


Article

Economic Benefits of Using Essential Oils in Food Stimulation Administrated to Bee Colonies

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Abstract: Bees are the most important pollinators of agricultural plants. The decline of bee colonies is caused by a multitude of factors of which diseases, pesticides, and climate change seem to be the most important. Losses can be huge when several factors act together. In recent years, there has been a growing interest in the use of alternative sources such as medicinal plants in the form of extracts or essential oils. The purpose of our research was to evaluate the economic efficiency of the use of essential oils of basil (*Ocimum basilicum*), cinnamon (*Cinnamomum veruuum*), clove (*Syzygium aromaticum*), juniper (*Juniperus communis* L.), oregano (*Oreganum vulgare*), mint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), and thyme (*Thymus vulgaris* L.) in the additional feeding of bee colonies in the spring. Ninety colonies of *Apis mellifera* bees were used, which were additionally fed with sugar syrup and one of the essential oils. Our results showed strong positive correlations between the total number of germs in the gut of worker bees and the number of brood cells when using the essential oil of oregano (*Oreganum vulgare*) ($R^2 = 0.786$) and thyme (*Thymus vulgaris* L.) ($R^2 = 0.729$), and between the total number of germs and the yield of honey obtained at the first harvest in the case of the essential oil of basil (*Ocimum basilicum*) ($R^2 = 1$), mint (*Mentha piperita*) ($R^2 = 0.718$), oregano (*Oreganum vulgare*) ($R^2 = 0.621$), and Thyme (*Thymus vulgaris* L.) ($R^2 = 0.859$). The best profit from the sale of honey was obtained in the case of the use of essential oils of mint, oregano, thyme, and basil, in a range of EUR 139.16–144.73/bee colony.

Keywords: economic benefits; essential oils; *Apis mellifera*



Citation: Pătruică, S.; Lazăr, R.N.; Buzamăt, G.; Boldea, M. Economic Benefits of Using Essential Oils in Food Stimulation Administrated to Bee Colonies. *Agriculture* **2023**, *13*, 594. <https://doi.org/10.3390/agriculture13030594>

Academic Editors: Bartosz Piechowicz and Anna Koziarowska

Received: 25 January 2023

Revised: 25 February 2023

Accepted: 27 February 2023

Published: 28 February 2023



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1. Introduction

Bees have an important role in maintaining the biodiversity of ecosystems, by pollinating plants, contributing to the generation of agricultural financial income [1,2]. Through the pollination process, the pollen is transferred from the anthers to the stigmas of the flowers, leading to their fecundation [3]. It is estimated that over 80–85% of flowering plants rely on pollination, mainly performed by insects [4,5] of which bees play a major role.

The relationship of bees with the pollinated plant is due to the social behavior of bee colonies. Thanks to their collective activities, bees manage to carry out all their activities in the hive and outside it in such a way as to ensure the survival of the colony: they build well-organized nests, regulate the microclimate in the hive, accumulate food deposits, ensure favorable conditions for the growth of the brood, jointly defend themselves from enemies, etc. This behavior has evolved over time, with bees developing a range of skills to identify nectar sources based on their aroma, which is transmitted to colony members through the mobilizing dance performed by gatherer scout bees [6]. The adaptations of bees for collecting pollen (the presence of bristles on the body, and brushes and corbicules on the legs) but also the vibrations of the wings during feeding facilitate the pollination process of plants [7]. There is a symbiotic relationship between melliferous plants and bees,

the plants provide nectar and pollen for the bees, and the bees contribute to the pollination of the plants [8].

From an economic point of view, globally, bees' pollination contribution to food production is estimated to be from 235 billion to 577 billion dollars [9] of which, in Europe, the annual contribution of bees is EUR 14.6 billion [10]. In the US, an annual dependence of the agricultural sectors on pollination is estimated at USD 14.2–23.8 billion [11].

Even though studies have shown an increase in the last 50 years worldwide in the number of bee colonies, there has been a decline in them in Europe and the United States during the last 10 years [12]. The decline of bee colonies is determined by a multitude of biotic and abiotic factors, a combination of which has been observed in recent years [13–17].

Parasites and pathogens (bacteria and viruses) are serious threats to bee colonies due to their location and rapid digestive transmission [18,19]. Worldwide, *Varroa* mites are an important cause of bee colony loss that can lead to their disappearance by compromising their immune system [20,21]. Heavily infested colonies, being weakened, die from the combination of parasitism and transmitted viral diseases (Deformed wing virus, Acute bee paralysis virus, Black queen cell virus) [22]. To prevent bee mortality caused by *Varroa*, beekeepers apply 5–7 treatments with acaricides annually [23]. Loss of bee colonies can also be attributed to *Nosema* infections in adult bees, affecting the midgut and causing reduced absorption of nutrients resulting in shorter longevity of adult bees [24,25]. Bacterial diseases American and European foulbrood can cause the collapse of bee colonies causing significant economic losses, and the only measure to avoid the spread of the disease remains the destruction of symptomatic colonies and the monitoring of neighboring beehives [26].

Climate changes in recent years have influenced the behavior and physiology of bees, with implications on the ability to feed, reproduce, and the health status of colonies [27,28]. In addition, weather changes in temperature, precipitation, and wind interact with bee diseases, increasing their incidence [29].

In addition to natural factors affecting bee colonies, exposure to pesticides causes neurotoxicity, immune deficiency, behavioral changes, and high mortality [30,31]. A decrease in flight performance and survival rate of gatherer bees was observed when exposed to pesticides, along with difficulties in protein digestion [32]. Pesticides found in beeswax negatively affect the quality of drones' semen and also the queens they mate with, with repercussions on the entire bee colony [33]. Research has shown that interactions between pesticides and pathogens can further increase bee mortality [34–36]. The adopted control measures such as the use of antibiotics against bee diseases are effective in vitro and in vivo, but are often ineffective for prophylactic purposes. In addition, some of them, such as the use of antibiotics in beekeeping, are prohibited in the European Union [37,38] because of the risks to human and bee health [39,40]. For these reasons, there is continued interest in identifying new effective means of preventing and combating bee diseases, such as the use of natural compounds [41–44].

