

# Research on the Use of Essential Oils on the Health of Bee Families

Lazăr Roxana Nicoleta<sup>1</sup>, Moț Daniela<sup>1</sup>, Simiz Eliza<sup>1</sup>, Pătruică Silvia<sup>1</sup>

<sup>1</sup> Banat's University of Agricultural Sciences and Veterinary Medicine „King Michael I of Romania” from Timișoara, Calea Aradului nr.119

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## Abstract

The use of essential oils (thyme, rosemary, basil, juniper, oregano, cloves, mint, cinnamon) in complementary feeding, applied to bee families in autumn, can have favourable effects in improving their health. The incorporation of essential oils in a dose of 2 drops / litre of sugar syrup had the effect of reducing the total number of germs in the intestine of the analysed worker bees. This was observed mainly 20 days after their administration, in all the experimental variants analysed, the best results being observed in the case of the batches in which the essential rosemary, oregano and clove essential oils were used ( $p < 0.05$ ).

**Keywords:** antimicrobial activity, bees, essential oils.

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## 1. Introduction

Bee diseases, followed by pollution, are the main causes of losses of bee families [1]. Worldwide, it has been shown by numerous studies that the main cause of failure in the treatment of infectious diseases is bacterial resistance [2]. The studies conducted by [3] and [4], highlighted that the excessive use of antibiotics leads to the development of bacterial resistance, increasing in recent years, thus decreasing their effectiveness on antimicrobial agents [5-6].

Plant extracts are represented by a variety of medicinal properties, which produce a relevant range of functional secondary metabolites [7]. [8], highlighted that medicinal plant extracts contain significant amounts of antimicrobial products. Multiple studies have highlighted their anti-

inflammatory, antifungal, antiviral and antibacterial properties [9-11].

The research conducted by [12], showed that the effectiveness of essential oils against pathogens, fungal, bacterial, viral agents is given by the presence of phenolic compounds, aldehydes and many other antimicrobial compounds, so the activity of essential oils depends largely on their components. Essential oils can be great alternatives for improving disease control [13], considering that the use of antibiotics influences negatively the quality of honey [14]. Authors like [15-17], showed that through antimicrobial activity, essential oils can be successfully used as an alternative method in the prophylactic treatment of bee diseases. The use of essential oils can reduce the risk of bacterial resistance due to the action of the components of these natural products [2]. Studies undertaken by [17], showed the capacity and mode of action of essential oils by penetrating the cell wall and cytoplasmic membranes, thus leading to the infiltration of intracellular compounds.

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\* Corresponding author: Lazăr Roxana Nicoleta, 0729374702, [roxanalazar@usab-tm.ro](mailto:roxanalazar@usab-tm.ro)

## 2. Materials and methods

The experiments were performed on 18 bee families maintained in Dadant and multi-storey hives. The bee families were fed between 7th-28th of September, with 1:1 sugar syrup, in an amount of 3 L/week. Essential oils were incorporated into the sugar syrup according to the scheme shown in Table 1.

**Table 1.** Experimental organization scheme

| Experimental group | Essential oil administered | Dose administered |
|--------------------|----------------------------|-------------------|
| Control lot        | -                          | -                 |
| Lot 1              | Thyme                      | 2 drops/L syrup   |
| Lot 2              | Rosemary                   | 2 drops/L syrup   |
| Lot 3              | Basil                      | 2 drops/L syrup   |
| Lot 4              | Juniper                    | 2 drops/L syrup   |
| Lot 5              | Oregano                    | 2 drops/L syrup   |
| Lot 6              | Clove                      | 2 drops/L syrup   |
| Lot 7              | Mint                       | 2 drops/L syrup   |
| Lot 8              | Cinnamon                   | 2 drops/L syrup   |

At the beginning of the experiment, at 10 and 20 days of feeding, the bee families with sugar syrup and essential oils, 10 worker bees from each experimental variant were collected in order to perform the microbiological examination.

In laboratory, the intestine and rectum of worker bees were aseptically transferred into sterile tubes with saline, using a fine forceps, to the flame of the gas bulb, so as not to be contaminated.

Biological material was sampled from each bee family in 3 separate tubes. The tubes were incubated at thermostat at 37°C for 24 hours.

