

EFFICIENCY OF SOME BOTANICAL PRODUCTS IN CONTROLLING *Varroa* MITE INFESTING HONEYBEE COLONIES

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By

S. I. Yousif-Khalil, A. M. Khater* and I. M. A. Ebadah**

Plant Protection Department, Faculty of Agriculture, Zagazig University, Egypt.

* *Plant Protection Research Institute. Agricultural Research Center, Dokki, Giza, Egypt.*

** *National Research Center, Dokki, Giza, Egypt.*

ABSTRACT

The present investigation was carried out in the apiary of Beekeeping Research Department, Plant Protection Research Institute at Zagazig, Sharkia Governorate during 2007 season, to evaluate the efficiency of camphor salt, peppermint extract, thymol and formic acid (60 %) as control agents against *Varroa* mite infesting honeybee colonies. Data obtained could be summarized as follows:

- 1- Mite infestation was reduced by 69.01, 48.92, 75.95 and 90.12 % when the colonies were treated with camphor salt, peppermint extract, thymol crystals and formic acid, respectively.
- 2- The infested workers suffered 21.87 % reduction in body weight while treating infested colonies with camphor salt, peppermint extract, thymol crystals and formic acid decreased the reduction in body weight to 11.84, 13.87, 5.91 and 2.63 %, respectively.
- 3- *Varroa* infested colonies showed severe reduction (47.96 %) in the sealed brood area reared as compared to that of healthy ones. Treating infested colonies with the test control agents decreased the reduction taken place in sealed brood area, as it attained 20.85, 29.58, 15.69 and 13.11 % for the treated colonies with test agents, respectively.
- 4- The mean annual honey yield (citrus and clover) of healthy, *Varroa* infested (non treated) colonies and those treated with camphor salt, peppermint extract, thymol crystals and formic acid recorded 8.06, 3.01, 5.77, 4.94, 6.53 and 7.15 kg/colony, respectively. The respective percentages of reduction in honey yield were 62.66, 28.41, 38.71, 18.98 and 11.29 %.

Key words: *camphor salt, formic acid, honeybee colonies, peppermint extract, thymol, varroa mite.*

1. INTRODUCTION

Varroa mite, *Varroa destructor (jacobsoni)* Oud. is considered one of the most dangerous beekeeping pests due to its capability of rapid and wide spreading and its power of destruction (Matheson, 1995). Therefore, many research works have been directed to control causative of this disease using different acaricides such as fluvalinate, Apitol, amitraz, etc. (Elzen *et al.*, 1999 and Norberto, 1999).

However, the pollution of bee products, the developing of resistant strains of *Varroa* mite and other complications obligated the researchers and beekeepers to look for alternative more safer control measurements.

Therefore, the present work was designed to evaluate the efficiency of some control agents of botanical origin (camphor salt, peppermint extract, thymol crystals and formic acid) in controlling *Varroa* mite.

2. MATERIALS AND METHODS

The present investigation was carried out in the apiary of Beekeeping Research Department, Plant Protection Research Institute at Zagazig, Sharkia Governorate during 2007 season to evaluate the efficiency of some botanical materials in controlling *Varroa* mite infesting honeybee colonies.

2.1. Materials used

The following materials were used to control *varroa* mite infesting honey bee colonies. They were 6 secured from El-Gomhoreia Company for medical supplies and tools, zagazig Branch, Egypt.

2.1.1. Camphor powder

Powdered camphor (one tablet) was mixed with 20 g sugar powder to be dusted on the brood combs covered with bees/colony. Four colony treatments were applied at 7 day intervals.

2.1.2. Peppermint oil

The peppermint (*Mentha peperita*) oil extract was obtained by boiling 500 g of leaves in 1.5 litres of water for 15 minutes. After filtration, the

extract (5 ml) was added to 500 ml of sugar syrup (1:1) per colony. Four colony treatments were applied at 6-day intervals.

2.1.3. Thymol

Thymol crystals (0.75 g/colony) were kept in a perforated saccule to be hung between brood combs. Two colony treatments were applied at 10-day intervals.

2.1.4. Formic acid

Formic acid, at the concentration of 60 % was applied on absorbent cardboard plates (5 × 20 cm) at the rate of 15 ml per colony. The treated cardboard plates were placed over the top bars of the frames at the evening time. Four colony treatments were applied at 4-day intervals.

