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Research article

Investigation of inhibition of the pathogen *Varirmorpha ceranaea* (Nosema) in *Apis mellifera* using plant extracts

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ABSTRACT

This study was applied at the College of Agriculture, University of Wasit, from September 16, 2025, to March 18, 2026. Three treatments were used: a control, a second treatment (*Lavandula spica*), and a third treatment (*Vitellaria paradoxa*). The study aimed to inhibit *Nosema ceranaea* disease in bees using pastes of *L. spica* and *V. paradoxa* extracts and to investigate their effects on bee colony members (number of bees, frame weight, honey production, brood area, number of eggs, and pollen area). The results showed the area of the pollen tubes showed that the first test revealed no significant differences between the treatments, with the mean values for the first, second, and third treatments being 174 and 99, respectively. However, the results of the second test showed no significant advantage for any of the treatments, with the first treatment having a mean of 441 and the second treatment having a mean of 286. The first treatment showed a significant decrease, with a mean value of 102.33, significantly lower than the other treatments. First test showed that the mean value for the first treatment was 941.66, the second treatment 360, and the third treatment 126.66. Significant differences were observed, with the third treatment outperforming the first and second treatments. In the second test, the results showed that the third treatment outperformed the first and second treatments, with no significant differences. The results for the first test regarding egg area were clear, with the average values being (141.66) for the first treatment, (128.33) for the second treatment, and (188.33) for the third treatment. The third treatment was observed to be superior to the first and second treatments. In the second test, the third treatment also outperformed the first, while the average value for the second treatment decreased to (178.33), the third treatment to (699), and the first treatment to (336.66). Third treatment outperformed the first treatment with an average of (4500), the second treatment to (7816.66), and the third treatment to (14133.33) in the first test. However, the results of the second test showed a clear difference, with a significant difference. The third treatment recorded the highest average (17567.66), followed by the first treatment with an average of (6016.66), and the second treatment with an average of (5883.33).

1. Introduction

Bees play a vital role in the ecosystem, both natural and agricultural, as they are important pollinators. This helps maintain the ecosystem, preserve biodiversity, and ensure crop abundance (Potts et al., 2016). Honeybee colonies face numerous threats, including chemicals, climate change, other pests, and pathogens such as microorganisms like the fungus *Nosema ceranaea* (Potts et al., 2016). Infection with *Nosema ceranaea* leads to a 22-44% reduction in worker bee lifespan, weakening the colony, reducing honey production, and increasing bee mortality (Pasca et al., 2019).

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Other effects of *Nosema ceranaea* pose a serious threat to honeybee colonies worldwide (Cristina et al., 2020). The text abruptly ends here, so the translation stops as well. This parasite enters the digestive system, specifically the midgut, thus affecting metabolism and reducing energy expenditure through fat metabolism, leading to physiological disturbances in honeybee colonies (Gillbert et al., 2024). This infection causes weakness, malnutrition, shortened lifespan, and increased mortality (Ho et al., 2024).

There is a safe and alternative treatment to chemicals: plant extracts, such as lavender and chia, are considered a safe and sustainable alternative to chemicals for combating the pathogen *V. ceranaea*, which infects bee colonies. Plant extracts can significantly reduce the pathogen, decrease spores, and mitigate the resulting risks. This suggests a trend toward using these substances due to their multifaceted therapeutic applications (Garrido et al., 2024). Therefore, the aim of study was to investigate of inhibition of the pathogen *Varirmorpha ceranaea* (*Nosema*) in *Apis mellifera* using plant extracts.

2. Materials and Methods

The study was applied in the Apiary Laboratory at the College of Agriculture, University of Wasit, starting from [date missing] to determine the effect of protein and carbohydrate feedstuffs on the performance, development, growth, and honey production of honeybee colonies. The study included the following:

Feeding with a Paste

Paste ingredients: (6 kg powdered sugar, 1 kg dried pollen, 75 g soybean meal, 75 g corn starch, 1 kg honey, 50 ml natural lemon juice).

