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CONTROL OF *VARROA DESTRUCTOR* WITH DIFFERENT COMBINATIONS OF ESSENTIAL OILS

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ABSTRACT

The parasitic mite, *Varroa destructor* is a major agent responsible for the decline of honey bee colonies *Apis mellifera* worldwide. In recent years, various essential oils, alone or in combination, have been used against varroosis. The aim of this study was to test the effectiveness of some of the essential oils used in beekeeping (peppermint, pine and cedar) for the control of *V. destructor*. The experiments included 11 apiaries located in three regions of the country. Nine (9) different combinations of the listed essential oils were prepared and tested. During the treatment, were monitored the effectiveness against the mite, tolerance and safety in the treated colonies. After evaluating the results obtained, we settled on the combination of essential oils (CEO)-9 / SetOil. This combination can be used as a method in integrated pest management and also when introducing/receiving a new queen bee by unifying the smell in the bee colony.

Key words: *Apis mellifera, Varroa destructor,* control, essential oils.

Introduction

The parasitic mite *Varroa destructor* is a major damaging pest of the western honey bee *Apis mellifera* worldwide. The development of acaricide resistance in *Varroa* populations is a global issue (Rinkevich, 2020).

Essential oils and their components are generally known for their acaricidal effects and are used as an alternative to control the population of the *Varroa destructor* instead of synthetic acaricides (Conti *et al.*, 2020; Hýbl, 2021). Recent studies have shown that essential oils of plants such as thyme, clove, peppermint, lemon grass, cinnamon, grapefruit, rosemary, marigold, eucalyptus, tea tree have positive effects against some mites, bacteria and fungi (Topal *et al.*, 2020).

Varroa mite causes many physical and physiological harmful effects at the individual bee and colony levels. Repeated feeding of the *Varroa* mite on the fat body and hemolymph of adult bees and pupae damages the bees physically, leads to a decrease in their protein content and weight (wet and dry), and interferes with organ development. In addition, the parasitic mite and the viruses it transmits contribute to morphological deformities, such as small body size, shortened abdomen, and deformed wings (Khan, 2019).

Based on literature data from scientific and practical experience in the use of essential oils against *Varroa destructor*, various combinations of essential oils (oleum menthae, oleum pini, oleum cedri) were developed and tested (Gurgulova *et al.*, 2004; Colin *et al.*, 2019; Begna et al, 2023). The compatibility, solubility, homogeneity and stability of the combinations were investigated.

From all the tested combinations, nine with different essential oils content were selected. During the preliminary laboratory studies, we found that three of them are the most stable and promising: No 3 (CEO 3), No 6 (CEO 6) and No 9 (CEO 9).

Materials and Methods

I. Materials

Tolerance

Tests on the tolerance of bee colonies to veneer strips dripped with combinations of essential oils were carried out in an apiary, which consists of 100 bee colonies in 12-frame Dadant Blatt hives.

The experiments were carried out to test the tolerance of bees to essential oils and to report the initial effect after 24 h. At our discretion, we left the strips until the 4th day, in order to monitor the residual effect of the oils. From all the tested combinations, we selected No. 9 (CEO9) dropped in different amounts of oil on the strip – 1, 2, 3 or 4 drops per one veneer strip, each applied to 3 bee colonies. The application was carried out immediately before placing the strips on the frames in the hives.

Effectiveness

The trials were conducted according to a previously prepared work program. Evaluating all the characteristics of the three combinations, we settled on CEO9, which has all the qualities to be successfully applied as an acaricide in the integrated pest management to control varroosis, together with other methods and registered products. As a control product was used Apiguard® Vita Bee Health Ltd, England, applied according to the manufacturer's instructions.

The experiments on the effectiveness of the combinations of essential oils were carried out on 208 bee colonies from 11 apiaries located in the following three regions: Sofia, Montana and Pernik.

Information of the essential oil combinations (CEO) used in the trial experiment: cedar oil, peppermint oil and pine oil.

The experiments were carried out in 10 colonies from the group.

We have formed the following groups of bee colonies in each apiary:

O1 – experimental group treated with CEO3 – 10 bee colonies.

O2 – experimental group treated with CEO6 – 10 bee colonies.

O3 – experimental group treated with CEO9 – 10 bee colonies.

K- control group – 10 bee colonies, untreated.

II. Methods

1. Determination of colony development by generally accepted zoo technical methods for measuring strength of bees in kg and brood amount in number of brood cells.