Due to their antioxidant and antimicrobial properties, essential oils (EOs) are being used more and more recently both in human therapy and in bees, being able to even represent an alternative source to the use of antibiotics [45–47]. Essential oils and other natural compounds have been evaluated for their toxicity on larvae and adult worker bees (*Apis mellifera* L.). Gashout and Guzmán-Novoa [48] tested eight dilutions of menthol, cloves oil, oregano oil, and thymol on larvae and adult worker bees using an estimation test of LC50. The results showed that thymol had the lowest LC50 for adult bees (210.3 µg/bee), and menthol had the highest LC50 (523.5 µg/bee). Thymol was the most toxic product for larvae, with an LC50 of 150.7 µg/larva, while menthol was the least toxic, with a LC50 of 382.8 µg/larva. The authors believe that in addition to thymol, the oregano oil, cloves oil, and menthol are products that can represent an alternative in the control of *V. destructor*. Ebert et al. [49] tested clove oil, cineole, formic acid, marjoram oil, menthol, oregano oil, oxalic acid, sage oil, thymol, and wintergreen on the toxicity of adult bees. Each product was incorporated in different concentrations (100, 500, 1000, 5000, 10,000, and 100,000 ppm) in the sugar syrup used in bee feeding for 8 and 14 days. The oxalic acid was the most toxic

of the products tested. The authors observed that menthol and cineole had low toxicity levels, close to the group fed only sugar syrup, so the tested products could be safely used in bee feed if the dose is carefully managed.

The purpose of our research was to evaluate the existing statistical correlations between the use of essential oils (basil, thyme, juniper, cloves, mint, cinnamon, oregano, rosemary) in feeding bee colonies on their productivity and health with implications on the resulting economic efficiency.

2. Materials and Methods

2.1. Experimental Conditions

The research was carried out on 90 *Apis mellifera* bee colonies divided into 9 experimental groups, each group consisting of 10 bee colonies. Between 23 March and 14 April 2021, each bee colony was additionally fed with 3 L of sugar syrup and EOs according to Table 1. The sugar syrup 1:1 (*w:w*) was prepared and administered weekly at 1 l/week, separately for each experimental batch, calculating the essential oil (EO) dose of 0.1 mL/L syrup. The dose of administered EOs was established starting from the doses used in the specialized literature to avoid the toxic effect on bee colonies [48]. The essential oils were added to the sugar syrup just before it was introduced into the hive. These were administered in 1 l plastic bags and placed on top of the frames. We tried, in this way, to reduce the evaporation of the essential oils and, at the same time the quick access of the bees to the sugar syrup.

Table 1. Experiment organization scheme.

Experimental Variants	Extra Feeding/Week/Bee Colony			Extra Feeding/Whole Period/Bee Colony	
	Type of EOs	Sugar Syrup (L)	EOs (mL/L)	Sugar Syrup (L)	EOs (mL)
Experimental group 1 (EG ₁)	Basil	1	0.1	3	0.3
Experimental group 2 (EG ₂)	Cinnamon	1	0.1	3	0.3
Experimental group 3 (EG ₃)	Clove	1	0.1	3	0.3
Experimental group 4 (EG ₄)	Juniper	1	0.1	3	0.3
Experimental group 5 (EG ₅)	Mint	1	0.1	3	0.3
Experimental group 6 (EG ₆)	Oregano	1	0.1	3	0.3
Experimental group 7 (EG ₇)	Rosemary	1	0.1	3	0.3
Experimental group 8 (EG ₈)	Thyme	1	0.1	3	0.3
Control group (CG)	-	1	-	3	-

One group = 10 bee colonies.

Eight experimental groups (EG₁, EG₂, EG₃, EG₄, EG₅, EG₆, EG₇, EG₈) were fed sugar syrup containing one essential oil, and one group was fed only sugar syrup, representing the control group for comparison. The essential oils were purchased from the company Bionovativ, Braşov, Romania. From the 120 existing bee colonies in the apiary, we chose for the experiment the 90 colonies that had 10 combs occupied by bees and approximately 25,000 worker bees. The bee colonies were maintained in multi-story hives and had a one-year-old queen. The permanent apiary was located in the locality of Murani, Timiş County, Romania (45°55'23'' N 21°18'15'' E), 800 m from the rapeseed crop whose flowering began on 20 April 2021. All bee colonies were treated on 10 March 2021 against varroosis with the product Varrachet (Beekeeping Research and Development Institute of Bucharest, Bucharest, Romania) which contains Amitraz 150 mg/mL and tauflualinat 60 mg/mL, by fumigations applied through the hives with the anti-varroa smoker. The treatment was

repeated after 7 days. During the treatment with Varachet, there was little nectar which was used by the bees only for their consumption.

The start of the experiment was delayed by 3 weeks due to weather conditions that did not allow the activities to be carried out to thoroughly check the state of the bee colonies and prepare them for the study.

2.2. Microbiological Analysis

The purpose of the microbiological analyses was to analyze the influence of essential oils on the total number of pathogenic germs in the gut of worker bees that influence the health of bee colonies. These analyses were necessary to establish the existing correlations between the health status of bee colonies, their development, and honey production. At the beginning (23 March) and end of the period (14 April) of additional feeding with sugar syrup and essential oils, 10 worker bees were collected from each experimental variant and transferred to the microbiology laboratory of the University of Life Sciences “King Mihai I” from Timisoara. The bees were processed according to the method described by study [41]. From each colony of bees, biological material which consisted of the small intestine of worker bees was collected in 3 separate tubes which were then incubated at a thermostat, at 37 °C for 24 h. Dilutions of 10^{-1} , 10^{-2} , 10^{-3} were made and they were seeded on 3 Petri dishes for each colony of bees, over which nutritive agar was poured. The Petri plates were placed in the thermostat at 37 °C for 24 h. From the 10^{-1} dilution, Gram-stained smears were made, which were examined under a microscope, and the bacterial colonies were counted with the device Nitech LKB 2002 (Nitech, Bucharest, România) [41].