Paste Preparation Method

- 1- All the feed ingredients were weighed according to the previously mentioned quantities.
- 2- The pollen was sterilized by placing it in a special container in an electric oven with continuous stirring for 10 minutes at 80°C, then allowing it to cool to eliminate any diseases.
- 3- Lavender was prepared by adding it to 1 kg of sterilized pollen and 2 kg of boiling water. 5 cups of powdered sugar in a pot, cover it, and heat it until it evaporates to preserve the volatile oils. Then let it cool, add 75% cornstarch and 75% soybean meal.
- 4- Prepare 1 kg of sterilized pollen, then add a sugar solution of 5 cups of powdered sugar to 2 cups of water. Add chia seeds, then 75% cornstarch and 75% soybean meal to a container and seal it.
- 5- Prepare 1 kg of sterilized pollen, then add a sugar solution of 2/5, then 75% soybean meal and 75% cornstarch to a container and seal it.
- 6- Prepare a concentrated sugar solution (2 parts sugar to 1 part water). This is used during the autumn and winter so that bees can transport it to the frames with minimal effort, thus avoiding the need to evaporate a large amount of water before storage (excessive humidity inside the hives causes many problems and diseases). After dissolving the powdered sugar, add it to hot water and stir until dissolved. Place it in a container and add lemon juice to prevent crystallization. Then let the solution

cool and add the remaining ingredients are mixed well to form a dough, then it is kept in the refrigerator for 24 hours. After preparation, it is left to homogenize and for the materials to fully dissolve for one day. Then 250 grams of the mixture are weighed and wrapped in food-grade plastic bags (vacuumed air) and stored for use according to the feeding meal and to monitor the health of the colony as a whole. It is presented to the bees after being placed on parchment paper on top of the wax frames.

Prepare the Colonies

Nine (9) hives were selected from hybrid bee colonies *Apis mellifera* (the local Iraqi strain). Measurements were taken for each colony. Queens were of a single strain. Modern Lancstroth hives were selected, identical in construction, wood type, shape, and frame type, all of the same shape and dimensions. The hives were free from diseases and pests to minimize or eliminate any negative factors or variations affecting their activity and vitality inside or outside the hive. Brood area, pollen, and honey were measured. Frames were added to the colonies as needed. All hives were randomly distributed and then numbered to indicate the treatment number.

Disease Infection with the Pathogen *V. Ceranaea* For honeybee colonies:

After preparing the honeybee colonies, it induces artificial infection by preparing a sugar solution (1 part sugar to 1 part water). Then, we add 10 ml of spore solution (approximately 10 million spores) to the sugar solution to infect the colonies with the pathogen. We use 250 ml of this solution for each colony. Three days after infecting the colonies, we take samples from worker bees in each colony and examine them to confirm infection.

Studying the Effect of Different Treatments on Bee Activity Internally and Externally:

Calculating Brood Area

To demonstrate the effect of different treatments on brood area, the brood area (workers and drones) for each hive was calculated in square inches (Al-Naji, 1980) using a transparent glass frame divided into squares (each square measuring 2 inches). The brood area was calculated based on the method of Jeffrey (1958). This involved placing a brood frame and measuring its area on each side of the glass frame. The process was carried out at the beginning of the experiment before implementing the treatments and before each feeding period to determine the differences between them.

Calculating Foraging Activity

To demonstrate the effect of field treatments on foraging activity, the number of foraging workers will be counted six days after the last treatment in the morning. The number of workers entering the hive will be counted for one hour, with one reading per hive at 9:00-10:00 AM, using high-resolution digital cameras installed near the hive entrance.

Studying Overall Growth of Treated Hives

To study the effect of the treatments on overall hive growth, the study was conducted in two parts as follows:

Calculating the Average Number of Frames Added to the Hives

The number of frames added in the hives was calculated. Each colony was weighed based on the original number of frames in each hive at the beginning of the experiment (for each hive and in all treatments). The number of wax frames added was calculated (according to the hive's needs). By calculating the total number of frames at the end of the experiment for each colony, the resulting growth in each hive was determined and compared with the number of frames in the control colonies. The rate at which the bees took up the food substitutes was calculated in days.

