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Effectiveness of Oxalis bee and Ecostop for prophylaxis and control of varroosis in honey bees (Apis mellifera L.)

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Abstract. The objective of the present study is to determine the effectiveness of Ecostop (plates) and Oxalis Bee - plant-based products for the prevention and control of varroosis in bees (Apis mellifera L.). The study was conducted at the end of the 2017 Beekeeping Season of the Beekeeping Experimental Training Center at the Faculty of Agriculture, Trakia University, Stara Zagora. Two products were used: Ecostop containing peppermint oil (2 ml/plate) and timol (5 g/plate), and Oxalis Bee - zootechnical feed additive for bees, including plant extracts, organic acids and invert solution from bio-sugar. The dosing of the products is in accordance with the instructions of the producers Primavet-Soﬁa Ltd., Bulgaria and the company Vechni pcheli Ltd., Bulgaria. The development and extensivization of bee colonies at the beginning and the end of the study and the effectiveness of the applied products was determined. It has been established that the development of bee colonies is normal for the end of the beekeeping season. The comparative analysis of the acaricidal effect of the test products against Varroa destructor shows 98.55±0.30% for Ecostop and 78.15±8.76% for Oxalis Bee. The reported difference in efficacy of both preparations is reliable at p≤0.05.

Keywords: honey bees, Varroa destructor, alternative products, effectiveness

Introduction

Proceeding from the fact that the bee colony is a biological (economic) unit, the diseases, regardless of whether on the brood, the queen bee, the bees or the drones are diseases of the entire family. In the course of honeybee diseases there are some peculiarities which distinguish them from the diseases of the other agricultural animals (Gurgulova et al., 2000): bee diseases are manifested faster and are more persistent; bee and brood diseases spread very quickly not only in the bee colony but also in the other colonies in the apiary and in other apiaries in the area of useful bee flying.

Varroosis is a brood (worker, drone) disease and adult bees (worker bees, queen bee, drones) disease. The cause is an ectoparasite – the Varroa destructor mite. This is the most widespread disease causing extremely large economic damage to beekeeping worldwide. Practice shows that control of the disease requires regular annual prophylactic and healing measures. According to the data from Agrostatistics of the Ministry of Agriculture and Foods in 2016, 747,676 bee colonies in Bulgaria were treated against varroosis, which is 99.15% of the total number of colonies raised in the country (Bulletin No. 322, 2017).

For the prevention and control of varroosis at this stage a number of complex measures are applied: physical methods (using a temperature of 40-46°C for 30 minutes, which kills the mites, does not have a harmful effect on the bees but damages the brood – therefore only the bees are treated and only once in a season); mechanical biological methods (including the use of a construction frame - cutting the sealed drone brood and removing it from the hive); chemical methods (based on the use of chemical agents applied in various ways - by fumigation, mist, dripping, sprinkling, spraying, evaporation, supplemental feeding); alternative methods (use of organic acids, essential oils, plant extracts, etc.).

In the chemical methods chemical agents are used (of different chemical structure - amitraz, fluvinate, flumethrin, cumafos, etc.) having an acaricidal effect of 80-99% (Takeuchi and Sakai, 1986; Kostecki et al., 1987; Ellis et al., 1988; Kamburov et al., 1989; Watherell et al., 1990). The chemical products and their analogs such as Apistan, Varotom, Bayvarol, Perizin, etc. have been known for a long time and are being applied in almost all countries, incl. in Bulgaria. The Bulgarian product is Varostop with active substance flumethrin, analogous to Bayvarol. The product has a proven efficiency in time 99% (Gurgulova et al., 2008; 2011). Up to now, no product has been created which is harmless to the bees, highly effective and easy to apply, without residual quantities in the bee products, at low cost.

The disadvantages of conventional means of control of varroosis require that alternative methods be used to combat this disease. At the present stage, alternative products are particularly relevant, as in many countries Varroa destructor shows resistance to the various conventional acaricides. Alternative therapy and prevention do not create or rarely produce resistance to natural products - essential oils, acids, etc. The risk of contamination of bee products is also reduced because the alternative substances are of natural origin, not toxic to bees and humans and are contained in bee honey.

Thymol derived from thyme is most commonly used against the Varroa destructor mite. There are no published reports of resistance of Varroa to thymol. Nonetheless, Knight (2015) suggests as good practice thymol treatment to be alternated with other products. An example of this are the products: Api-Life-Var (76% thymol, 16.4% eucalyptol, 3.8% menthol and 3.8% camphor) with high acaricidal
efficiency of 95-98.6% (Moosbeckhofer, 1994); APIGUARD - a gel with a patented formula containing thymol and other natural substances; CAS-81 - a combination of thymol with a bitter wormwood infusion and pinetree tips (Kantar, 2007).