2.3. Assessment of Bee Colony Development

The influence of essential oils on the degree of development of bee colonies was measured with the Netz frame at the beginning of feeding, after 10 days and 21 days, by assessing the area occupied by hatched and unhatched broods. The Netz frame was built on the 450 × 380 × 245 mm multi-story beehive by vertical and horizontal wiring resulting in a 50 × 50 mm square grid [50]. The estimation of the number of cells occupied by brood was made according to the formula:

$$Nc = np \times 100$$

where:

- Nc—number of cells with hatched and unhatched brood;
- np—number of squares occupied by brood;
- 100—number of cells in a square.

2.4. Evaluation of Honey Production

Rapeseed honey production was assessed at the end of the harvest by individually weighing the resulting honey from each bee colony. Then we calculated the average honey production achieved by each experimental variant [50].

2.5. Statistical Analysis

The statistical processing of the obtained results was carried out with the SPSS IBM 20 program, and the ANOVA test completed by the LCD post hoc was used, for the thresholds $p < 0.001$ and $p < 0.05$. The degrees of correlation are expressed by the value of R^2 .

2.6. Economic Calculation

The calculation of the expenses for the additional feeding of the bee colonies was made starting from the price of one kg of sugar in March 2021 of 3.5 lei equivalent to EUR 0.72, resulting in a cost of one liter of sugar syrup of EUR 0.43. The cost of essential oils for one liter of syrup was EUR 0.07 for EG1 (basil), EUR 0.05 for EG2 (cinnamon), EG4 (juniper), EG6 (oregano), EG8 (thyme), and EUR 0.06 for EG3 (clove), EG5 (mint), and EG7 (rosemary). The cost of treatments against Varroa was EUR 0.84/bee colony. There were no

fuel costs for transporting bee colonies because the permanent apiary was located 800 m from the rapeseed crop. The selling price of one kg of rapeseed honey was EUR 4.48, of which EUR 0.8 represented the costs of packaging and distribution. We did not take into account the labor performed with the work in the beehive, as it was performed by the beekeeper's family.

3. Results

3.1. Health Status of Bee Colonies and Bioproductive Parameters

Our research has highlighted that the use of essential oils in the supplementary feeding of bee colonies applied in the spring has a positive influence on the development of the bee colonies, the state of their health, and the production of rapeseed honey. We found a strong positive correlation between the total number of germs in the gut of bees and honey production ($R^2 = 1$, $p < 0.001$) when using basil essential oil (Table 2), and a weak correlation between the total number of germs and bee colony development ($R^2 = 0.119$, $p > 0.05$). Weakly positive correlations were recorded with the use of cinnamon essential oil, both when comparing the total number of germs with the number of brood cells ($R^2 = 0.390$, $p > 0.05$) as well as when compared with the production of rapeseed honey obtained ($R^2 = 0.482$). Similar results were recorded with the use of essential oils of clove ($R^2 = 0.295$; $R^2 = 0.241$, $p > 0.05$) and juniper ($R^2 = 0.331$; $R^2 = 0.200$, $p > 0.05$). When using peppermint essential oil, we observed a high positive correlation between total germ count and honey production ($R^2 = 0.718$, $p < 0.001$), and a weak positive correlation with bee colony development ($R^2 = 0.175$, $p > 0.05$).

Table 2. Correlations between total worker bee gut germ counts, bee colony development, and rapeseed honey production following sugar syrup and essential oil feedings.

Analyzed Parameters/Essential Oil	No. of Brood Cells (R^2)	Honey Production (R^2)
Total number of germs	Basil (EG ₁)	0.119 ($p > 0.05$)
	Cinnamon (EG ₂)	0.390 ($p > 0.05$)
	Clove (EG ₃)	0.295 ($p > 0.05$)
	Juniper (EG ₄)	0.331 ($p > 0.05$)
	Mint (EG ₅)	0.175 ($p > 0.05$)
	Oregano (EG ₆)	0.786 ($p < 0.001$)
	Rosemary (EG ₇)	0.108 ($p > 0.05$)
	Thyme (EG ₈)	0.729 ($p < 0.001$)

Basil essential oil (EG₁); Cinnamon essential oil (EG₂); Clove essential oil (EG₃); Juniper essential oil (EG₄); Mint essential oil (EG₅); Oregano essential oil (EG₆); Rosemary essential oil (EG₇); Thyme essential oil (EG₈).

The use of oregano essential oil led to a significant reduction in the total number of germs in the gut of worker bees, the development of bee colonies, and a significant increase in honey production when harvesting rapeseed ($p < 0.001$), leading to strongly positive correlations between the total number of germs and the number of brood cells ($R^2 = 0.786$, $p < 0.001$), respectively, honey production ($R^2 = 0.621$, $p < 0.001$). For rosemary oil, when comparing the total number of germs with the other two analyzed parameters, we observed slightly positive correlations both in terms of the development of bee colonies ($R^2 = 0.108$, $p > 0.05$) as well as the production of honey obtained ($R^2 = 0.410$, $p > 0.05$). When using thyme essential oil, we found the strongest correlation both between the total number of germs and the development of the studied bee colonies ($R^2 = 0.729$, $p < 0.001$), as well as with the production of harvested honey ($R^2 = 0.859$, $p < 0.001$).

3.2. Economic Efficiency

The cost of additional feeding during the 3 weeks of a colony of bees from the control group fed only with sugar syrup was EUR 1.29. In the case of the bee colonies that had one

essential oil incorporated into the syrup, the cost of additional feeding was EUR 1.50 /bee colony in EG1 (basil essential oil); EUR 1.44/bee colony in the case of EG2 (cinnamon essential oil), EG4 (juniper essential oil), EG6 (oregano essential oil), and EG8 (thyme essential oil), and for the experimental groups EG3 (clove essential oil), EG5 (mint essential oil), and EG7 (rosemary essential oil) EUR 1.47/bee colony (Table 3).

Table 3. The economic benefits of using essential oils as supplements in the stimulation feed administered to bee colonies.