2. Methods for establishing the levels of *Varroa destructor* infestation in the bees and brood were used according to Manual of diagnostic tests and vaccines for terrestrial animals of OIE, 2021 (18).

3. Experimental treatment

In each group we applied CEO9 in an amount of 4 drops on one strip of the investigated combinations of essential oils, three times in interval of seven days placed horizontally on the frames in the brood chamber.

4. As a control product we used Apiguard gel (50 g with content of 12.5 g Thymol). The products were administered according to the manufacturer's instructions.

5. Counting the number of fallen mites in the control and experimental groups on:

- I treatment – 1st, 4th and 7th day,
- II treatment - 8th, 11th and 14th day,
- III treatment – 15th, 18th and 21st day,
- on 22nd day – 24 hours after the control treatment.

6. Calculate the effectiveness of the three tested combinations of essential oils (CEO) using the following formula:

$$\frac{\text{Effectiveness}}{(\%\ \text{Reduction})} = \frac{-\text{mites killed by CEO}}{\text{killed by CEO} + \text{remaining mites}} \text{ x } 100$$

- Mites killed by CEO are all mites collected during exposure to the tested product;
- The remaining mites are those that are not killed by the CEO and fall off after treatment with the control product.

The treated colonies were monitored throughout the test period for normal development, the occurrence of adverse more harmful effects of the test substances on the queen, bees, and brood - death of pupae and brood ejection, bees leaving the hive, bee robbery, and replacement of the bee queen.

Animal research ethics: The authors confirm that their studies performed on bees were carried out according to internationally recognized guidelines for animal welfare.

Results

The bee colonies showed good tolerance during the treatment with the essential oil combinations. No negative phenomena were observed in the queens, bees and brood on the 7th, 24th hour and 4th day. No bees were observed leaving the entrance to the hives, robbing bee colonies and attacking bees. No smell of essential oils was detected in the apiary around the treated hives during the stay of the strips. The initial effectiveness for 4 days depends on the infestation level of the bee colonies and the amount of essential oils on the strips - the highest effect was observed when dosing with 4 drops per strip.

The results of our research showed a high effect when applying the combination of essential oils CEO9 in the Montana region. The effectiveness exceeds 90%, which is actually a requirement for registered acaricides (Fig. 1).

When comparing the results of applying CEO9 in apiaries from the three regions, we found that the lowest efficiency was 87.51% and maximum – 91,89%, which is absolutely acceptable for essential oils as acaricides (Council Directive 81/852/EEC (2001); Guideline on veterinary medicinal products controlling Varroa destructor parasitosis in bees (2008)).

When studying the effectiveness of CEO6, we found a large variability in the results.

The average efficiency was 89.05%, the minimum - 84.42%, and the maximum – 93.67%.

This is probably due to the different level of Varroa infestation in different regions, different beekeeping practices and different hive systems;