The use of formic acid in control of varroasis in European countries is widely accepted. It can be applied in beehive nests and when there is brood. In Bulgaria is registered product Formitom (plagues soaked with 66% formic acid).

Oxalic acid has been authorized as a means of controlling varroasis as early as 1983 in Russia. Later, it became of great importance in the control of varroasis in Europe, too. By Council Regulation/EC No. 1804 (1999), the use of oxalic acid is permitted in the European Union member states. When used correctly, oxalic acid is not harmful to the bees and it does not pollute honey and the other bee products. Oxalic acid is very efficient against mites, and when there is no brood efficiency reaches 97-99%.

In recent years, due to the high requirements of the EU countries, the production of quality and safe (without pollutants) bee products is imperative. The preservation of the valuable nutritional qualities and the biological value of bee honey, as well as the protection of the bee colonies from diseases, in particular varroasis, require the application of biological, zoohygienic and technological methods for control of varroasis by using organic acids (formic, oxalic, lactic), essential oils (thymol, menthol, eucalyptol, etc.) and plant extracts. In this regard, studies on the testing of various natural products as means for control of varroasis are very topical.

The objective of the present study is to determine the effectiveness of Ecostop (plates) and Oxalis Bee, plant-based products for the prevention and control of varroasis in bees (Apis mellifera L.).

Material and methods

Study area

The experiment was conducted in the period September – October 2017 on a training apiary of the Apiculture section at the Department of Animal husbandry – non-ruminants and other animals, Faculty of Agriculture, Trakia University, Stara Zagora. For the beekeeping practice this period is extremely important as the bee colonies are preparing for wintering and it is considered as the beginning of the beekeeping year.

Bee colonies settled in 10-combs Dadant-Blatt hives were used. Throughout the entire study period the treated colonies were monitored for normal development, occurrence of side effects and adverse effect of the tested products on the queen bee, the bees and the brood.

Products used for control of varroasis

For the purpose of the study, two products for control of varroasis were used:

- Ecostop – plates (veterinary-medical product), composition: 5g thymol and 2 ml mint oil/plate, produced by Primavet Ltd., Sofia, Bulgaria;
- Oxalic acid – used for the control treatment. All products were used as per the manufacturer’s instructions.

Groups of bee colonies

The experiment was conducted with 13 bee colonies, allocated in the following groups:

- O1 – experimental group of 5 bee colonies, treated with 2 plates of Ecostop;
- O2 – experimental group of 5 bee colonies treated with Oxalis Bee at a dose of 20ml per bee colony of normal strength. Oxalis Bee was applied by dripping on the upper laths of the frames and in the interframe spaces with bees;

The experiment started since 18 September 2017: Ecostop – left in the hive for 42 days (18 September - 30 October 2017); Oxalis Bee – three treatments at 7 days' intervals (18 September – 10 October 2017).

The recording of the mites dropped was done on: 24th hour, on 7th, 11th, 15th, 20th, 25th, 30th, 35th and 42nd days.

Determining the strength of bee colonies and the quantity of sealed brood

The development of bee colonies has been determined by generally accepted zootechnical methods. The strength (number of bees in the bee nest) is presented in number of interframe spaces tightly covered with bees and the conversion in kg at an estimate of 0.250kg of bees in a Danant-Blatt beehive interframe space. The quantity of brood is measured with a measurement frame with square sizes 5x5cm and is presented as number of cells (in 1cm² of the comb there are 4 worker cells).

Determining the extentive invasion (EI) of bees and brood

Methods for determining the EI of Varroa destructor in bees and brood have were used according to the Office International Epizootic Terrestrial Manual, 2008, Section 2.2.7. For this purpose, from a randomly selected comb, a few bees are swept in a polyethylene bag with alcohol. Then it is shaken well and the alcohol is poured out. Bees and fallen mites are counted and the infestation rate is determined in percentage (%).

Brood EI is determined by taking out 25 larvae and pupae from a brood comb and counting the mites on them. The calculation is made in percentages (%). Infestation rate (EI) of bees and brood is determined twice - at the beginning of the experiment before treatment and at the end before the control treatment.

Determining the number of fallen mites

The number of fallen mites has been traced during specific time periods. For the purpose greasy paper was used placed on the bottom of the hive. At the end of the experiment the data have been summed up and the total number of mites fallen as a result of the treatment has been obtained.

Control treatment

The control treatment was performed at the end of treatment with the tested products. For this purpose, oxalic acid 3% (dose 7ml per interframe space) was used. The product is applied by dropping into the interframe spaces covered with bees. To determine the number of mites fallen from the control treatment, greasy paper was used placed on the bottom of the hive.

Calculating the effectiveness of the applied products for control of Varroa destructor
The effectiveness of the applied products has been calculated by using the following formula:

$$\text{Effectiveness} (%) = \frac{\text{MFFTP} - \text{MFFCT}}{\text{MFFTP} \times \text{MFFCT}} \times 100.$$  

Where:
- MFFTP = all mites fallen from the tested products collected during treatment with the tested product.
- MFFCT = all mites fallen from the control treatment.