Weight of Hives

To demonstrate the effect of different treatments on the overall growth of the hives, the wooden hives (without bees) were weighed first. At the end of the experiment, the wooden hives with all their components, including bees, brood, honey, and pollen, were weighed to determine the weight gain in the treated hives and compare it with the control hives, which were weighed using the same method and at the same time.

3. Results and Discussion

The results of the statistical analyses using Fisher's Least Significant Difference (LSD) test at a significance level of (0.05) showed no statistically significant differences between the three experimental treatments (1, 2, and 3) regarding the number of bees in the first and last examinations, as shown below in Figure 1. The mean values for treatments 1, 2, and 3 were (736.66, 520.66, and 1193.33), respectively. However, treatments 1 and 3 were observed to be superior to treatment 2, with no significant differences at a significance level of (0.05) between treatments 1 and 2. The mean values for the number of bees were 1425 for treatment 1, 830 for treatment 2, and 2145 for treatment 3. The reason may be due to (increased feeding by the house bees, thus providing a larger quantity of royal jelly to the queen and a larger quantity of eggs).

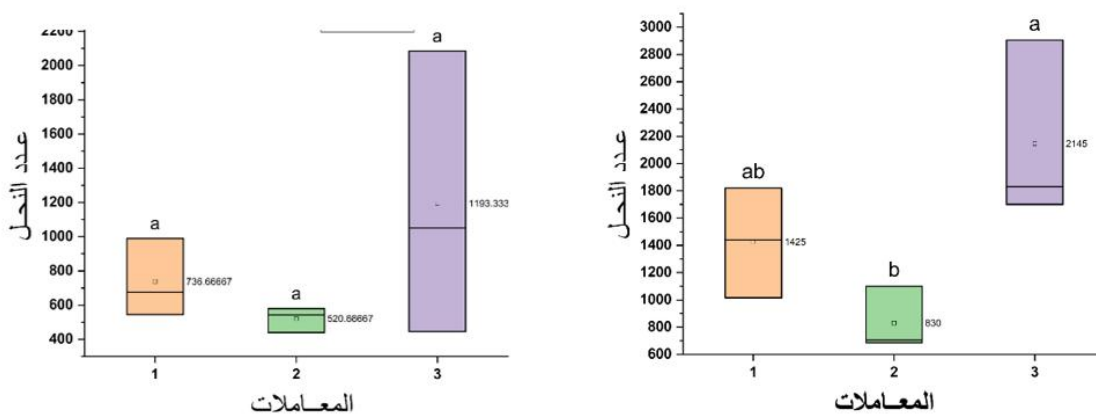


Figure (1) average values of the number of bees for the experimental treatments

Statistical results showed no significant differences between the three treatments at a significance level of 0.05. Treatments 1 and 3 recorded the highest mean values (300 and 221.66, respectively), while treatment 2 recorded the lowest mean value (128.33) for the first test. The results of the second

test differed, indicating significant differences between the experimental treatments at a significance level of 0.05. Treatment 3 significantly outperformed the other treatments, achieving the highest mean value for brood area (968.33), compared to treatment 1, which recorded the lowest mean value (513.33). Treatment 2 (853) did not show any significant differences compared to treatments 1 and 3. This is because the brood originates from eggs, as shown in the previous table, which hatch into brood. The availability of good nutrition, both in terms of quantity and quality, leads to an increase in brood area (Figure 2).

