

EFFECT OF FEEDING WITH NATURAL PRODUCT IMUNOSTART HERB ON LYSOZYME CONTENT AND BIOLOGICAL DEVELOPMENT OF THE BEE COLONIES

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Abstract

The aim of this study was to determine the lysozyme content in the haemolymph of worker bees and biological development of the bee colonies after supplementation with the herbal product IMMUNOSTART HERB. The experiment included local honey bees *Apis mellifera* L., settled in the Langstroth-Rut hives. The study was conducted at the experimental apiary of the Research Centre of Stockbreeding and Agriculture – Smolyan in the active beekeeping season of 2020. The experiment involved one control (10 bee colonies) and one experimental group (10 bee colonies). The experimental group was fed with IMMUNOSTART HERB at a dose of 10 ml in 100 ml sugar solution (sugar/water 1:1) 4 times at intervals of 7 days. The control group received only sugar solution without additives. Supplemental feeding with IMMUNOSTART HERB increases the lysozyme content in the experimental group bee colonies two months after the application of the product. Consumption of this product significantly enhances strength of the bee colonies, sealed worker brood, honey and pollen. IMMUNOSTART HERB has positive effects on bee colonies' development and it is beneficial to honey bee survival.

Keywords: honeybees, lysozyme content, IMUNOSTART HERB, bee colony

Introduction

Over the past few decades, significant bee colony losses have been observed worldwide (Decourtaye et al., 2019; Osterman et al., 2021; Gray et al., 2022). Numerous factors and stressors including environmental contaminants, climatic changes and poor nutrition negatively affect the biological development and bee health. Honeybees collect pollen and nectar from flowers. These products provide bees with carbohydrates, proteins, lipids, essential mineral elements, vitamins and other nutrients (Dolezal and Toth, 2018; Dolezal et al., 2019; Negri et al., 2019; Di Noi et al., 2021). In many cases honey bees are affected by the combined effects of multiple stressors (Goulson, et al., 2015). The efficiency of beekeeping depends on the richness of flora around the apiary. The abundance or availability of honey plants in a particular region is stable for a long period of time but it can vary from year to year and from some climate changes. The scarcity of honey plants and insufficient food supplies within the bee colonies affect brood rearing, honey production and development of the bee colonies (Paray et al., 2021). The honey bees must be provided with early blooming plants for fast spring development of the brood and the bee colony as a whole (Stanimirović et al., 2019). Additionally, in the absence of sufficient, naturally occurring nectar and pollen, commercially available sugar and protein supplements can be provided to the honey bees. Sometimes beekeepers perceive pollen to be limited or of low quality

in the environment (Mortensen et al., 2019). On the other hand, some herbal supplements can be used for different purposes to honey bees. From ancient times, herbs and medicinal plants have been used feeding bee colonies (Patruica, 2012). They have biologically active substances that may control honey bee disease and stimulate honey bee immunity. Plant extracts were used as an effective method for controlling varroa mite (El-Bolak and Mahfouz, 2021). Since honey bee immunity depends largely on the protein and lysozyme content of the bee haemolymph, we decided to investigate lysozyme content as suitable markers of the activity of the humoral part of the immune system after administration of the herbal extracts. Lysozyme is a part of the innate immune system of the bees (Zaobidna et al., 2017). Besides, very little is known about the relationship between supplementary feeding and immunity response of the honey bees. Therefore, the aim of this study was to determine the lysozyme content in the haemolymph of worker bees and biological development of the bee colonies after supplementation with the herbal product IMMUNOSTART HERB.

Material and methods

The experiment included local honey bees *Apis mellifera* L., settled in the Langstroth-Rut hives. The study was conducted at the experimental apiary of the Research Centre of Stockbreeding and Agriculture – Smolyan (41°3507.01” N 24°41030.98” E) in the active beekeeping season of 2020. The experiment involved one control and one experimental group. Each group contained 10 bee colonies. All bee colonies were equal in regards to the amount of bees (strength), brood and food supplies. The experimental group was fed with IMMUNOSTART HERB at a dose of 10 ml in 100 ml sugar solution (sugar/water 1:1) 4 times at intervals of 7 days. The control group received only sugar solution (sugar/water 1:1) without additives. IMMUNOSTART HERB is a Bulgarian natural product produced by Extract Pharma Ltd. It contains herbal extracts. All bee colonies received a total of 5 L sugar solution during the experimental period, at intervals of 2 – 3 days.