Experimental Variants	Rapeseed Honey Yield (kg/Bee Colony)	Income (EUR/Bee Colony)	Feeding Costs (EUR/Bee Colony)
Basil (EG ₁)	31.68	141.92	1.50
Cinnamon (EG ₂)	26.98	120.87	1.44
Clove (EG ₃)	27.15	121.63	1.47
Juniper (EG ₄)	26.49	118.67	1.44
Mint (EG ₅)	33.00	147.84	1.47
Oregano (EG ₆)	32.16	144.07	1.44
Rosemary (EG ₇)	30.01	134.44	1.47
Thyme (EG ₈)	31.75	142.24	1.44
Control group (CG)	25.09	112.40	1.29

Income from the sale of rapeseed honey were higher in the case of additional feeding of bee colonies with the addition of essential oils compared to the control group fed only sugar syrup (Table 3). Very good results have been observed with the addition of essential oils of mint (EG5), oregano (EG6), thyme (EG8), basil (EG1), and rosemary (EG7) where the revenues from the sale of rapeseed honey increased by 19.60–31.53% compared to the control group (CG). Income from the sale of rapeseed honey obtained from experimental groups EG2 (cinnamon), EG3 (clove), and EG4 (juniper) were 5.57–8.21% compared to the control group fed only with sugar syrup (CG).

The profit obtained in the case of using the essential oils studied by us was in the range of EUR 109.47–144.73/bee colony (Figure 1). The highest profit from the sale of rapeseed honey was obtained from bee colonies fed sugar syrup and mint essential oil (EG5), 32.20% higher compared to the control group (CG). Very good results compared to the control batch were obtained when incorporating the oils of oregano (EG6—31.60%), thyme (EG8—29.93%), basil (EG1—29.64%), and rosemary (EG7—22.80%). In the case of using the essential oils of cinnamon (EG2), clove (EG3), and juniper (EG4), the profit was higher by 5.59–8.26% compared to that obtained by bee colonies that were supplemented with only sugar syrup (CG).

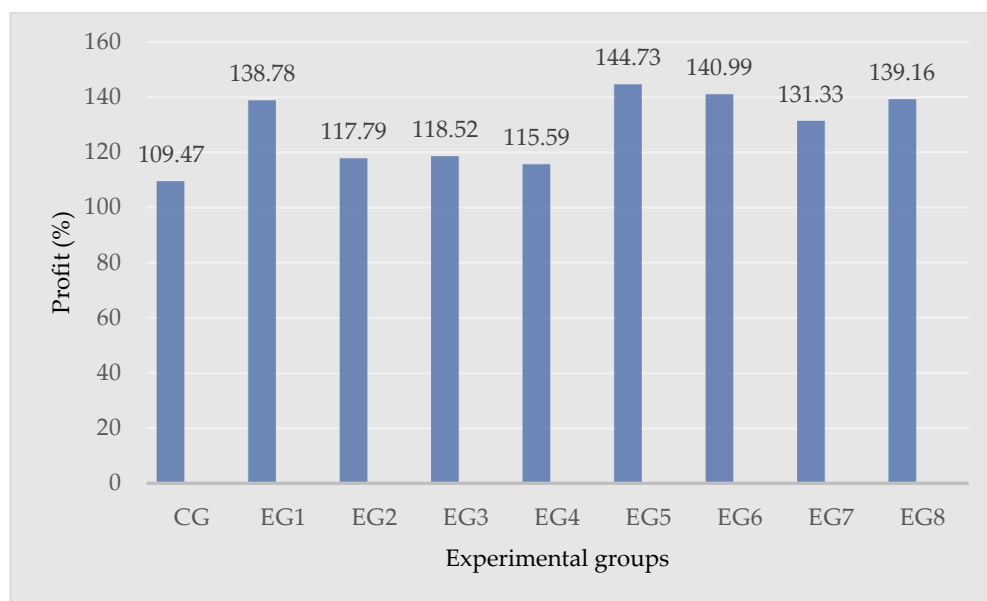


Figure 1. Comparison of profit obtained by using essential oil supplements in spring administered stimulation feeds of bee colonies. CG—control group (no essential oil supplement); EG1—Basil essential oil; EG2—Cinnamon essential oil; EG3—Clove essential oil; EG4—Juniper essential oil; EG5—Mint essential oil; EG6—Oregano essential oil; EG7—Rosemary essential oil; EG8—Thyme essential oil.

4. Discussion

Additional bee stimulation feeding is a technological measure that is applied at the end of winter in order to stimulate queen laying and colony development for superior utilization of the harvests in honey plants. Moreover, due to climate change, winter temperatures suffer large fluctuations, the consumption of food in the hive increases, so the additional feeding of bee colonies at the end of winter has become a mandatory measure. The trophallactic behavior of the bee colony in which permanent exchanges of food take place inside the hive make it possible to increase the efficiency of the use of additives by incorporating them into the additional food administered.

The aim of our study was to evaluate the existing correlations between the administration of the 8 essential oils in relation to the development of bee colonies, their health status, the honey production achieved, and finally, the economic efficiency of their use. The effectiveness of essential oils is influenced by the type of oil used, and how it is obtained and administered. The chemical composition of the oils analyzed by us in this study was evaluated by Lazăr et al. 2022 [47]. The essential oils used in the current study contain mainly estragol 55.73% and linalool 38.64% (basil essential oil); cinnamaldehyde (E) 69.28% and cinnamaldehyde-o-methoxy 14.30% (cinnamon essential oil); eugenol 85.17% and carryophillene 8.15%, (clove essential oil); alfa-pinene 49.75% and beta-pinene 16.70% (juniper essential oil); menthone 30.73% and neomenthol 17.37% (mint oil); karvacrol 33.42%, o-cymene 22.98%, gamma terpinene 17.44% (oregano essential); 52.82% eucalyptol and alfa pinene 17.56% (rosemary essential oil), and thyme essential oil which contains thymol 39.44%, borneol 17.15%, and camphene 14.41%, but also other compounds in variable proportions.

During the feeding of bee colonies with sugar syrup and essential oils, no changes in bee behavior were observed, but we found an increase in attractiveness towards sugar syrup with OEs compared to the control group.

Our study highlighted the favorable influence of the use of essential oils in bees by stimulating the laying of the queen correlated with a better development of the colonies materialized in the increasing of honey production. When administering the 8 essential oils, research has shown an increase in the number of sprouted cells by 12.2–50% with the best

results being reported for thyme and basil essential oils ($p < 0.001$), followed by oregano and mint ($p < 0.01$).