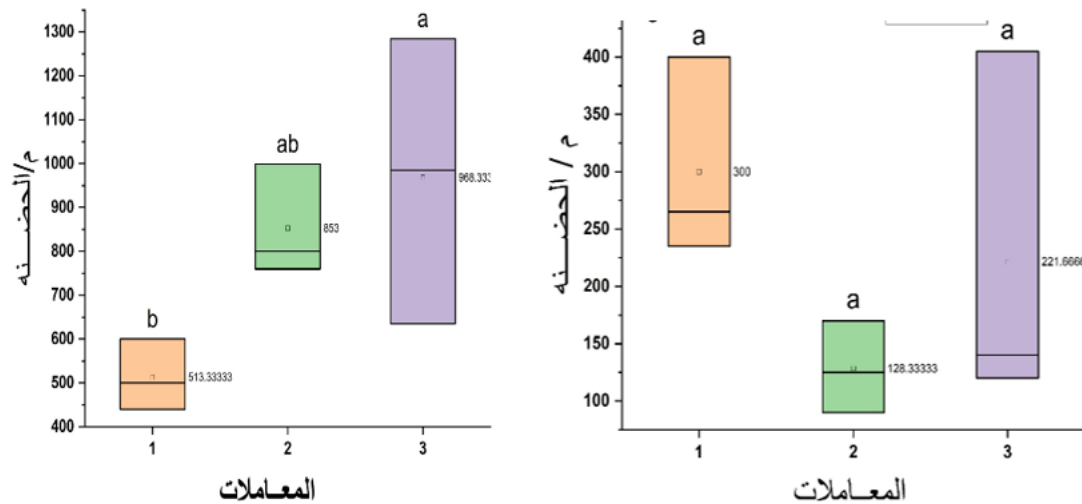


Figure (2) average values of the brood area for the experimental treatments.

The results of the first test showed no significant differences between the three treatments at a significance level of (0.05) in pollen grain area. The mean values for the first, second, and third treatments were (99, 74, and 100), respectively, as shown in Figure 3 below. However, the results of the second test showed a significant advantage at a significance level of (0.05) for both the third treatment (mean 441) and the second treatment (mean 286), indicating a significant effect with no significant differences between them. Meanwhile, the first treatment declined significantly, recording the lowest mean (102.33), a clear and significant difference from the other treatments.

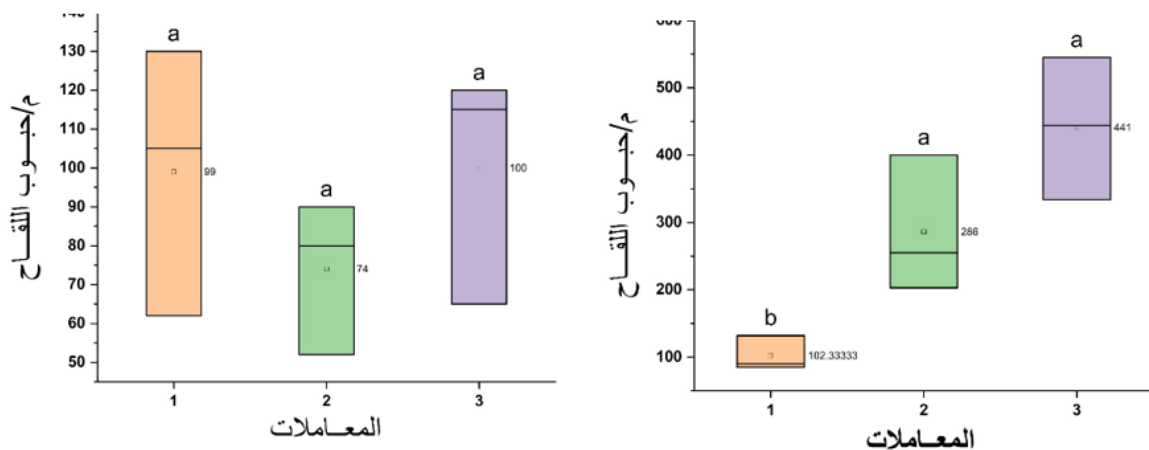


Figure (3) average values of the pollen grain area trait for the experimental treatments

Figure (4) shows the measurement of the quantity of honey. The mean values for the first treatment were 941.66, for the second treatment 360, and for the third treatment 126.66 in the first test. Significant differences were found between the experimental treatments at a significance level of (0.05). The third treatment outperformed the other treatments, with no significant differences between the first and second treatments. The results of the second test were identical, with the third treatment outperforming the first and second treatments, again with no significant differences between the two treatments at a significance level of (0.05). The mean values for the first, second, and third treatments were 244.33, 360, and 740.66, respectively. The concept may be reversed due to the honey being used to feed the brood and queen as a result of its use in making royal jelly.

