/ 2weeks/ colony for (4weeks) at high temperature greater than 27ºC.

INTRODUCTION

The parasitic mite *Varroa destructor* (Anderson and Truemane, 2000) is the most devastating pest affecting in honeybee (*Apis mellifera L*.) colonies worldwide. Varroa damage immature and adult bees by feeding on bee hemolymph and also by transmitting harmful viruses.

In the past years, the primary chemicals has been the pyrethroide fluvalinate, followed by the organophosphate and then by formic acid. Each compound has negative associated with their use. It is impact has been compounded because these Varroa mites quickly became resistance to the chemicals fluvalinate (Elzen *et al.*, 1998, 2000). Research on alternative chemical controls such as formic acid thymol has shown some promising results Feldloufer *et al.* (1997) and Mattila *et al.* (2000) but problems such as the effect of temperature on the timing of application, variable efficacy results, labor costs and the need for multiple applications have showed the advancement of these control methods for beekeeper use. Many products both chemical and natural are currently used in the control of this mite infestation. Among these products, different components of the essential oils were tested in laboratory and in particular, thymol (5-methyl- 2-(1- methyl ethyl) phenol) demonstrated the highest Varroa cidal activity at concentration well tolerated by the bees (Imdorf *et al.* 1995). The Varroacidal activity of

thymol was experimented not only in laboratory assays, but also in field in Europe (Imdrof *et al.* 1999) and in North America (Ellis *et al.* 2001, Melathopoulos and Gates 2003). Different dosages, ways and times of application have been tested on colonies in the post, often reporting highly satisfactory results (range of mean effectiveness 66-99.5%) Imdrof *et al.* (1999). Emara *et al.* (1994) Concerned with the formulation and evaluation of certain plant extracts for controlling varroa mites.

Thymol, which is a volatile monoterpenoid and a natural constituent of thyme (*Thymus vulgaris*) is widely used in honeybee colonies as a treatment against Varroa (Gregorc and Jelenc 1996; Imdorf *et al.* 1999). Thymol residues, which can be found in the honey after treatments, do not affect its taste if applied following the harvest (Bogdanov *et al.* 1998). Apiguard (Vita Europe Ltd., Basingstoke, England), which comes in gel form, is a registered Thymol-based fumigant that is used against Varroa mites in honeybee colonies. The present work aimed to evaluate of some natural substances for controlling *Varroa destructor* and their effects on activity of honeybee colonies.

MATERIALS AND METHODS

The present work was carried out at branch Beekeeping Research Department at Qalubia governorate is that administration, Agriculture Research Center. The aim of investigation evaluate the efficacy of some local natural substances for controlling *Varroa destructor* in honeybee colonies and their comparison with Apiguard 25% thymol as material import was recommended.

1- Preparation of the experimental colonies:

Eighteen honeybee colonies first hybrid Carniolan were infested with Varroa mite, the experimental colonies were divided into six groups each composed of three colonies, relatively similar strength, colonies in each group were insignificant of infested with Varroa mite in adult. The treatment of experiment was carried out from winter season, 2005 to clover season, 2006.

2- Preparing the natural substances:

Such groups were treated as follows:

Group (A): Apiguard on the shape plates of aluminum as material import was recommended (vita Europe limited), which contained 50g gel, 25% thymol.

Group (B): Crystal thymol 25%, which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of talc powder + Vaseline (as carrier material) containing 25% thymol (12.5g) and put in aluminum foil inside Petri dish as past form.

Group (C): Menthol 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of (talc powder + Vaseline) containing 25% Camphor oil (12.5g) and put in aluminum foil inside Petri dish as past form.

Group (D): Camphor 25% which was prepared on the shape plates from aluminum foil as follow: A mixture of 50g of (talc powder + Vaseline) containing 25% Camphor (12.5g) and put in aluminum foil inside Petri dish as past form.

Group (E): A mixture of natural product (Camphor + Menthol + Thymol) which was prepared on the shape of plates from aluminum foil. The plate was prepared as a mixture of 50g. of talc powder + Vaseline containing 25% of mixture natural product (12.5g.) as a ratio 4.17g. of each.

The Thymol and Camphor were obtained from El Gomhouria Co. whereas the six Menthol was obtained from El Nasr Pharmacutical chemicals Co. Abou Zabal.

Group (F): untreated colonies (Control).

The plates were placed on the top of board facing the brood chamber after open cover the plate to allow the bees to enter the plate and remove the product.