Mesbah et al. [51] reported increased brood surface area values and a greater number of frames covered with bees after feeding bee colonies with diets containing essential oils of mint, camphor, thyme, and garlic, the best results being obtained by the variant fed with mint essential oil, followed by the one fed with thyme essential oil.

Lazăr et al.'s [44] studies showed that after 3 weeks of administration of the 8 essential oils, there was a significant reduction in the total number of germs ($p < 0.05$), in the case of using the essential oils of thyme, basil, oregano, juniper, and cinnamon, by 8.43–34.25% compared to the batch fed only with sugar syrup.

There are many studies that provide information on the antimicrobial activity of essential oils [52–55]. Essential oils can play an important role in prevention and treatment of American foulbrood disease [56,57]. The studies carried out by Alippi et al. [58], on the use of essential oils of savory, lavandin, eucalyptus, lemon grass, mint, oregano, rosemary, and thyme, in order to test antimicrobial activities against *Paenibacillus larvae*, the causative agent of American foulbrood disease (AFB), highlighted the effect of lemon grass and thyme oils against the 8 strains analyzed.

In addition, there are many studies that provide information about antifungal and acaricidal effects of OEs. Following in vitro evaluation of the antifungal activity of 27 essential oils against two strains of *Ascosphaera apis*, the authors in [59] observed that mountain pepper oil, Kala Bhangra oil, and spearmint oil prove strong antifungal activity. Similar results were obtained by study [60], when using the essential oils of *Thymus herba-barona*, *Thymus capitata*, and *Cinnamomum zeylanicum* in the control of *Ascosphaera apis*.

Hýbl et al. [61] studied the acaricidal effect of 30 essential oils as an alternative measure to control the populations of *Varroa destructor*. The best results were obtained with the essential oils of peppermint and manuka, followed by oregano, litsea, carrot, and cinnamon. Dumitru et al. [62] reported the effectiveness of a blend of essential oils of lemon balm (*Melissa officinalis*), summer savory (*Satureja hortensis*), peppermint (*Mentha piperita*), and coriander (*Coriander sativum*), against *Nosema* spores in infested bees. Research by [63] showed a 40% reduction in *N. ceranae* spores after 17 days of thymol administration, which could represent an alternative to the use of the antibiotic Fumagilin B. Similar results regarding the reduction of *Nosema* spores were obtained by study [64] when using oregano essential oil and thymol. The authors believe that in the case of oregano essential oil, the results can be attributed to the high concentration in carvacol and thymol.

The effect of paper impregnated with essential oils of mint, camphor, thyme, garlic, and mustard on *Varroa* was evaluated by the authors of [51] during the two beekeeping seasons of 2020 and 2021. The study results showed an effectiveness of 62.5–77.84% in the first year and between 79.59–83.95% in the second year.

The toxicity of essential oils to worker bees depends on the method of application, the doses administered, and the bee species [65]. The authors observed that the toxicity of essential oils administered in doses of 0.5 to 25% (v/v), in descending order, were oregano, thyme, peppermint, and ginger.

In Romania, honey production varies between 18,000–30,000 tons annually [66], honey representing the main source of income for Romanian beekeepers. Improving the health status of bee colonies following the use of essential oils of basil, cinnamon, clove, juniper, oregano, mint, rosemary, and thyme is correlated with their development and honey production. The profit made by the beekeeper after rapeseed gathering from a bee colony varied between EUR 5.59 (juniper essential oil) and EUR 32.20 (mint essential oil), with very good results also being obtained in the case of oregano essential oils (EUR 31.60), thyme (EUR 29.93) and basil (EUR 29.64). These results are close to those obtained with the use of prebiotics and probiotics [67].

Our research shows that the analyzed essential oils can be used in supplementary feeding at the end of the winter period, and that depending on the biologically active components they contain, they can improve the health and bioproductive potential of bee

colonies and the economic efficiency of the apiary. Depending on weather conditions, we recommend feeding sugar syrup and EOs as early as possible in the spring to avoid honey contamination. Further research is required to establish the effect of administering essential oils over a longer period of time, i.e., during a beekeeping season, possibly correlated with a second administration in the fall when the bee colony is preparing for the rest period.

5. Conclusions

The strong correlations fully documented in our study between the reduction of the total number of germs in the gut of worker bees and the increase of honey production obtained at the first harvest, in the case of the use of essential oils of basil ($R^2 = 1$, $p < 0.001$), thyme ($R^2 = 0.859$, $p < 0.001$), mint ($R^2 = 0.718$, $p < 0.001$), and oregano ($R^2 = 0.621$, $p < 0.001$) shows their effectiveness in supplemental feeding of bee colonies in early spring. In our opinion, the effects of the use of essential oils are mainly due to the phenolic components contained which have a favorable effect on the stimulation of queen laying and the health of the entire bee colony but these aspects will have to be studied in the future.

The profit resulting from the sale of honey obtained after the first harvest was higher by using mint, oregano, thyme, and basil essential oil, which means a very good economic efficiency.

Author Contributions: Conceptualization and original draft preparation, S.P.; methodology, formal analysis, S.P., R.N.L. and G.B.; software analysis, M.B.; review, editing, and validation, S.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research is published from internal funds of the University of Life Sciences “King Mihai I” from Timisoara, Romania.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data used in this study are available by email request to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