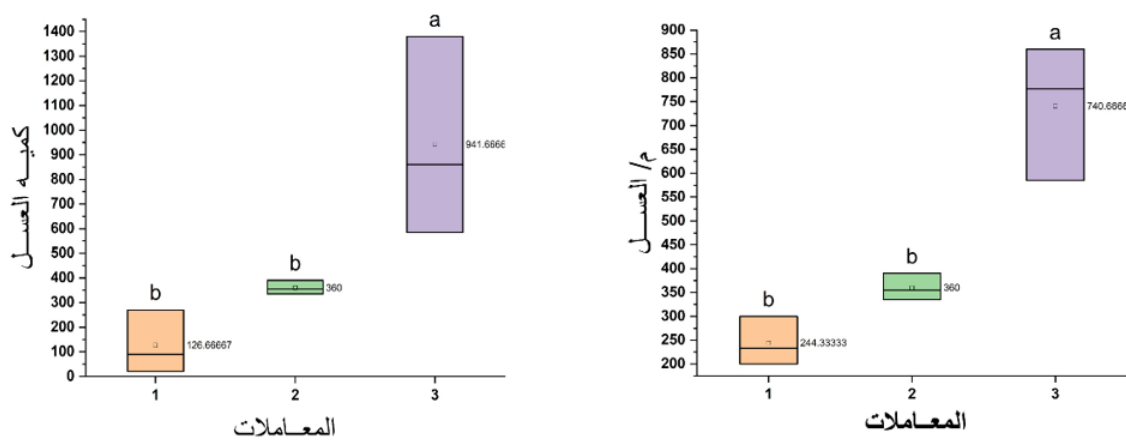


Figure (4) average values of the quantity of honey for the experimental treatments

The results indicate no significant differences between the three treatments at a significance level of 0.05 in the egg area trait for the first test. The mean values were 188.33, 128.33, and 141.66 for the first, second, and third treatments, respectively, in the same order as before. The results differed significantly for the second test. A significant advantage was found at a significance level of 0.05 for both the third treatment (339) and the first treatment (336.66), with no significant difference between them. The second treatment, however, showed a marked decrease, recording the lowest mean (178.33), a clear and significant difference from the other treatments. After increasing the queen's activity following the administration of royal jelly, she lays a large number of eggs, thus increasing the egg area (Figure 5).