The parameters strength of the honey strength of the bee colonies estimated based on mass (kg), sealed worker brood area (cm²), stored capped honey (kg), stored pollen area (cm²) characterizing the biological development of the bee colonies were determined by the method described by Shumkova and Balkanska (2021).

Bee hemolymph were collected from young non-flying worker bees by suction with a pasteur pipette at the border between the 2nd and 3rd abdominal tergites. The lysozyme levels in the haemolymph were determined by a Lysozyme Detection Kit (Cat. No, LY0100, Sigma-Aldrich), according to the manufacturer's instructions. The hemolymph samples were stored at -20°C until the analysis.

The data was expressed as mean ± standard deviation. Significant differences were considered at $P < 0.05$ by Student's t-test.

Results and Discussion

The haemolymph of worker bees were taken in June and in September 2020 and the results are shown in Figure 1.

Haemolymph from worker bees was obtained in June but no significant difference in lysozyme concentration between experimental and control groups were found. Our study determined that the concentration of lysozyme in the hemolymph of worker bees that consumed IMMUNOSTART HERB was significantly higher ($p < 0.05$) in the haemolymph samples in September, compared to those that were provided with a sugar-only diet in the control group (Fig. 1). Our data suggest that the increase in lysozyme activity can be viewed as a mechanism induced in the presence of

adequate nutrition with IMMUNOSTART HERB. The results of Gorzin et al. (2017) showed that *Echinacea purpurea* herbal extract had a positive effect on the immune system of bees.

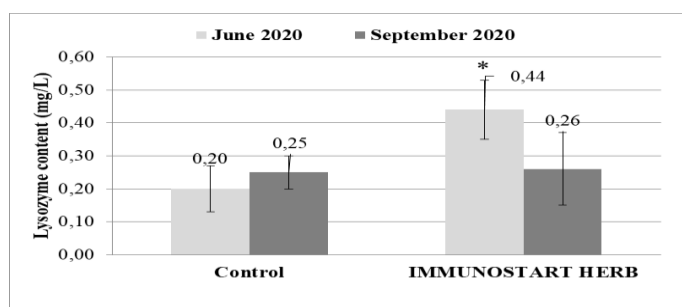


Figure 1. Average ($\pm$ SD) values of lysozyme content (mg/L) in haemolymph of worker bees

We determined the change in strength of the bee colonies throughout the feeding period from the beginning of April to the beginning of June. On average, colonies in each of the two groups experienced an increase in adult bee mass during the feeding period, which is typical of colonies during spring. Nevertheless, bee colonies that received IMMUNOSTART HERB significantly increased the strength of the bee colonies than the control colonies. The results are shown in Fig. 2. Charistos et al. (2015) showed that using a mixture of algae extracts and *Cymbopogon spp.*, increased colony worker bee population size by 89%.

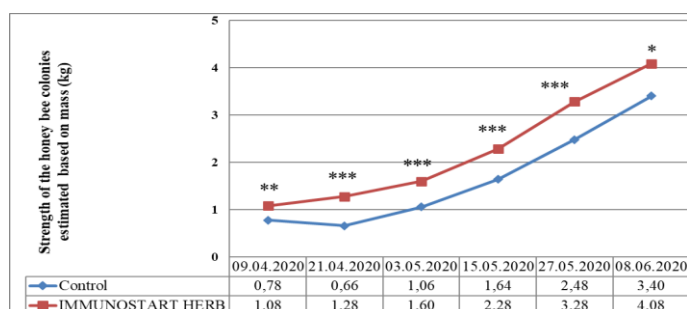


Figure 2. Average values of the strength of the bee colonies (kg)