The colonies of experimental colonies were treated with (2 plates /colony) one plate at fortnight. The total treatment period was four weeks from the beginning of the fourth week of December 2005 till the end of the third week of January, 2006. The honeybee colonies were fed on sugar syrup (66.6% Conc.) one /week. The bottom board of the hive was covered with a plastic sheet coated with raw Vaseline to capture the fallen mites. The died Varroa mites were counted and removed at the end of each treatment. The day temperature and relative humidity were recorded daily allover the experiment.

3- Determination of Varroa infestation:

a- On workers:

The percent infestations of Varroa mite on workers before and after treatments were determined according to Komeili (1988) Accordingly, samples of hundred bees/ colony were collected randomly in vial partially filled with water containing few drops of detergent. The samples were shaken and the bees were washed in a strainer, individual mites that fell off from worker bees were found at the bottom of the white container (Ritter, 1981). All worker bees and mite were counted for each sample, where the number of mites / 100 workers was calculated.

b- In brood cells:

The infestation percent of Varroa mites in brood cells was determined by using forty five worker cells that were opened and the Varroa mite occurring with these cells were counted. Reduction percentage in mite infestation was calculated according to Henderson and Tilton (1955).

4- Effects of tested materials:

a- On individual honeybee colonies:

After treatment of the experimental colonies, the average daily of queen and adult bees were counted until the end of experimental, also odour and disturbance of honeybee colonies were observed compared with untreated colonies.

b- On brood rearing activity:

The daily worker sealed brood cells was counts after treated honeybee colonies at 13 day intervals, on December 22, 2005 until March, 22, 2006.

c- On clover honey production:

The clover honey produced by test colonies was evaluated for each colony individually as a difference between the weight of bee honey combs before and after extraction in clover season 2006.

RESULTS AND DISCUSSION

1- Effect of the natural substances against Varroa mite:

Table (1) and Fig. (1) Show that, the Apiguard (Vita Europe limited) caused reduction of infestation being 85.27% and 88.29% for brood cells and adult, respectively. The mean reduction of infestation reached 86.78% for both brood and adult. Crystal Thymol on the shape plates as past form caused reduction of infestation being 76.93% and 86.03% for brood cells and adult, respectively. The mean reduction of infestation reached 81.48% for both brood and adult.

Menthol on the shape plates as pest form caused reduction of infestation being 66.83% and 71.08% for brood cells and adult, respectively. The mean reduction of infestation reached 68.96% for both brood and adult. Camphor on the shape plates as past form, caused reduction of infestation being 71.15% and 79.51% for brood cells and adult, respectively. The mean reduction of infestation reached 75.33% for both brood and adult.

Mixture of (Thymol, Menthol and camphor) on the shape plates as past form caused reduction of infestation being 75.87% and 82.79% for brood cells and adult, respectively. The mean reduction of infestation reached 79.33% for both brood and adult.

Table (1): Reduction percentages of Varroa mites on brood and adult honeybee.

Treatments	%Infestation in brood cells		% Reduction	%Infestation on adult bees		% Reduction	Mean
	Before treat.	After treat.		Before treat.	After treat.		
Apiguard 25% thymol	32.0 ±2.31	5.33 ±1.33	85.27	25.12 ±3.62	3.61 ±1.41	88.29	86.78
Crystal thymol 25%	30.67 ±3.53	8.0 ±2.31	76.93	23.38 ±2.72	4.01 ±1.52	86.03	81.48
Menthol 25%	32.0 ±4.61	12.0 ±2.33	66.83	26.67 ±3.85	9.47 ±2.72	71.08	68.96
Camphor 25%	38.33 ±2.31	10.87 ±3.33	71.15	24.69 ±3.79	6.21 ±1.71	79.51	75.33
25%Mix. of(Camphor + Thymol + Menthol)	29.33 ±3.53	8.0 ±2.31	75.87	29.01 ±2.63	6.13 ±1.77	82.79	79.33
Untreated	30.67 ±4.09	34.67 ±3.53	--	21.09 ±3.19	25.89 ±3.47	--	--

2- Fallen Varroa mite in honeybee colonies:

Table (2) indicated that, treating Varroa mites infesting honeybee colonies with the tested material caused significantly higher number of fallen

Varroa mites as compared to that of untreated colonies. Mean total of fallen Varroa (788 mites) was recorded after using plate of Apiguard followed by crystal thymol on the shape plates (768 mites), mixture of (Thymol, Menthol and camphor) on the shape plates (566 mites), Camphor on the shape plates (496 mites) and Menthol on the shape plates (485 mites). The least number of fallen Varroa was recorded in untreated colony (84 mites). It can be concluded that, 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.