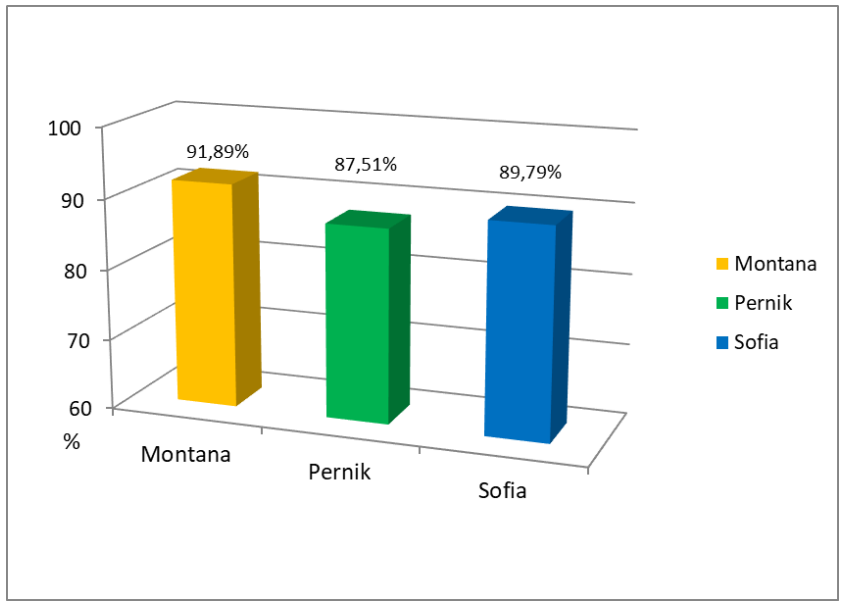

Figure 1: The effectiveness of CEO 9 in the three tested districts

When comparing the effectiveness of the combination used in bee colonies in which less than 5 mites fell after the control treatment with those in which more than 5 mites fell (Fig. 2), we found that the effectiveness did not change significantly. (Fig.2). Further analysis included detailed tracking of mite fall and infestation rates following both initial and control treatments. Notably, the impact of the essential oil combinations varied not only between regions but also among individual apiaries within the same region, underlining the importance of local conditions and hive management practices. The comparative study highlighted the potential for targeted application strategies based on the degree of infestation and regional beekeeping practices.

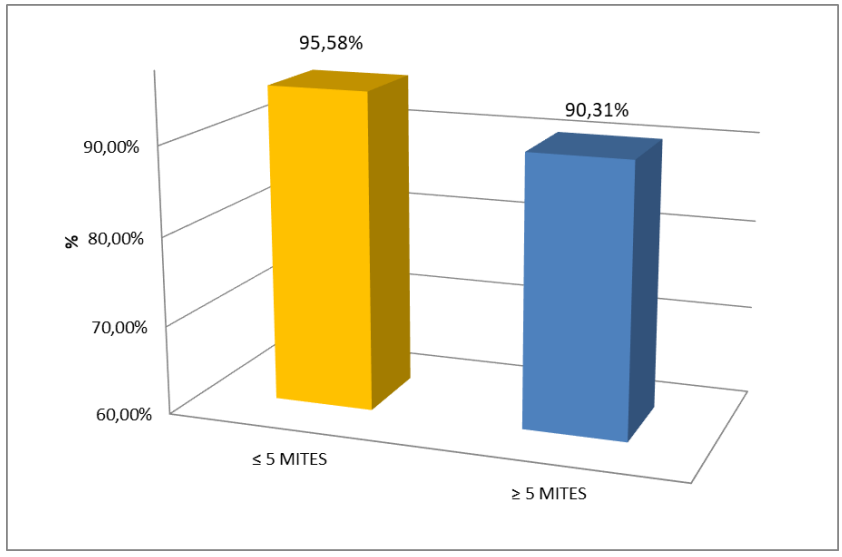

Figure 2: Effectiveness of CEO 9 in different levels of *Varroa* infestation

The average number of remaining mites in the colonies that fell after the control treatment with Apigard is presented in Fig. 3

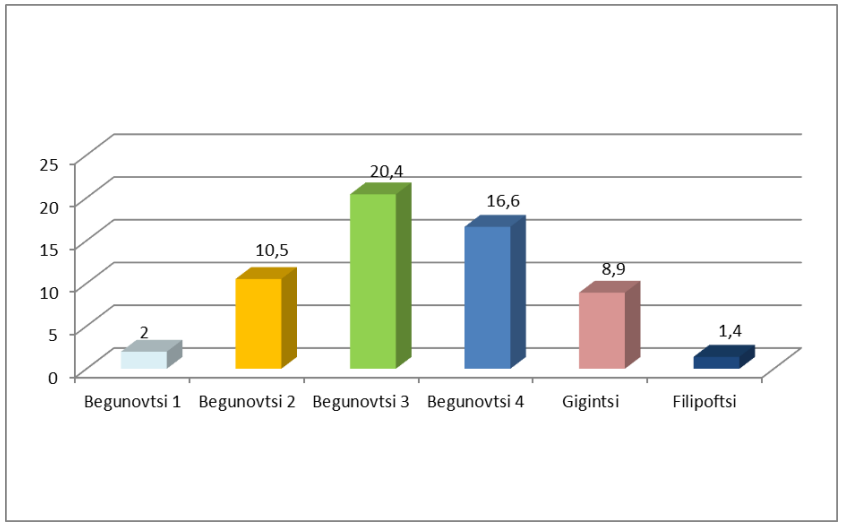

Figure 3: Average number of fallen mites in Pernik region during the control treatment

The study of CEO9 / SetOil was conducted in several apiaries in Pernik region. The data showed a great diversity in the different apiaries. This was due to the different infestation of the bee colonies. On average, the largest number of mites fell in apiary No. 3 in the village of Begunovtsi - 20.4 mites. Comparing these data with the effectiveness in infested-colonies with >5 mites (Fig. 2), we can say that the effectiveness of CEO9 at a higher level of infestation reached almost 90%. The smallest number of fallen mites was found in the apiary in the village of Filipovtsi, respectively 1.4 mites. We were impressed that in some of the colonies no mites fell in the control treatment, which indicates a low infestation level. The number of fallen mites under the action of CEO9 in most treated colonies was significant (Fig. 3).