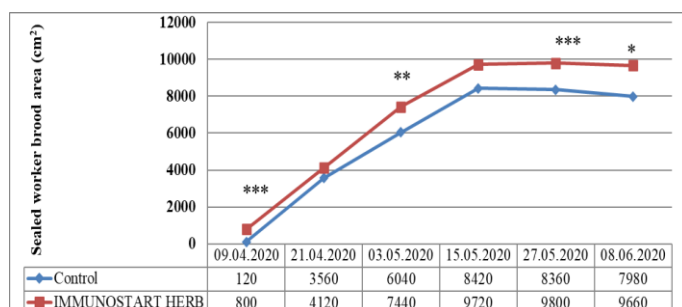


Figure 3. Average values of sealed worker brood area (cm²)

The bee colonies which received IMMUNOSTART HERB had significantly higher sealed worker brood compared to the control colonies (Fig. 3). Similar results are received by Eshbah et al. (2018). The researchers examined the impact of natural substances, including anise (*Pimpinella anisum*), coriander (*Coriandrum sativum*) and nuckthorn (*Zizphus spina Christi*), when added to sugar syrup. The addition of these substances improved both brood rearing and colony growth

(Eshbah et al., 2018). Furthermore, Pătruică et al. (2017) conclude that the use of the plant extracts as supplementary feeds for honeybees had a positive effect on egg-laying activity of the bee queen and respectively worker brood area.

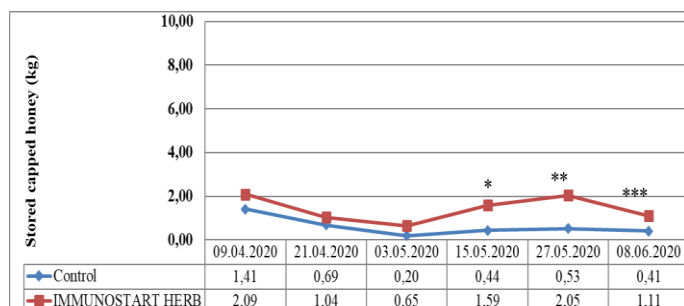


Figure 4. Average values of stored capped honey (kg)

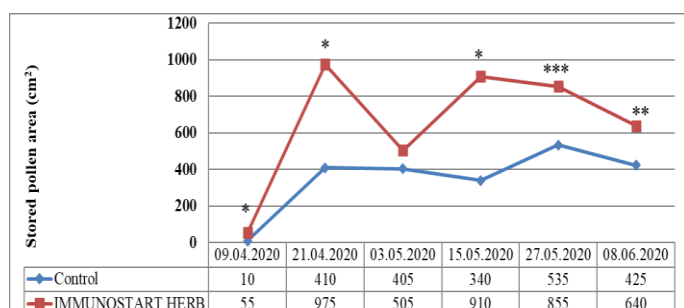


Figure 5. Average values of stored pollen area (cm²)

In recent years, interest in herbal extracts for the treatment of bee diseases has increased because the contents are natural and do not leave a residue in bee products (Bravo et al., 2017; Özkırım and Küçüközmen, 2021). The results for honey and bee pollen collection after feeding with IMMUNOSTART HERB are presented in Fig. 4 and 5. The last three measurements of the stored capped honey showed significant differences in the experimental group compared to control group bee colonies (Fig. 4).

Conclusion

Supplemental feeding with IMMUNOSTART HERB increases the lysozyme content in the experimental group bee colonies two months after the application of the product. Consumption of this product significantly enhances strength of the bee colonies, sealed worker brood, honey and pollen. IMMUNOSTART HERB has positive effects on bee colonies' development and it is beneficial to honey bee survival.

References

- Bravo, J., V. Carbonell, B. Sepúlveda, C. Delporte, C. E. Valdovinos, R. Martín-Hernández, M. Higes (2017): Antifungal activity of the essential oil obtained from *Cryptocarya alba* against infection in honey bees by *Nosema ceranae*, Journal of Invertebrate Pathology, 49: 141-147.
- Charistos, L., N. Parashos, F. Hatjina (2015): Long term effects of a food supplement HiveAlive™ on honey bee colony strength and *Nosema ceranae* spore counts, Journal of Apicultural Research, 54: 420-426.