From the results obtained in Table (3) and Fig (1) it could be concluded that, the reduction of infection could be arranged into the following descending orders as follows: Apiguard plates, Crystal thymol plates, mixture of (Thymol, Menthol and Camphor) plates, Camphor plates and Menthol plates for both brood cells and adult.

Table (2): Number of fallen Varroa mites after daily sequence from the treatments.

Treatments	Mean number of fallen Varroa mites after treatment							Total
	1	2	3	7	14	21	28	
Apiguard 25% thymol	273 ±6.24	152 ±15.3	129 ±11.79	133 ±8.95	69 ±14.11	25 ±6.17	7 ±1.55	788
Crystal thymol 25%	196 ±9.17	168 ±19.12	114 ±14.52	183 ±11.85	60 ±7.86	36 ±8.12	11 ±1.78	768
Menthol 25%	141 ±5.17	87 ±12.51	91 ±7.18	105 ±10.81	39 ±8.74	10 ±3.18	12 ±1.72	485
Camphor 25%	98 ±9.49	121 ±12.5	95 ±6.19	66 ±10.9	93 ±16.12	16 ±5.29	7 ±1.53	496
25%Mix. of (Camphor + Thymol + Menthol)	161 ±11.21	85 ±9.14	131 ±12.15	61 ±7.25	82 ±9.51	37 ±6.11	9 ±2.14	566
Untreated	8 ±1.73	10 ±1.78	9 ±1.21	14 ±4.33	12 ±5.19	15 ±2.64	16 ±3.36	84

Generally, a thymol product should be applied when the treatment ranges between 18.5 ºC and 27 ºC. This results similarity with Imdorf *et al* (1995).

It is advisable to use Apiguard, Crystal thymol, Menthol, Camphor and mixture of (Thymol, Menthol and Camphor) on the shape plates on strong colony at high temperature greater than 27 ºC enhance the distribution of thymol in the hive through ventilation but it was recommended not to use on weak colonies at high temperature greater than 18.5 ºC this results coincide with Alessandra *et al* (2004).

Table (3): Natural substances in descending orders for % reduction of adult and brood honeybee.

Individual	Apiguard	Crystal Thymol	Mix. of Camphor+ Thymol+Menthol	Camphor	Menthol	L.S.D.
Adult	88.29	86.03	82.79	79.51	71.08	8.14
Brood	85.27	76.93	75.87	71.15	66.83	9.12

3- Effect of tested substances on odour and individual colony status:

As shown in Table (4) show that, there was no effect of tested substances on odour and individual colony statues as compared to that of untreated colonies.

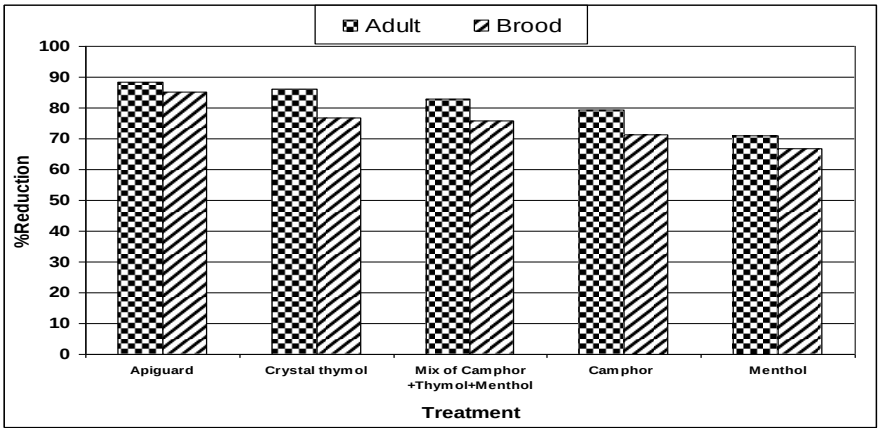

Fig. (1): Efficiency of some natural substances in controlling Varroa mite on adult and brood honeybees.

4- Effect of tested substances on worker sealed brood cells:

From the results obtained in table (4), the highest average daily worker brood cells was 478.38 cells with increase of 13.18% when using Apiguard, followed by 472.46 cells with increase 11.77% when using thymol, 457 cells with increase 8.12% when using mixture of (Thymol, Menthol and Camphor), 439.38 cells with increase 3.95% when using menthol and 434.46 with increase 2.78% when using Camphor, respectively.