2.2. Experimental honeybee colonies

Three *Varroa* free-(healthy) and fifteen *Varroa* infested honeybee colonies (infestation range between 9 - 13 %) were used in the present study. The *Varroa* infested colonies were divided into 5 groups of 3 colonies each as follows:

Group 1: consisted of 3 healthy colonies used as +ve control

Group 2: consisted of 3 untreated *Varroa* infested colonies used as -ve control

Group 3, 4, 5 and 6 consisted of 3 infested colonies each and treated with camphor, peppermint, thymol and formic acid (60 %), respectively.

The tested colonies (except group 4) were fed with the same quantity of sugar syrup (1:1) during the period of study. Onset of the experimentation started on January 15 until the end of February, 2007.

The (pre-and post-treatment) *Varroa* infestation was assessed according to Shabanov *et al.* (1981). Thereafter, the rate of reduction in % infestation was calculated according to Henderson and Tilton (1955) formula:*

*% Reduction=

$$1 - \left(\frac{\% \text{ Infestation after treatment}}{\% \text{ Infestation before treatment}} \times \frac{\% \text{ Infestation in the control before}}{\% \text{ Infestation in the control after}} \right) \times 100$$

2.3. The development of workers body weight

The development of workers body weight was followed and recorded according to Yousif-Khalil (1992) method. This study was performed during March 2007.

2.4. Evaluating brood rearing activity

The areas of workers sealed brood cells present in the test colonies were measured separately, using Hoffman frame and divided into square inches at 12-day intervals from March to September, 2007.

2.5. Estimating honey production

The surplus honey combs were taken from their respective colonies after discarding the bees covering and marked with colour paint. Thereafter, honey yield was estimated for each colony separately (in kg/colony) by calculating the difference between the weight of honey combs before and after citrus and clover honey extraction.

Data obtained were statistically analysed according to Snedecor (1957) .

3. RESULTS AND DISCUSSION

3.1. Efficiency of the test control agents in reducing *Varroa* mite infestation

Data presented in Table (1) clear that the pre-treatment mean rate of *Varroa* infestation recorded 12.66, 9.33, 11.66, 11.33 and 10.66 %. The respective post-treatment % infestation attained 4.66, 5.66, 3.33, 1.33 and 12.66 % after treating the colonies with camphor powder, peppermint extract, thymol, formic acid (60 %) and those untreated (control), respectively. The calculated rate of reduction in *Varroa* mite infestation recorded 69.01, 48.92, 75.95 and 90.12 % for camphor, peppermint, thymol and formic acid (60 %) treatments, respectively.

Analysis of variance revealed that formic acid was the most potent control agent followed by thymol whereas peppermint was the least effective agent. These results are in agreement with those of Clark (1994) and Mutinelli *et al.* ,(1997).

Table (1): Effect of the test control agents in reducing the rate of *Varroa* infestation in honeybee colonies during 2007 season.

| Treatments | Pre-treatment infestation % | Post-treatment infestation % | Reduction % |
|------------------------------|-----------------------------|------------------------------|-------------|
| Camphor salt | 12.66 | 4.66 | 69.01 |
| Peppermint extract | 9.33 | 5.66 | 48.92 |
| Thymol crystals | 11.66 | 3.33 | 75.95 |
| Formic acid (60 %) | 11.33 | 1.33 | 90.12 |
| Control (untreated colonies) | 10.66 | 12.66 | |
| L.S.D. | 0.05 | 0.68 | |
| | 0.01 | 0.96 | |

3.2. Development of worker body weight

Obtained results clear that the mean weight of newly emerged workers recorded 93.45, 91.35, 89.0, 86.75, 82.8 and 70.05 mg/worker arised in healthy colonies and those exposed to formic acid 60 %; thymol; camphor; peppermint extract and infested untreated colonies, respectively (Table 2). Analysis of variance indicated that *Varroa* infested (untreated) workers showed the least significant mean body weight. Formic acid and thymol caused noticeable positive effect while peppermint proved to be the least potent.

Thereafter, workers body weight increase

was reported by Yousif-Khalil (1992) and Abd-Alla (1999).

It is a well known fact that the adult *Varroa* females invade worker cells, containing grown larvae after larval feeding has been ceased and just before cell capping. *Varroa* mite (adult and immatures) usually feed (by sucking) on larval and pupal haemolymph containing digested food staffs and secretions needed for the development and metamorphosis of bees. Therefore, weight loss is expected, even if an efficient control measure is applied. De Jong and De Jong (1983) stated that the weight loss of newly emerged bees

Table (2): Development of workers body weight (mg) in different ages of healthy and *Varroa* mite infested honeybee colonies as affected with the tested control agents during 2007 season.