- Potts, S.G.; Imperatriz-Fonseca, V.; Ngo, H.T.; Aizen, M.A.; Biesmeijer, J.C.; Breeze, T.D.; Dicks, L.V.; Garibaldi, L.A.; Hill, R.; Settele, J. Safeguarding pollinators and their values to human well-being. *Nature* **2016**, *540*, 220–229. [[CrossRef](#)]
- Garibaldi, L.A.; Carvalheiro, L.G.; Vaissiere, B.E.; Gemmill-Herren, B.; Hipolito, J.; Freitas, B.M.; Ngo, H.T.; Azzu, N.; Saez, A.; Astrom, J.; et al. Mutually beneficial pollinator diversity and crop yield outcomes in small and large farms. *Science* **2016**, *351*, 388–391. [[CrossRef](#)] [[PubMed](#)]
- Wu, S.; Liu, J.; Lei, X.; Zhao, S.; Lu, J.; Jiang, Y.; Xie, B.; Wang, M. Research Progress on Efficient Pollination Technology of Crops. *Agronomy* **2022**, *12*, 2872. [[CrossRef](#)]
- Ollerton, J.; Winfree, R.; Tarrant, S. How many flowering plants are pollinated by animals? *Oikos* **2011**, *120*, 321–326. [[CrossRef](#)]
- Gardi, T.; Berta, F.; Fabbri, C.A.; Marchetti, C. Operation Pollinator: A New Way for the Protection and Implementation of Insect Pollinators in Different Agroecosystem—Results of Seven Years of Experiment in Italy. *AgroLife Sci. J.* **2015**, *4*, 70–73.
- Seeley, T.D. The tremble dance of the honey bee: Message and meanings. *Behav. Ecol. Sociobiol.* **1992**, *31*, 375–383. [[CrossRef](#)]
- Winston, M.L. *The Biology of the Honey Bee*; Harvard University Press: Cambridge, MA, USA, 1991.
- Hung, K.L.J.; Kingston, J.M.; Albrecht, M.; Holway, D.A.; Kohn, J.R. The worldwide importance of honey bees as pollinators in natural habitats. *Proc. R. Soc. B Biol. Sci.* **2018**, *285*, 20172140–20172147. [[CrossRef](#)] [[PubMed](#)]
- FAO. *Pollinators Vital to Our Food Supply under Threat*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2016.
- Leonhardt, S.D.; Gallai, N.; Garibaldi, L.A.; Kuhlmann, M.; Klein, A.M. Economic gain, stability of pollination and bee diversity decrease from southern to northern Europe. *Basic Appl. Ecol.* **2013**, *14*, 461–471. [[CrossRef](#)]
- Chopra, S.S.; Bakshi, B.R.; Khanna, V. Economic dependence of U.S. industrial sectors on animal-mediated pollination service. *Environ. Sci. Technol.* **2015**, *49*, 14441–14451. [[CrossRef](#)]
- Van Engelsdorp, D.; Meixner, M.D. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. *J. Invertebr. Pathol.* **2010**, *103*, S80–S95. [[CrossRef](#)] [[PubMed](#)]
- Potts, S.G.; Biesmeijer, J.C.; Kremen, C.; Neumann, P.; Schweiger, O.; Kunin, W.E. Global pollinator declines: Trends, impacts and drivers. *Trends Ecol. Evol.* **2010**, *25*, 345–353. [[CrossRef](#)]

