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 case 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 pyrethroid 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
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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 Jelenč 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 Pharmaceutical 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 |
|-----------------------------|------------------------------|-------------|-----------------------------|-------------|
| Apiguard 25% thymol         | 32.0 ±2.31     | 5.33 ±1.33  | 25.12 ±3.62     | 3.61 ±1.41  | 88.29         | 86.78         |
| Crystal thymol 25%          | 30.67 ±3.53    | 8.0 ±2.31   | 23.38 ±2.72     | 4.01 ±1.52  | 86.03         | 81.48         |
| Menthol 25%                 | 32.0 ±4.61     | 12.0 ±2.33  | 26.67 ±3.85     | 9.47 ±2.72  | 71.08         | 68.96         |
| Camphor 25%                 | 38.33 ±2.31    | 10.87 ±3.33 | 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 | 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 | -- |

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.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean number of fallen Varroa mites after treatment</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apiguard 25% thymol</td>
<td>788 ±6.24</td>
<td>788</td>
</tr>
<tr>
<td>Crystal thymol 25%</td>
<td>768 ±9.17</td>
<td>768</td>
</tr>
<tr>
<td>Menthol 25%</td>
<td>485 ±5.17</td>
<td>485</td>
</tr>
<tr>
<td>Camphor 25%</td>
<td>496 ±9.49</td>
<td>496</td>
</tr>
<tr>
<td>25% Mix. of (Camphor + Thymol + Menthol)</td>
<td>566 ±11.21</td>
<td>566</td>
</tr>
<tr>
<td>Untreated</td>
<td>84 ±1.73</td>
<td>84</td>
</tr>
</tbody>
</table>

Generally, a thymol product should be applied when the treatment ranges between 18.5 °C and 27 °C. 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 °C 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 °C this results coincide with Alessandra et al (2004).

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

<table>
<thead>
<tr>
<th>Individual</th>
<th>Apiguard Thymol</th>
<th>Crystal Thymol</th>
<th>Mix. of Camphor + Thymol + Menthol</th>
<th>Camphor</th>
<th>Menthol</th>
<th>L.S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>88.29</td>
<td>86.03</td>
<td>82.79</td>
<td>79.51</td>
<td>71.08</td>
<td>8.14</td>
</tr>
<tr>
<td>Brood</td>
<td>85.27</td>
<td>76.93</td>
<td>75.87</td>
<td>71.15</td>
<td>66.83</td>
<td>9.12</td>
</tr>
</tbody>
</table>

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.

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

Acknowledgement

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REFERENCES

تقييم بعض المواد الطبيعية في مكافحة طفيل الفاروا وتاثيرها على أفراد وأنشطة طوائف نحل العسل

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1- قسم بحوث النحل - مركز بحوث وقاية النباتات - مكرم البحوث الزراعية.
2- قسم وقاية النبات - كلية الزراعة جامعة عين شمس.

اجري هذا البحث في منحل قسم بحوث النحل بمحافظة القليوبية بهدف تقييم فاعلية بعض المنتجات الطبيعية المحلية ودراسة تأثيرها على أفراد طائفة نحل العسل وكذلك على أنشطة الطوائف (تربيه الحضنة وانتاج العسل). وكانت المواد الطبيعية المستخدمة هي اطباق الابيجارد، الثيمول، الكافور و مخلوط من (الثيمول، المنتول و الكافور) وهذه المواد تم إعدادها وتجهيزها في صورة اطباق بعد إضافة مواد حاملة مناسبة لتوزيعها داخل طوائف نحل العسل عند الاستخدام.

واضحت النتائج ما يلي:
- أعطت اطباق الابيجارد أعلى معدل خفض في الإصابة لطفيل الفاروا حيث وبلغت إلى 88.86% كما أعطت اطباق الثيمول المرتبة الثانية حيث بلغ النسبة المئوية للانخفاض في الإصابة 81.48% وحقق المركب الطبيعي المخلوط من (الثيمول، المنتول، الكافور) في صورة اطباق المرتبة الثالثة حيث بلغت النسبة المئوية للانخفاض في الإصابة 79.33% بينما أعطت اطباق الكافور-المنتول كل منهما علي حدة المرتبة الرابعة والخامسة حيث بلغت النسبة المئوية للانخفاض في الإصابة 75.33% و 68.96% على الترتيب.
- وحققت طوائف نحل العسل التي تم معاملتها بكافور زائدة ملاحظة في انتاجها، حيث بلغ متوسط الإنتاج في معدل نسبية الحضنة وانتاج العسل 74.92% و 77.69% على الترتيب عند استخدام اطباق المقارنة، هذا بالإضافة إلى عدم تأثير المواد المستخدمة في مكافحة الفاروا أو انشطة طوائف نحل العسل.

الوصولية:
- توصي النتائج باستخدام اطباق الابيجارد – الثيمول – مخلوط مركب من (الثيمول + المنتول + الكافور) حسب طبق واحد/خلاياً وتسهيل المعاملة مرتين وتستمر فترة العلاج 28 يوم مع مراعاة الأ زيد درجة الحرارة أثناء المعاملة عن 27.5 م تحت لا تؤثر على انشطة أفراد طوائف نحل العسل.