| Treatments | Age | Workers body weight (mg/individual) | | | | | Weight* index % | Reduction % |
|--------------------|------|-------------------------------------|-----------|------------|------------|--------|-----------------|-------------|
| | | Newly emerged | 7 day old | 14 day old | 21 day old | Mean | | |
| Healthy colonies | | 93.45 | 105.65 | 110.4 | 99.1 | 102.15 | 100 | - |
| Formic acid 60 % | | 91.35 | 100.72 | 104.36 | 101.4 | 99.46 | 97.37 | 2.63 |
| Thymol crystals | | 89.0 | 98.05 | 102.4 | 95.0 | 96.11 | 94.09 | 5.91 |
| Camphor salt | | 86.75 | 97.0 | 99.0 | 85.65 | 92.10 | 90.16 | 11.84 |
| Peppermint extract | | 82.8 | 91.8 | 95.8 | 81.5 | 87.98 | 86.13 | 13.87 |
| Infested colonies | | 70.05 | 84.1 | 88.1 | 77.0 | 79.81 | 78.13 | 21.87 |
| L.S.D. | 0.05 | 2.94 | 2.21 | 2.71 | 3.08 | 3.30 | | |
| | 0.01 | 3.82 | 3.59 | 3.61 | 4.50 | 4.15 | | |

* Based on healthy colonies as 100 %

progressively by time, in all treatments until recording the heaviest body weight for 14-day old workers. The recorded body weight on that age reached 110.4, 104.36, 102.4, 99.0, 95.8 and 88.1 mg/worker for healthy, formic acid; thymol; camphor; peppermint extract treated colonies and infested untreated colonies, respectively. Workers of healthy colonies showed the heaviest significant mean body weight. On the other hand, infested workers possessed the lightest significant one. Formic acid 60% and thymol proved to be the most potent control agents, meanwhile peppermint was the least. Twenty one day old workers of all treatments suffered noticeable decrease in body weight.

Weight index data {calculated basied upon healthy workers (the heaviest) are as 100 %} clear that body weights of the treated workers with formic acid, thymol, camphor and peppermint and untreated workers are 97.37, 94.09, 90.16, 86.13 and 78.31% as heavy as healthy workers.

In general, it could be concluded that the infested workers manifested the lightest significant body weights at all tested ages. However, the weight within the same group differed from one age to another, being the lightest for newly emerged and the heaviest for 14 day old worker in all treatments. The same trend

may be due to the haemolymph sucked by the mite during pupal stage. Glinski and Jorosz (1984) and Ball (1993) found that the total protein content was lower in parasitized brood, probably due to either protein depletion in the host larvae, or as a result to biochemical changes following the release of toxic substances by the mite into the host's blood.

3.3. Brood rearing activity

Obtained results manifested relatively higher brood rearing activity starting from the beginning of March followed by a progressive increase by time until recording the first peak during the first week of April where the sealed brood area reached 246.6, 125.1, 189.6, 176.3, 206.8 and 211.2 inch²/colony in healthy, *Varroa* infested (untreated colonies) and those infested and treated with camphor, peppermint extract, thymol and formic acid, respectively. A gradual decrease was then taken place until May, 13, followed by irregular increase till recording a second peak detected during the 2nd half of June. The third and the highest peak was detected on August, 17 (Table 3).

Analysis of data revealed that healthy colonies reared the highest significant sealed brood area on all peaks. On the other hand,

Table (3): Brood rearing activity of healthy and Varroa infested honeybee colonies as affected by the test control agents during the active season of 2007.