### Processing the obtained results

The obtained results have been processed statistically by using Statistica computer software.

### Table 1. Development of bee colonies

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Strength-kg</td>
<td>Brood-No of cells</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\bar{x}\pm S_x)</td>
<td>min/max</td>
</tr>
<tr>
<td>O - Ecostop</td>
<td>5</td>
<td>1.65±0.15</td>
<td>1.50/2.25</td>
</tr>
<tr>
<td>O - Oxalis Bee</td>
<td>5</td>
<td>1.70±0.18</td>
<td>1.25/2.00</td>
</tr>
<tr>
<td>C-control</td>
<td>3</td>
<td>1.58±0.08</td>
<td>1.50/1.75</td>
</tr>
</tbody>
</table>

*Brood rearing is over

At the end of the experiment the strength of the colonies in the three groups declined, and brood rearing ceased which is normal for the season. The least is the decrease in the number of bees in O group treated with Oxalis Bee - by 2.9%. In the control group and group O, the decrease was by 10.1% and 9.1%, respectively. The observed differences in the strength of the colonies at the end of the survey are unreliable.

The obtained values for extense invasion (EI) of the mite before and after treatment are given in Table 2.

### Table 2. Extense invasion (EI) of brood and bees (%)

<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>El brood-%</td>
<td>El bees-%</td>
</tr>
<tr>
<td></td>
<td>(\bar{x}\pm S_x)</td>
<td>min/max</td>
</tr>
<tr>
<td>O - Ecostop</td>
<td>12.80±6.37</td>
<td>0/36</td>
</tr>
<tr>
<td>O - Oxalis Bee</td>
<td>5.60±2.04</td>
<td>0/12</td>
</tr>
<tr>
<td>C-control</td>
<td>1.33±1.33</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*Brood rearing is over

Prior to treatment the highest values for brood EI were found in the experimental group O, (12.80±6.37%) and the lowest for the control group (1.33±1.33%). The infestation rate of bees in the three groups has close values and varies from 3.20±1.07% to 5.63±2.25%. The reported differences in EI values for brood and bees in the groups are unreliable. As can be seen from the Table 2, at the beginning of the experiment great number of the mites are concentrated in the sealed brood.

After treatment with the products, in group O, (Ecostop) plates for 45 days and in group O, (Oxalis Bee) for 22 days, only the infestation rate of the bees is determined. The rearing of brood in the colonies in all three groups was terminated, indicating that in October the parasite gradually transferred to the bees.

The comparative analysis of the date for EI of bees at the beginning and at the end of the study shows that the infestation rates is reduced at the greatest extent in the group treated with Ecostop (6.2 times - from 4.09±0.81% to 0.66±0.32%) and in the control group (9.4 times - from 3.20±1.07% to 0.34±0.34%), and at the smallest extent in group O, (Oxalis Bee) - 1.7 times from 5.63±2.25% to 3.23±1.74%. The reported differences are statistically unproven. These results can be accounted for by the change in the strength of the bee colonies (greater decrease in the number of bees in the bee nests (strength) in the control and group O,) and the chasing away of the drones during that period (Table 1).

Table 3 and Figure 1 present the results from the monitoring of the mite fall dynamics in the experimental and the control groups during treatment. The data show that the greatest number of mites fall off in group O, under the effect of Ecostop, which also has the highest infestation rate - 458.60±68.70 pcs. At the end of the period the smallest number of mites fell off in the control group, resulting from natural mortality. No statistically significant differences have been found.

Results and discussion

In the experiment were used bee colonies with strength prior to the treatment (18 September 2017) between 1.58 and 1.70 kg of bees and quantity of brood varied from 4033 to 5060 cells with sealed worker brood (Table 1). Higher averages for the studied parameters (strength and quantity of brood) were defined in group O, where Oxalis Bee is to be applied. The reported differences between the groups are unreliable.
Table 3. Quantity of fallen mites (number) during the treatment period

<table>
<thead>
<tr>
<th>Group</th>
<th>Dates of reporting (start of treatment 18 Sep 2017)</th>
<th>Total mites fallen from the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁-Ecostop</td>
<td>108.00±32.10</td>
<td>102.00±26.15</td>
</tr>
<tr>
<td>min/max</td>
<td>34/191</td>
<td>30/180</td>
</tr>
<tr>
<td>O₂-Oxalis Bee</td>
<td>91.40±29.48</td>
<td>37.00±16.78</td>
</tr>
<tr>
<td>min/max</td>
<td>31/202</td>
<td>5/85</td>
</tr>
<tr>
<td>C-control</td>
<td>40.67±26.74</td>
<td>43.33±29.63</td>
</tr>
<tr>
<td>min/max</td>
<td>2/92</td>
<td>0/100</td>
</tr>
</tbody>
</table>

The graphic presentation of the results (Figure 1) shows that in the experimental group O₁ (Ecostop) during the first 24 hours and until the end of the first week, 45.8% of the mites fell from the total number of fallen mites. In the same group the number of fallen mites decreased sharply after the 27th day of treatment - between 8.00±1.38 and 14.00±3.32 per reading (Table 3).