14. Morimoto, T.; Kojima, Y.; Toki, T.; Komeda, Y.; Yoshiyama, M.; Kimura, K.; Nirasawa, K.; Kadowaki, T. The habitat disruption induces immune-suppression and oxidative stress in honey bees. *Ecol. Evol.* **2011**, *1*, 201–217. [[CrossRef](#)] [[PubMed](#)]
15. Goulson, D.; Nicholls, E.; Botías, C.; Rotheray, E.L. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science* **2015**, *347*, 1255957. [[CrossRef](#)]
16. Gray, A.; Brodschneider, R.; Adjlane, N.; Ballis, A.; Brusbardis, V.; Charriere, J.-D.; Chlebo, R.; Coffey, M.F.; Cornelissen, B.; Amaro da Costa, C. Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources. *J. Apic. Res.* **2019**, *58*, 479–485. [[CrossRef](#)]
17. Havard, T.; Laurent, M.; Chauzat, M.-P. Impact of stressors on honey bees (*Apis mellifera*; Hymenoptera: Apidae): Some guidance for research emerge from a meta-analysis. *Diversity* **2020**, *12*, 7. [[CrossRef](#)]
18. Cox-Foster, D.L.; Conlan, S.; Holmes, E.C.; Palacios, G.; Evans, J.D.; Moran, N.A.; Quan, P.-L.; Briese, T.; Hornig, M.; Geiser, D.M. A Metagenomic survey of microbes in honey bee colony collapse disorder. *Science* **2007**, *318*, 283–287. [[CrossRef](#)]
19. Raymann, K.; Moran, N.A. The role of the gut microbiome in health and disease of adult honey bee workers. *Curr. Opin. Insect Sci.* **2018**, *26*, 97–104. [[CrossRef](#)]
20. Guzmán-Novoa, E.; Eccles, L.; Calvete, Y.; McGowan, J.; Kelly, P.G.; Correa-Benítez, A. *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie* **2010**, *41*, 443–450. [[CrossRef](#)]
21. Van Dooremalen, C.; Gerritsen, L.; Cornelissen, B.; Van der Steen, J.; Van Langevelde, F.; Blacquiere, T. Winter survival of individual honey bees and honey bee colonies depends on level of *Varroa destructor* infestation. *PLoS ONE* **2012**, *7*, e36285. [[CrossRef](#)]
22. Highfield, A.C.; El Nagar, A.; Mackinder, L.C.; Noël, L.M.L.; Hall, M.J.; Martin, S.J.; Schroeder, D.C. Deformed wing virus implicated in overwintering honeybee colony losses. *Appl. Environ. Microbiol.* **2009**, *75*, 7212–7220. [[CrossRef](#)] [[PubMed](#)]
23. Chen, J.; De Grandi-Hoffman, G.; Ratti, V.; Kang, Y. Review on mathematical modeling of honeybee population dynamics. *Math. Biosci. Eng.* **2021**, *18*, 9606–9650. [[CrossRef](#)]
24. Paris, L.; El Alaoui, H.; Delbac, F.; Diogon, M. Effects of the gut parasite *Nosema ceranae* on honey bee physiology and behavior. *Curr. Opin. Insect Sci.* **2018**, *26*, 149–154. [[CrossRef](#)]
25. Snow, J.W.; Ceylan Koydemir, H.; Karınca, D.K.; Liang, K.; Tseng, D.; Ozcan, A. Rapid imaging, detection, and quantification of *Nosema ceranae* spores in honey bees using mobile phone-based fluorescence microscopy. *Lab Chip* **2019**, *19*, 789–797. [[CrossRef](#)]
26. Forsgren, E.; Locke, B.; Sircoulomb, F.; Schäfer, M.O. Bacterial Diseases in Honeybees. *Curr. Clin. Microbiol. Rep.* **2018**, *5*, 18–25. [[CrossRef](#)]
27. Le conte, Y.; Navajas, M. Climate change: Impact on honey bee population and diseases. *Rev. Sci. Tech. Int. Off. Epizoot.* **2008**, *27*, 499–510.
28. Pătruică, S.; Peț, I.; Simiz, E. Beekeeping in the context of climate change. *Sci. Pap. Ser. D Anim. Sci.* **2021**, *LXIV*, 277–280.
29. Rowland, B.W.; Rushton, S.P.; Shirley, M.D.F.; Brown, M.A.; Budge, G.E. Identifying the climatic drivers of honey bee disease in England and Wales. *Sci. Rep.* **2021**, *11*, 21953. [[CrossRef](#)]
30. Woodcock, B.A.; Isaac, N.J.B.; Bullock, J.M.; Roy, D.B.; Garthwaite, D.G.; Crowe, A.; Pywell, R.F. Impacts of neonicotinoid use on long-term population changes in wild bees in England. *Nat. Commun.* **2016**, *7*, 12459. [[CrossRef](#)] [[PubMed](#)]
31. Tosi, S.; Nieh, J.C.; Sgolastra, F.; Cabbri, R.; Medrzycki, P. Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, 20171711–20171719. [[CrossRef](#)]
32. Fisher, A., II; De Grandi-Hoffman, G.; Smith, B.H.; Johnson, M.; Kaftanoglu, O.; Cogley, T.; Fewell, J.H.; Harrison, J.F. Colony field test reveals dramatically higher toxicity of a widely-used mito-toxic fungicide on honey bees (*Apis mellifera*). *Environ. Pollut.* **2021**, *269*, 115964. [[CrossRef](#)]
33. Fisher, A.; Rangel, J. Exposure to pesticides during development negatively affects honey bee (*Apis mellifera*) drone sperm viability. *PLoS ONE* **2018**, *13*, e0208630. [[CrossRef](#)] [[PubMed](#)]
34. Vidau, C.; Diogon, M.; Aufauvre, J.; Fontbonne, R.; Viguès, B.; Brunet, J.-L.; Texier, C.; Biron, D.G.; Blot, N.; El Alaoui, H.; et al. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. *PLoS ONE* **2011**, *6*, e21550. [[CrossRef](#)] [[PubMed](#)]
35. Straub, L.; Williams, G.R.; Vidondo, B.; Khongphinitbunjong, K.; Retschnig, G.; Schneeberger, A.; Chantawannakul, P.; Dietemann, V.; Neumann, P. Neonicotinoids and ectoparasitic mites synergistically impact honeybees. *Sci. Rep.* **2019**, *9*, 8159. [[CrossRef](#)]
36. Tesovnik, T.; Zorc, M.; Ristanić, M.; Glavinić, U.; Stevanović, J.; Narat, M.; Stanimirović, Z. Exposure of honey bee larvae to thiamethoxam and its interaction with *Nosema Ceranae* infection in adult honey bees. *Environ. Pollut.* **2020**, *256*, 113443. [[CrossRef](#)] [[PubMed](#)]
37. Reybroeck, W.; Daeseleire, E.; De Brabander, H.F.; Herman, L. Antimicrobials in beekeeping. *Vet. Microbiol.* **2012**, *158*, 1–11. [[CrossRef](#)]
38. Mutinelli, F. European legislation governing the authorization of veterinary medicinal products with particular reference to the use of drugs for the control of honey bee diseases. *Apiacta* **2003**, *38*, 156–168.
39. World Health Organization (WHO). Antimicrobial Resistance: Global Report on Surveillance. 2014. Available online: https://apps.who.int/iris/bitstream/handle/10665/112647/WHO_HSE_PED_AIP_?sequence=1 (accessed on 6 December 2022).
40. Raymann, K.; Shaffer, Z.; Moran, N.A. Antibiotic exposure perturbs the gut microbiota and elevates mortality in honeybees. *PLoS Biol.* **2017**, *15*, e2001861. [[CrossRef](#)]