| Date Treatments | Sealed brood area (inch ² /colony) | | | | | | | | | | | | | | | | | Total | Mean | Reduc. % of brood area |
|--------------------|---|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|---------------------------------|
| | 1/3 | 13/3 | 25/3 | 7/4 | 19/4 | 1/5 | 13/5 | 25/5 | 7/6 | 19/6 | 1/7 | 13/7 | 25/7 | 7/8 | 17/8 | 29/8 | 11/9 | | | |
| Healthy colonies | 233.4 | 232.8 | 222.3 | 246.6 | 204.6 | 223.1 | 189.3 | 237.3 | 256.6 | 260.8 | 258.6 | 200.3 | 245.5 | 250.8 | 261.8 | 219.2 | 211.9 | 3954.9 | 232.6 | - |
| Infested colonies | 128.8 | 134.8 | 117.1 | 125.1 | 140.8 | 122.6 | 122.3 | 123.5 | 113.6 | 119.8 | 120.8 | 109.3 | 105.3 | 117.8 | 125.6 | 120.1 | 110.3 | 2057.6 | 121.04 | 47.96 |
| Camphor salt | 158 | 180.1 | 177.6 | 189.6 | 179.8 | 187.8 | 169.8 | 190.6 | 184.3 | 185.8 | 188.6 | 174 | 188.5 | 209.3 | 217 | 182.3 | 167.1 | 3130.1 | 184.1 | 20.85 |
| Peppermint extract | 150.3 | 160.1 | 166.15 | 176.3 | 163 | 160.6 | 143 | 158.2 | 166.5 | 170.1 | 169 | 160.8 | 174.1 | 176.1 | 183.3 | 162.3 | 145.3 | 2785.1 | 163.8 | 29.58 |
| Thymol crystals | 186.6 | 188.5 | 192 | 206.8 | 175.3 | 190.3 | 179.8 | 192.5 | 202.3 | 201.8 | 201 | 183.1 | 215.1 | 218.8 | 233.3 | 188.9 | 178.3 | 3334.4 | 196.1 | 15.69 |
| Formic acid(60%) | 198.4 | 201.2 | 191.2 | 211.2 | 186.4 | 197.5 | 184.6 | 198.1 | 207.5 | 200.3 | 204.1 | 190.6 | 220.3 | 224.1 | 237.2 | 191.4 | 183.5 | 3436.1 | 202.1 | 13.11 |
| Total | 1055.5 | 1097.5 | 1074.8 | 1155.6 | 1049.9 | 1081.9 | 988.8 | 1100.2 | 1130.8 | 1138.6 | 1142.1 | 1018.1 | 1148.8 | 1196.9 | 1258.2 | 1064.2 | 996.4 | | | |
| Mean | 144.8 | 182.9 | 179.1 | 192.6 | 174.9 | 180.3 | 164.8 | 183.3 | 188.4 | 189.7 | 190.3 | 169.6 | 191.9 | 199.4 | 209.7 | 177.3 | 166.0 | | | |
| L.S.D. 0.05 | | | | | | | | | | | | | | | | | | | 37.44 | |
| 0.01 | | | | | | | | | | | | | | | | | | | 47.37 | |

Efficiency of some botanicals products in controlling

Varroa infested colonies reared the least significant area of sealed brood. Treating infested colonies with the test agents affected positively this activity. Formic acid proved to be the most potent agent followed by the thymol. The

that the *Varroa* mite infestation affected clearly honey production of the diseased honeybee colonies.

In this connection, Milne (1981) and Woyke (1984) reported that body weight, average daily brood production, workers longevity and

Table (4): Honey yield (kg/colony) of healthy and *Varroa* infested honeybee colonies as affected by the test control agents during 2007 season.

| Treatments | Honey yield (kg/colony) | | | |
|--------------------|-------------------------|--------------------|--------------------|---------------------------|
| | Citrus honey | Clover honey | Total honey yield | Reduction % of production |
| Healthy colonies | 2.75 ^a | 5.31 ^a | 8.06 ^a | - |
| Infested colonies | 0.95 ^c | 2.06 ^c | 3.01 ^c | 62.66 |
| Camphor salt | 1.91 ^{ac} | 3.86 ^b | 5.77 ^b | 28.41 |
| Peppermint extract | 1.56 ^{bc} | 3.38 ^b | 4.94 ^{bc} | 38.71 |
| Thymol crystals | 2.24 ^{ba} | 4.29 ^b | 6.53 ^{ab} | 18.98 |
| Formic acid (60 %) | 2.37 | 4.78 ^{ba} | 7.15 ^{ab} | 11.29 |
| L.S.D 0.05 | 1.06 | 0.53 | 2.40 | |
| 0.01 | 1.33 | 0.68 | 3.56 | |

reduction in the realed sealed brood area due to mite infestation attained 47.96, 20.85, 29.58, 15.69 and 13.11 % in infested untreated colonies and those treated with camphor, peppermint extract, thymol and formic acid, respectively.

The side effect of the chemicals used to control *Varroa* mite infesting honeybee colonies is doubtless and was reported by many authors such as Henderson (1984), Milani and Borbattine (1988) and Herbert *et al.* (1988). Also Dimetry *et al.* (1995) reported that the amount of sealed and unsealed worker brood and the number of combs covered with bees were decreased in Folbex-VA treated colonies as egg laying activity of the queens was stopped for 3-4 days after each treatment.

3.4. Honey production

Data obtained cleared that the mean citrus honey yield recorded 2.75, 0.95, 1.91, 1.56 and 2.37 kg/colony, for healthy, *Varroa* infested untreated and treated colonies with camphor, peppermint, thymol and formic acid, respectively. The respective clover honey yield attained 5.31, 2.06, 3.86, 3.38, 4.29 and 4.72 kg/colony. The corresponding mean annual honey yield reached 8.06, 3.01, 5.77, 4.94, 6.53 and 7.15 kg/colony for the forementioned treatments (Table 4).