In group O₂ (Oxalis Bee), the greatest number of mites fell at the 24th hour of treatment (27.1% of the total number) (Figure 1). A minimum number of fallen mites was observed at the reporting at the end of the first week, then an increase in the number and again a decrease after the 22nd day. In the control group the main number of mites fell up to the 15th day - 82.9% of the total number. After this period at each reading the number of fallen mites was between 10 and 30 pcs. (Table 3 and Figure 1).

The comparative analysis of the data on the number of dead mites resulting from the control treatment shows that the lowest number of mites (1.93% of all fallen mites) fell in group O₁ (Ecostop), and in group O₂ (Oxalis Bee) - 20.3% of all fallen mites (Table 4). In the control group the mites that fell as a result of the control treatment were 16%.

The effectiveness of the tested products is presented in Table 4.

Table 4. Effectiveness (%) of the products used

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mites fallen from the treatment (pcs.)</th>
<th>Mites fallen from the control treatment (pcs.)</th>
<th>Total mites fallen (pcs.)</th>
<th>Effectiveness (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁-Ecostop</td>
<td>5</td>
<td>458.60±68.70 280/681</td>
<td>6.00±0.84 4/9</td>
<td>464.60±68.28 286/685</td>
<td>98.55±0.30 97.90/99.42</td>
</tr>
<tr>
<td>min/max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O₂-Oxalis Bee</td>
<td>5</td>
<td>337.80±74.37 174/604</td>
<td>86.00±31.72 10/200</td>
<td>423.80±66.08 325/684</td>
<td>78.15±8.76 46.52/97.45</td>
</tr>
<tr>
<td>min/max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-control</td>
<td>5</td>
<td>214.00±118.70 32/437</td>
<td>40.67±34.17 6/109</td>
<td>254.67±117.42 39/443</td>
<td></td>
</tr>
<tr>
<td>min/max</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reliability of differences between groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>O₁/O₂*</td>
</tr>
</tbody>
</table>

* p<0.05
The established effectiveness of Ecostop shows that the product has high acaricidal activity - 98.55±0.30%. The fact that in the control treatment of colonies in the group treated with Ecostop the smallest number of mites fell, confirms its effectiveness. The efficacy in the second experimental group (O2 - Oxalis Bee) is much lower than the expected, 78.15±8.76%, respectively. The observed differences in the effectiveness values between the two experimental groups have low level of reliability (p<0.05).

Observation on the treated groups shows that the tested products do not affect adversely the bees and the brood. No self-change of queen bees in the experimental and the control groups have been established. The development of bee colonies at the end of the experiment was normal for the season, according to their initial strength.

The results obtained in the present study concerning the antiacaridial effect of Ecostop (plates) confirms the data from previous studies in our country, where this product showed effectiveness of over 90% (Gurgulova et al., 2004, 2008, 2011). The advantage of the product is that its application does not change the quality of honey. This product has no quarantine period. Its main ingredients (thymol and peppermint oil) are known for their remedial and antiparasitic action.

Oxalis Bee is a new product (zootechnical supplement for bees with anti-acaridial and stimulating effect), conforming the EU standards – Council Regulation/EC No. 834 (2007). It is composed of natural ingredients only (plant extracts, organic acids, inverted syrup). The data from the present study show lower acaridial effect, but as a zootechnical supplement it could be a good stimulant for the development of bee colonies. In this connection, it is necessary to deepen studies with this product as an anti-acaridial product for spring treatment and as a stimulant for spring and autumn supplemental feeding of bee colonies.

Conclusion

It was found that: a) the development of bee colonies included in the study is normal for the season (October 2017) as the number of bees in the bee nests gradually decreased and brood rearing is terminated; b) the extense invasion (EI) at the beginning and at the end of the study decreased to the largest extent in the control group (9.4 times) followed by the group O2 treated with Ecostop (6.2 times) and the group O2 treated with Oxalis Bee (1.7 times); c) for the entire treatment period with the tested products the largest number of mites fell into group O2 under the action of Ecostop (with high acaridial activity effectiveness 98.55±0.30%) and the lower one into group O2 (Oxalis Bee) - 78.15±8.76% (p<0.05); d) it is necessary to deepen the studies with Oxalis Bee (a new product) as an anti-acaridial product for spring treatment and as a stimulant for spring and autumn supplemental feeding of bee colonies.

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