From these tubes, the next day, serial dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  were made. After that, inoculations were made on 3 Petri dishes for each bee family, from 3 dilutions, over which nutritious agar was poured. This was followed by incubation of the Petri dishes on a thermostat at 37°C for 24 hours. Depending on how the bacterial colonies developed, it was agreed to read the samples at a dilution of  $10^{-1}$ , because the colonies were neither confluent nor very rare, being the optimal dilution for a correct reading. Gram-stained smears were taken from each sample and examined under a microscope. The count of developed bacterial colonies was performed using the Nitech LKB 2002 (the working method was used by [18]). The Sabouraud's medium was used separately for inoculation, too, in order to identify possible species of microscopic fungi in the intestine of the bees studied. From the smears performed and examined, as well as from the inoculations on special culture media, it was found that the inoculated bacteria, coming from the intestine of the bees studied belong to the genera: *Escherichia*, *Pseudomonas*, *Bacillus*, *Proteus*, *Staphylococcus*, *Lactobacillus*, and *Candida* was identified of the microscopic fungi (first sampling), *Pseudomonas*, *Bacillus*, *Proteus*, *Staphylococcus*, *Lactobacillus* (the second sampling) and *Bacillus*, *Proteus*, *Staphylococcus*, *Lactobacillus* (sampling number three).

**Table 2.** Determination of the number of germs after the administration of essential oils

| Experimental group | Moment of determination                     |                                   |                                   |
|--------------------|---|-----------------------------------|-----------------------------------|
|                    | Beginning of experiment<br>$\bar{x} \pm SD$ | After 10 days<br>$\bar{x} \pm SD$ | After 20 days<br>$\bar{x} \pm SD$ |
| Control            | 493.17 ± 127.196 <sup>a</sup>               | 451.80 ± 67.807 <sup>a</sup>      | 466.70 ± 67.335 <sup>a</sup>      |
| Thyme              | 535.50 ± 119.212 <sup>a</sup>               | 526.50 ± 79.043 <sup>a</sup>      | 399.33 ± 138.785 <sup>a</sup>     |
| Rosemary           | 502.50 ± 132.128 <sup>a</sup>               | 485.00 ± 123.140 <sup>a, b</sup>  | 366.33 ± 56.344 <sup>b</sup>      |
| Basil              | 457.83 ± 120.907 <sup>a</sup>               | 422.67 ± 89.997 <sup>a</sup>      | 400.33 ± 79.645 <sup>a</sup>      |
| Juniper            | 485.50 ± 201.379 <sup>a</sup>               | 444.17 ± 145.740 <sup>a</sup>     | 354.00 ± 124.704 <sup>a</sup>     |
| Oregano            | 499.33 ± 140.829 <sup>a, b</sup>            | 508.67 ± 152.479 <sup>a</sup>     | 336.83 ± 113.731 <sup>b</sup>     |
| Cloves             | 530.17 ± 91.287 <sup>a</sup>                | 525.50 ± 84.025 <sup>a</sup>      | 397.17 ± 124.926 <sup>b</sup>     |
| Mint               | 455.67 ± 167.940 <sup>a</sup>               | 411.17 ± 109.314 <sup>a</sup>     | 319.17 ± 182.762 <sup>a</sup>     |
| Cinnamon           | 358.50 ± 153.838 <sup>a</sup>               | 387.17 ± 184.151 <sup>a</sup>     | 297.00 ± 70.088 <sup>a</sup>      |

<sup>a-a</sup>p>0,05; <sup>a-b</sup>p<0,05.

The statistical processing of the obtained results was performed with the IBM 20 SPSS program, and the ANOVA test completed by post hoc LCD was used, for the threshold of  $p < 0.05$ .

### 3. Results and discussions

The use of essential oils in the sugar syrup administered to bee families in autumn led to changes in the total number of germs in the intestine during the analysed period. If at the beginning of the experiment the total number of germs was close to all experimental variants, after 10 days of administration of essential oils in sugar syrup we observed a reduction in the number of germs in all experimental variants, especially in the case of bees fed on sugar syrup with addition of peppermint essential oil, of 9.77%, being followed by the experimental group fed on sugar syrup and juniper essential oil, of 8.52%. 20 days after the administration of essential oils, major differences can be observed regarding the total number of germs compared to the beginning of the experiment. The most effective oils in terms of reducing germs in the intestine was oregano essential oil which reduced the number of germs by 32.54%; peppermint oil which caused a reduction of 29.95%; rosemary oil reduced the total number of germs by 27.09%. The same situation was observed in the case of thyme essential oil, which reduced the number by 25.43%. Differences were also found in the case of the clove essential oil where the reduction in the total number of germs was 25.08% after 20 days of administration in feeding bee families.

Reducing the total number of germs in the gut is an important criterion for assessing the health of bee families with implications for increasing productive performance [1].

### 4. Conclusions

The essential oils studied can improve the health and development of bee families. The positive effects of the studied essential oils were highlighted, especially those of thyme, rosemary, oregano, cloves, mint, which determined the reduction of the total number of germs in the intestine of working bees by 0.88 - 9.77%, compared to the first control, and by 25.08 -

32.54% compared to the second control. Oregano essential oil showed the best results 20 days after the first administration, significantly reducing the total number of germs.

It is necessary to continue researches in order to evaluate these essential oils on their bio-productive potential.

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