5- Effect of tested substances on honey production:

As shown in Table (4), the highest average of honey production was 6kg. With increase 84.62% as compared to that untreated colonies when using mixture of (Thymol, Menthol and Camphor) for controlling Varroa mites, followed by 5.75kg. with increase 76.92% when using Thymol, 5.5kg with increase 69.23% when using Apiguard, 5.25kg. with increase 61.54% when using Camphor and 4.75kg with increase 46.15% as compared to that untreated colonies when using Menthol for controlling Varroa mites.

Table (4): Effect of tested substances on individual colony status, brood rearing activity and honey production on honeybee colonies.

Treatments	No of queenless	Average daily of adult bees died after treat.	Average daily of sealed brood cells after treat.	% increase of sealed brood	Clover honey production (kg)	% increase of honey
Apiguard 25% thymol	0.0	2.93 ±0.121	478.38 ±32.08	13.18	5.5 ±0.55	69.23
Crystal thymol 25%	0.0	3.9 ±0.101	472.46 ±33.0	11.77	5.75 ±0.61	76.92
Menthol 25%	0.0	4.04 ±0.181	439.38 ±28.38	3.95	4.75 ±0.71	46.15
Camphor 25%	0.0	4.0 ±0.14	434.46 ±31.92	2.78	5.25 ±0.86	61.54
25%Mix. of (Camphor + Thymol + Menthol)	0.0	3.06 ±0.16	457.0 ±30.62	8.12	6.0 ±0.75	84.62
Mean	0.0	3.59	456.34	7.96	5.45	67.69
Untreated	0.0	4.37 ±0.191	422.69 ±36.38	0.0	3.25	0.0

There was no effect of natural tested substances on physical shape, color and odour of harvest honey, it is preferred advice to use of natural tested substances for controlling Varroa mites in winter and after the honey harvest. This results coincide with Mattilla and Otis (2000).

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تقييم بعض المواد الطبيعية في مكافحة طفيل الفاروا وتاثيرها علي افراد وانشطة طوائف نحل العسل

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اجري هذا البحث في منحل قسم بحوث النحل بمحافظة القليوبية بهدف تقييم فاعلية بعض المنتجات الطبيعية المحلية ودراسة تاثيرها علي افراد طائفة نحل العسل وكذلك علي انشطة الطوائف (تربية الحضنة وانتاج العسل) وكانت المواد الطبيعية المستخدمة هي اطباق الابيجارد ، الثيمول، المنتول ،الكافور و مخلوط من (الثيمول ، المنتول و الكافور) وهذة المواد تم اعدادها وتجهيزها في صورة اطباق بعد اضافة مواد حاملة مناسبة لتنظيم عملية تبخيرها داخل طوائف نحل العسل عند الاستخدام.

واوضحت النتائج ما يلي :-

- اعطت اطباق الابيجارد اعلي معدل خفض في الاصابة لطفيل الفاروا حيث وبلغت الي ٧٨.٨٦ % كما اعطت اطباق الثيمول المرتبة الثانية حيث بلغت النسبة المئوية للخفض في الاصابة ٨١,٤٨ % وحقق المركب الطبيعي المخلوط من (الثيمول ، المنتول والكافور) في صورة اطباق المرتبة الثالثة حيث بلغت النسبة المئوية للخفض في الاصابة ٧٩,٣٣ % بينما حققت اطباق الكافور – المنتول كل منهما علي حده المرتبة الرابعة والخامسة حيث بلغت النسبة المئوية للخفض في الاصابة ٧٥,٣٣ %، ٦٨,٩٦ % علي الترتيب.
- و حققت طوائف نحل العسل التي تم معاملتها لمكافحة طفيل الفاروا زيادة ملحوظة في انتاجيتها، حيث بلغ متوسط الزيادة في معدل تربية الحضنة وانتاج العسل ٧,٩٦ % ، ٦٧,٦٩ % علي الترتيب عن طوائف المقارنة، هذا بالاضافة الي عدم تاثير المواد المستخدمة في المكافحة علي افراد او انشطة طوائف نحل العسل.

التوصية:

توصي النتائج باستخدام اطباق الابيجارد – الثيمول – مخلوط مركب من (الثيمول + المنتول + الكافور) بمعدل طبق واحد/ خلية/ اسبوعين وتكرر المعاملة مرتين وتستمر فترة العلاج ٢٨ يوم مع مراعاة الا تزيد درجة الحرارة اثناء المعاملة عن ٢٧ °م حتي لا تؤثر علي انشطة وافراد طوائف نحل العسل.