The rate of reduction in honey yield due to *Varroa* infestation was 62.66 % as compared to that of healthy colonies. Treating the infested colonies with camphor, peppermint, thymol and formic acid (60 %) improved honey yield of infested colonies, as the reduction percentages recorded 28.41, 38.71, 18.98 and 11.29 %, respectively. In this respect, Matter (1996) stated

individual productivity of workers proved to play noticeable effects on honey production. The same conclusion was also reported by Mobus and Connor (1989), who stated that the diseased colonies have workers characterized with lower ability of flying to make collection tours, lower capacity of loading nectar and pollen and shorter life span.

In all cases control of *Varroa* mite using natural plant products are more recommended than other chemical acaricides to keep the social life of honey bee away from any harmful effects (Dimetry *et al.*,2005 and Abd El- Wahab and Ebadah , 2006).

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كفاءة بعض المنتجات النباتية في مكافحة طفيل الفاروا الذي يصيب طوائف نحل العسل

سعد إبراهيم يوسف خليل – على محمد خاطر* – إبراهيم محمد عبد المنعم عبادة**

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق - مصر
* معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الدقي – جيزة - مصر
** المركز القومي للبحوث – الدقي – جيزة – مصر

ملخص

أجريت هذه الدراسة بغرض تقييم فعالية كل من ملح الكافور ، زيت النعناع ، و الثيمول مقارنة بالمركب الكيميائي حامض الفورميك (60 %) في مكافحة طفيل الفاروا الذي يصيب طوائف نحل العسل. وأجريت هذه الدراسة في معمل بحوث النحل ، معهد بحوث وقاية النباتات بالزقازيق- الشرقية خلال موسم 2007 ويمكن تلخيص النتائج كالآتي:

- 1- بلغ معدل الانخفاض فى نسبة الإصابة بطفيل الفاروا نتيجة المعالجة بالمواد المختبرة ، 48.92، و 69.01، و 75.95 ، و 90.12 % فى طوائف النحل التي تم علاجها بزيت النعناع، وملح الكافور ، و الليمون وحامض الفورميك (60 %)، على الترتيب.
 - 2- بلغ معدل الإنخفاض في وزن جسم الشغالات نتيجة الإصابة بالفاروا 21.87 % بينما في الطوائف المصابة والتي تم علاجها بالمواد المختبرة فقد انخفض هذا المعدل ليصل وزن جسم الشغالات 13.87، و 11.84، و 5.91، و 2.63 % وذلك عند المعالجة بزيت النعناع، وملح الكافور ، و الليمون وحامض الفورميك، على الترتيب.
 - 3- بلغ معدل الإنخفاض في مساحة الحضنة في الطوائف المصابة بالفاروا 47.96 % مقارنة بالطوائف السليمة أما الطوائف المصابة والتي تم علاجها بالمواد المختبرة فقد كان معدل الإنخفاض فيها 29.58، و 20.85، و 15.69، و 13.11 % وذلك في الطوائف التي تم علاجها باستخدام زيت النعناع ، ملح الكافور، الليمون وحامض الفورميك على الترتيب.
 - 4- بلغ متوسط إنتاج عسل الموالح 2.75، و 2.37، و 2.24، و 1.91، و 1.56، و 0.95 كجم/طائفة من طوائف نحل العسل السليمة والتي تم علاجها باستخدام حامض الفورميك ، و الليمون، و ملح الكافور، ومستخلص النعناع، وكذلك المصابة بالفاروا والتي لم يتم علاجها على الترتيب. بينما كان ترتيب إنتاج عسل البرسيم 5.31، و 4.78، و 4.29، و 3.86، و 3.38، و 2.06 كجم/طائفة.
- بلغ متوسط الإنتاج السنوي من العسل (موالح + برسيم) 8.06، و 7.15، و 6.53، و 5.77، و 4.94 و 3.01 كجم/طائفة للطوائف السليمة وتلك المعالجة بحمض الفورميك، و الليمون، و ملح الكافور ، وزيت النعناع و كذلك الطوائف المصابة بالفاروا غير المعالجة على الترتيب. وهذا وقد سجل معدل الإنخفاض في محصول العسل السنوي في الخلايا المصابة غير المعالجة 62.65% بينما بلغ هذا المعدل 38.70 و 28.41 و 18.98 و 11.29 % في الخلايا التي تم علاجها باستخدام زيت النعناع ، و ملح الكافور، و الليمون وحامض الفورميك على الترتيب.