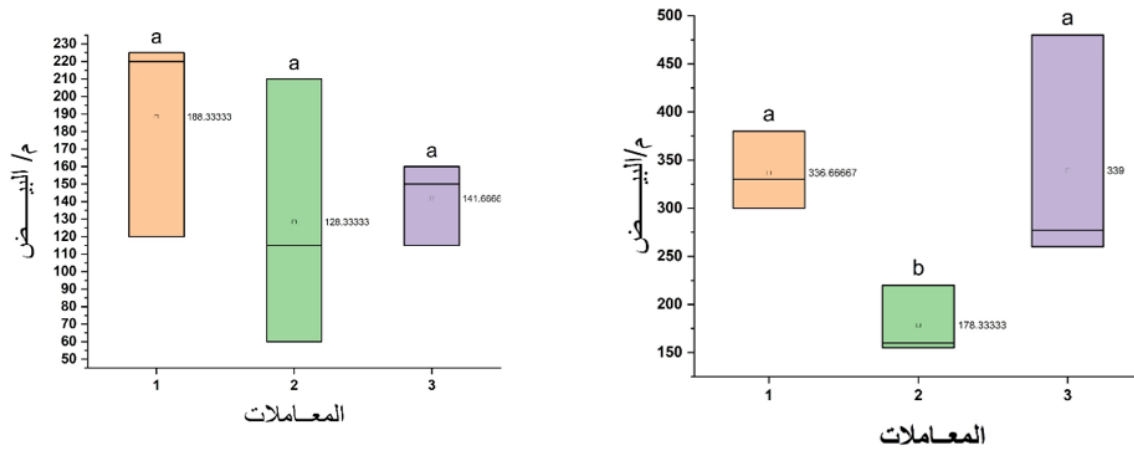


Figure (5) average values of the egg area for the experimental treatments

The results of the statistical analysis for the first test showed significant differences between the treatments regarding tire weight. Treatment 3 showed a significant advantage at the 0.05 level, recording the highest average weight (14133.33) compared to Treatment 1, which recorded the lowest average weight (4500). Treatment 2 had an average weight of (7816.66), but no significant differences were found between Treatments 1 and 3, as illustrated in Figure 6. The results of the second test showed a clear difference in tire weight, indicating a significant advantage at the 0.05 level for Treatment 3, which recorded the highest average weight (17567.66). Treatment 1 (average 6016.66) and Treatment 2 (average 5883.33) both recorded average weights, but no significant differences were found between them.

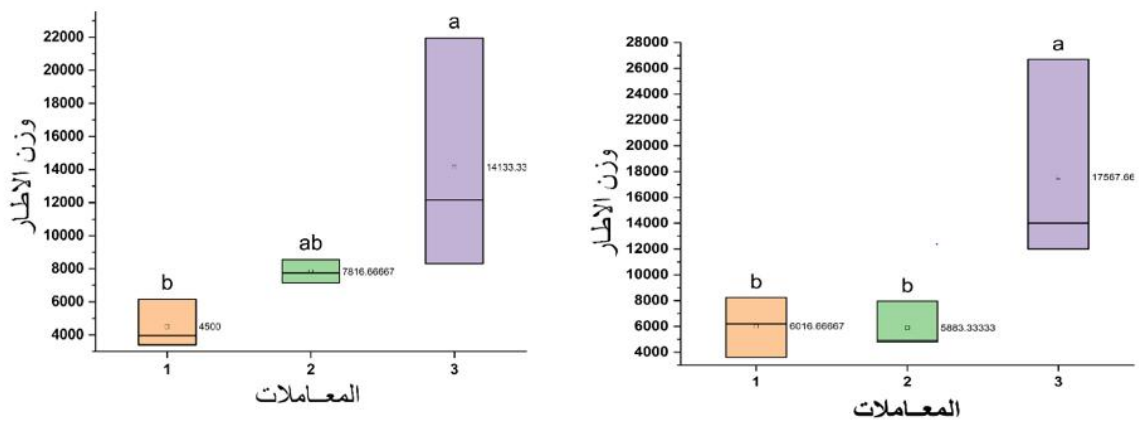


Figure (6) average values of the frame weight for the experimental treatment

Recent studies have also confirmed the above results regarding the reduced ability of lavender to inhibit the pathogen. When aqueous alcoholic extracts were prepared at doses of 2% thyme (2 g/100 ml) and 2% lavender (2 g/100 ml), and used in three replicate treatment groups, twenty adult bees were collected after 24, 36, 48, and 72 hours. The reduction in spore count was determined, and the thyme extract showed higher antispore activity against *V. ceranae* than lavender. It also inhibited *Nosema* spore growth after 72 hours of treatment. The study concluded that plant extracts may be effective alternatives for controlling *V. ceranae* in bee colonies (Keikhosrow et al., 2024). The study

concluded that plant extracts may be effective alternatives in controlling *V. destructor* and *V. ceranae* in bee colonies. Among the most beneficial extracts from medicinal plants were identified as Indian lilac (*Melia azadirachta*) with a minimum inhibitory concentration (MIC) ranging from 10 to 800 µg/ml, Ceylon cinnamon with an MIC ranging from 25 to 100 µg/ml, and lemongrass with an MIC ranging from 50 to 100 µg/ml (Ács et al., 2018). Otherwise, Lavender essential oil (*L. angustifolia*) rich in ethanol, 2-(2-ethoxyethoxy)- (13.05%), linalool (10.71%), alpha-terpenyl acetate (10.93%), and linalool acetate (9.60%) showed antibiotic efficacy in combating American foulbrood.