Discussion

The increase in the number of organic beekeepers and the requirements to obtain ecologically clean production has led to increased interest and widespread use of organic acids and essential oils as natural methods for disease control in beekeeping.

Our results on the use of essential oils for the control of *Varroa destructor* mites confirm the results, reported by other studies in recent years, which demonstrate their effectiveness in beekeeping.

Recent studies have shown that essential oils of plants such as thyme, clove, peppermint, lemon grass, cinnamon, grapefruit, rosemary, marigold, eucalyptus, tea tree have positive effects against some mites, bacteria and fungi. Furthermore, active ingredients such as sanguinarine, thymoquinone, capsaicin, carvacrol, citral, eugenol, thymol isolated from these plants have been reported to have these effects on organisms (Demirel *et al.*, 2019; Khan *et al.*, 2019).

However, although the products used are considered natural ingredients, the dosages for use should not be exceeded (Glavan *et al.*, 2020; Pinheiro *et al.*, 2019). In general, beekeepers use

essential oils in conventional beekeeping as an alternative to synthetic drugs for rotational purposes in order to avoid the creation of resistance and also to prevent residues in bee products.

It is important to control diseases and pests by using biological and organic methods because of suitable production models for safe food. As a result of the banned of some chemicals for animals breeding, medicinal and aromatic plants and oils have been commonly used. The compounds used against diseases and pests, as well as the dosage will reveal its positive effect on bee colony, as well as the environment. In many studies, it has been reported that alternative methods used in the organic control of both pest and diseases do not have toxic effects on larvae, pupae and adults and do not adversely affect colony development when they are used at appropriate doses. The herbal products used in *Varroa* control are generally inexpensive and most of them pose little health risk. Terpenes (mainly monoterpenes) are the main compounds that make up 90% of the essential oils. In laboratory screening tests, over 150 essential oils and their compounds were evaluated. The extract and leaves of many plants, such as tobacco, pine leaf, garlic, thyme, eucalyptus, juniper, peppermint, pear, walnut, citrus, teal and cumin are used against *Varroa*. However, while very few were successful in field experiments, thymol and thymol mixtures proved to be a promising exception (Imdorf *et al.*, 1999). Epling (peperina) have been shown to be highly effective against mites, and their applications did not have a negative effect on bees (Damiani *et al.*, 2011). All the essential oils in the Lamiaceae (peppermint) family can be used to help control varroa. The mint plant, which has antifungal characteristics, is also used for the treatment of Chalkbrood or for the prevention of other diseases (Kouache *et al.*, 2017; Tutkun, 2016).

To minimize the risk of residues in bee products, it is necessary to avoid treatment with acaricides during the harvesting period, when stores and honey bodies are on the hives. Depending on the period of treatment, the correct choice of the appropriate VMP should be made. Some of the products (e.g. Apiguard) require warm weather for maximum effectiveness. Others (oxalic and lactic acid) need broodless - periods to be effective and are usually applied in late autumn or early spring. Still others (e.g. Varostop, Bayvarol and CheckMite+) can be used quite safely in spring and autumn, when there is no commercial honey production).

Conclusion and recommendation

In the conclusion we can say that essential oils and their compounds can be successfully used as an alternative and/or complementary treatment model against *V. destructor*.

The application of the developed essential oil combination – CEO9, is a suitable method throughout the active season. CEO9 is a combination of essential oils that can meet the needs of conventional and especially organic beekeeping, where the application of acaricides is limited, providing an efficiency of over 80%, which, applied together with other methods for year-round control of *V. destructor*, will help in the integrated pest management practis.

Essential oils in various concentrations can be used on bees for many purposes, such as:

- control of parasitic mite *Varroa destructor*, tracheal mite *Acarapis woodi* and the bee louse *Braula caeca*;
- *Nosema* spp. control support;
- help with queen bee introduction;
- mould inhibitor incorporated in sugar syrup;
- use bait attractants in swarm traps;
- a substance that acts as a feeding stimulant;

- promotes hygienic behaviour in bees;
- for detoxification of bees in case of poisoning
- Increases resistance to oxidative stress.

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