41. Pătruică, S.; Mot, D. The effect of using prebiotic and probiotic products on intestinal micro-flora of the honeybee (*Apis mellifera carpatica*). *Bull. Entomol. Res.* **2012**, *102*, 619–623. [CrossRef]
42. Alonso-Salces, R.M.; Cugnata, N.M.; Guaspari, E.; Pellegrini, M.C.; Aubone, I.; De Piano, F.G.; Antunez, K.; Fuselli, S.R. Natural strategies for the control of PaeniBacillus larvae, the causative agent of American foulbrood in honey bees: A review. *Apidologie* **2017**, *48*, 387–400. [CrossRef]
43. Tauber, J.P.; Collins, W.R.; Schwarz, R.S.; Chen, Y.; Grubbs, K.; Huang, Q.; Lopez, D.; Peterson, R.; Evans, J.D. Natural product medicines for honey bees: Perspective and protocols. *Insects* **2019**, *10*, 356. [CrossRef]
44. Lazăr, R.N.; Mot, D.; Alexa, E.; Boldea, M.; Stef, L.; Pătruică, S. Influence of essential oils on bioproductive indices and health of bee families. *Sci. Pap. Ser. D Anim. Sci.* **2021**, *LXIV*, 247–253.
45. Kim, S.I.; Roh, J.Y.; Kim, D.H.; Lee, H.S.; Ahn, Y.J. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. *J. Stored Prod. Res.* **2003**, *39*, 293–303. [CrossRef]
46. Prabuseenivasan, S.; Jayakumar, M.; Ignacimuthu, S. In vitro antibacterial activity of some plant essential oils. *BMC Complement. Altern. Med.* **2006**, *6*, 39. Available online: <https://bmccomplementmedtherapies.biomedcentral.com/articles/10.1186/1472-6882-6-39> (accessed on 22 December 2022).
47. Lazăr, R.N.; Alexa, E.; Obiștioiu, D.; Cocan, I.; Pătruică, S. The effect of the use of essential oil in the feed of bee families on honey chemical composition and antimicrobial activity. *Appl. Sci.* **2022**, *12*, 1094. [CrossRef]
48. Hanan, A.; Gashout, E.G.-N. Acute toxicity of essential oils and other natural compounds to the parasitic mite, *Varroa destructor*, and to larval and adult worker honey bees (*Apis mellifera* L.). *J. Apic. Res.* **2009**, *48*, 263–269. [CrossRef]
49. Ebert, T.A.; Kevan, P.G.; Bishop, B.L.; Kevan, D.; Downer, R.A. Oral toxicity of essential oils and organic acids fed to honey bees (*Apis mellifera*). *J. Apic. Res. Bee World* **2007**, *46*, 220–224. [CrossRef]
50. Pătruică, S. Research on the Influence of Apicultural Biostimulators on Bee Family Feeding. Habilitation Thesis, Banat University of Agricultural Sciences and Veterinary Medicine, Timișoara, România, 2017.
51. Mesbah, H.A.A.; El-Sayed, N.A.; Mourad, A.K.; Abdel-Razik, B.A. Controlling *Varroa destructor* Infesting Honey Bee *Apis mellifera* using Essential Oils as Diet Supplements and as Impregnated Paper. *J. Plant Prot. Pathol.* **2021**, *5*, 381–387. [CrossRef]
52. Sivropoulou, A.; Papanikolaou, E.; Nikolaou, C.; Kokkini, S.; Lanaras, T.; Arsenakis, M. Antimicrobial and cytotoxic activities of Origanum essential oils. *J. Agric. Food Chem.* **1996**, *44*, 1202–1205. [CrossRef]
53. Hammer, K.A.; Carson, C.F.; Riley, T.V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985–990. [CrossRef] [PubMed]
54. Rota, M.C.; Herrera, A.; Martinez, R.M.; Sotomayor, J.A.; Jordan, M.J. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food Control* **2008**, *19*, 681–687. [CrossRef]
55. Doyle, A.A.; Stephens, J.C. A review of cinnamaldehyde and its derivatives as antibacterial agents. *Fitoterapia* **2019**, *139*, 104405. [CrossRef] [PubMed]
56. Maggi, M.; Gende, L.B.; Russo, K.; Fritz, R.; Eguaras, M. Bioactivity of Rosmarinus officinalis essential oils against *Apis mellifera*, *Varroa destructor* and *Paenibacillus larvae* related to the drying treatment of the plant material. *Nat. Prod. Res.* **2011**, *25*, 397–406. [CrossRef] [PubMed]
57. Ansari, M.J.; Al-Ghamdi, A.; Usmani, S.; Al-Waili, N.; Nuru, A.; Sharma, D.; Khan, K.A.; Kaur, M.; Omer, M. In vitro evaluation of the effects of some plant essential oils on *Paenibacillus larvae*, the causative agent of American foulbrood. *Biotechnol. Equip.* **2016**, *30*, 49–55. [CrossRef]
58. Alippi, A.M.; Ringuelet, J.A.; Cerimele, E.L.; Re, M.S.; Henning, C.P. Antimicrobial Activity of Some Essential Oils Against *Paenibacillus larvae*, the Causal Agent of American Foulbrood Disease. *J. Herbs Spices Med. Plants* **1996**, *4*, 9–16. [CrossRef]
59. Ansari, M.J.; Al-Ghamdi, A.; Usmani, S.; Khan, K.A.; Alqarni, A.S.; Kaur, M.; Al-Waili, N. In vitro evaluation of the effects of some plant essential oils on *Ascosphaera apis*, the causative agent of Chalkbrood disease. *Saudi J. Biol. Sci.* **2017**, *24*, 1001–1006. [CrossRef]
60. Pusceddu, M.; Floris, I.; Mangia, N.P.; Angioni, A.; Satta, A. In Vitro Activity of Several Essential Oils Extracted from Aromatic Plants against *Ascosphaera apis*. *Vet. Sci.* **2021**, *8*, 80. [CrossRef]
61. Hýbl, M.; Bohatá, A.; Rádsetoulalová, I.; Kopecký, M.; Hošťicková, I.; Vanícková, A.; Mráz, P. Evaluating the Efficacy of 30 Different Essential Oils against *Varroa destructor* and Honey Bee Workers (*Apis mellifera*). *Insects* **2021**, *12*, 1045. [CrossRef]
62. Dumitru, A.; Chioveanu, G.; Ioniță, M.; Dobre Mitrea, I.L. “In vitro” studies on using natural essential oils in treatment of Nosemosis in honey bees: Determination of the therapeutic dose. *Sci. Work. Ser. C Vet. Med.* **2017**, *63*, 165–170.
63. Van den Heever, J.P.; Thompson, T.S.; Otto, S.J.G.; Curtis, J.M.; Ibrahim, A.; Pernal, S.F. Evaluation of Fumagilin-B[®] and other potential alternative chemotherapies against *Nosema ceranae*-infected honeybees (*Apis mellifera*) in cage trial assays. *Apidologie* **2015**, *47*, 617–630. [CrossRef]
64. Borges, D.; Guzman-Novoa, E.; Goodwin, P.H. Control of the microsporidian parasite *Nosema ceranae* in honey bees (*Apis mellifera*) using nutraceutical and immuno-stimulatory compounds. *PLoS ONE* **2020**, *15*, e0227484. [CrossRef]
65. da Silva, I.M.; Zanoncio, J.C.; Brügger, B.P.; Soares, M.A.; José, A.; Zanoncio, V.; Wilcken, C.F.; Tavares, W.S.; Serrão, J.E.; Sediyaama, C.S. Selectivity of the botanical compounds to the pollinators *Apis mellifera* and *Trigona hyalinata* (Hymenoptera: Apidae). *Sci. Rep.* **2020**, *10*, 4820. [CrossRef] [PubMed]

66. FAO/STAT. 2021. Available online: <https://www.fao.org> (accessed on 13 January 2022).
67. Pătruică, S.; Huțu, I. Economic benefits of using prebiotic and probiotic products as supplements in stimulation feeds administered to bee colonies. *Turk. J. Vet. Anim. Sci.* **2013**, *37*, 259–263. [[CrossRef](#)]

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