4. Conclusions

The study concludes that the area of the pollen tubes showed that the first test revealed no significant differences between the treatments, with the mean values for the first, second, and third treatments being 174 and 99, respectively. However, the results of the second test showed no significant advantage for any of the treatments. The first treatment showed a significant decrease, significantly lower than the other treatments. First test showed that the mean value for the first treatment was 941.66, the second treatment 360, and the third treatment 126.66. Significant differences were observed, with the third treatment outperforming the first and second treatments.

References

- Ács, K., Balázs, V. L., Kocsis, B., Bencsik, T., Böszörményi, A., & Horváth, G. (2018). Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. *BMC Complementary and Alternative Medicine*, 18 (1), 227. <https://doi.org/10.1186/s12906-018-2291-9>.
- Al-Waili, N., Salom, K., Al-Ghamdi, A., & Ansari, M. J. (2012). Antibiotic, Pesticide, and Microbial Contaminants of Honey: Human Health Hazards. *The Scientific World Journal*, 2012, 1–9. <https://doi.org/10.1100/2012/930849>.
- Cristina, R. T.; Kovacevic, Z.; Dumitrescu, E.; Imre, K.; Mederle, N.; Hadaruga, N.; Cincovic, M.; Muselin, F.; Militaru, D.; Radulov, I. and Puvaca, N. 2020. composition of a natural phytotherapeutic blend against noseosis in honey bees. *sustainability*, 12(14): 58-68. [doi.10.3390/su12145868](https://doi.org/10.3390/su12145868).
- Garrido, P. M.; Porrini, M. P.; Alberoni, D.; Baffoni, L.; Scott, D.; Mifsud, D.; Eguaras, M. J. and Di Gioia, D. 2024. Beneficial bacteria and plant extracts promote honey bee health and reduce *Nosema ceranae* infection. *Probiotics and Antimicrobial Proteins*, 16(1): 259-274. [doi: 10.1007/s12602-022-10025-7](https://doi.org/10.1007/s12602-022-10025-7).
- Gilbert, J.; Paris, L.; Dubuffet, A.; Texier, C.; Delbac, F. and Diogon, M. 2024. *Nosema ceranae* infection reduces the fat body lipid reserves in the honeybee *Apis mellifera*. *Journal of Invertebrate Pathology*, 207:1-11 [doi: 10.1016/j.jip.2024.108218](https://doi.org/10.1016/j.jip.2024.108218).
- Ho, S. T.; Nai, Y. S.; Chang, Z. T.; Chang, J. C.; Hsu, W. C.; Ko, C. Y.; Chen, Y. W.; and Yang, Y. L. 2024. Dimethyl sulfoxide, an alternative for control of *Nosema ceranae* infection in honey bees (*Apis mellifera*). *Archives of Insect Biochemistry and Physiology*, 116(4): e22099.

doi: 10.1002/arch.22099<https://doi.org/10.4324/9780429056765>.
<https://doi.org/10.1016/j.plaphy.2015.12.004>
<https://doi.org/10.3117/plantroot.5.25>.

- Keikhosrow Sarhangzadeh, Mohammad Yakhchali, Mostafa Moradi et al.(2024). The effects of plants extracts of thyme (*Thymus vulgaris*) and lavender (*Lavandula angustifolia*) on *Varroa destructor* (Acarina: Varroidae) and *Nosema ceranae* (Dissociodihaplophasida: Nosematidae) in adult worker honey bees (*Apis mellifera*), 31 May 2024, PREPRINT (Version 1) available at Research Square [<https://doi.org/10.21203/rs.3.rs-4407998/v1>].
- Pasca, C.; Marghitas, L . A.; Bobis, O.; Buzura –Matei, I. A. and Dezmirean, D. S. 2019. a review of *Nosema cerane* and *nosema apis*: caracterization and impact for beekeeping. Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 76(2).77-87. doi:10.15835/buasvmcn-asb: 0018.19.
- Potts, S.; 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. and Vanbergen, A. J. 2016. Safeguarding pollinators and their values to human well-being. *Nature*, 540: 220–229. doi.org/10.1038/nature20588.