- Decourtye, A., C. Alaux, Y. Le Conte, M., Henry (2019): Toward the protection of bees and pollination under global change: present and future perspectives in a challenging applied science, *Current Opinion in Insect Science*, 35: 123-131.
- Di Noi, A., S. Casini, T., Campani, G. Cai, I. Caliani (2021): Review on sublethal effects of environmental contaminants in honey bees (*Apis mellifera*), knowledge gaps and future perspectives, *International Journal of Environmental Research and Public Health*, 18, 1863.
- Dolezal A. G., A. L. Toth, 2018. Feedbacks between nutrition and disease in honey bee health, *Current Opinion in Insect Science*, 26: 114-119
- Dolezal, A. G., J. Carrillo-Tripp, T. M. Judd, W. Allen Miller, B. C. Bonning, A. L. Toth (2019): Interacting stressors matter: diet quality and virus infection in honeybee health, *Royal Society Open Science*, 6:181803.
- El-Bolok, D. M. R., H. M. Mahfouz (2021): Efficacy of some plant extracts against *Varroa destructor* and their side effect on honeybee colonies, *Zagazig Journal of Agricultural Research*, 48(4): 1023-1032.
- Eshbah, H. M., A. A. Mohamed, A. R. Hassan, M. E. Mahmoud, M. M. Shaban (2018): Efficiency of feeding honey bee colonies, *Apis mellifera* L., with mixture of natural products and sugar syrup on brood and adult population, *Scientia Agriculturae*: 21(1): 14-18.
- Gorzin, Z., H. Moravej, G. A. N. Paghale, L. Tabrizi, A. Moradi (2017): Effect of *Echinacea purpurea* herbal extract on immune parameters in honey bee (*Apis mellifera*), *Iranian Journal of Animal Science*, 48(1): 101-107.
- Goulson, D., E. Nicholls, C. Botias, E. L. Rotheray (2015): Bee declines driven by combined stress from parasites, pesticides, and lack of flowers, *Science*, 347, 1255957.
- Gray, A., R. Brodschneider, N. Adjlane, A. Ballis, V. Brusbardis, J.-D. Charrière (2019): Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources, *Journal of Apicultural Research*, 58: 479-485.
- Mortensen, A. N., C. J. Jack, T. A. Bustamante, D. R. Schmehl, J. D. Ellis (2019): Effects of supplemental pollen feeding on honey bee (*Hymenoptera: Apidae*) colony strength and *Nosema* spp. infection, *Journal of Economic Entomology*, 112(1), 2019, 60-66.
- Negri, P., E. Villalobos, N. Szawarski, N. Damiani, L. Gende, M. Garrido, M. Maggi, S. Quintana, L. Lamattina, M. Eguaras (2019): Towards precision nutrition: a novel concept linking phytochemicals, immune response and honey bee health, *Insects*, 10(11), 401. doi: 10.3390/insects10110401
- Osterman, J., M. A. Aizen, J. C. Biesmeijer, J. Bosch, B. G. Howlett, D. W. Inouye, C. Jung, D. J. Martins, R. Medel, A. Pauw, C. L. Seymour, R. J. Paxton (2021): Global trends in the number and diversity of managed pollinator species, *Agriculture, Ecosystems & Environment*, 322, 107653, 1-13.
- Özkırım, A., B. Küçüközmen (2021): Application of herbal essential oil extract mixture for honey bees (*Apis mellifera* L.) against *nosema ceranae* and *Nosema apis*, *Journal of Apicultural Science*, 65(1): 163-175.
- Paray, B. A., I. Kumari, Y. A. Hajam, B. Sharma, R. Kumar, M. F. Albeshr, M. A. Farah, J. M. Khan (2021): Honeybee nutrition and pollen substitutes: A review, *Saudi Journal of Biological Sciences*, 28(1): 1167-1176.

Pătruică, S., D. Moț, D. Popovici (2017): The effect of using medicinal plant extracts upon the health of bee colonies, Romanian Biotechnological Letters, 1-4.

Pătruică, S (2012): Metode biotehnologice de hrănire a familiilor de albine, Ed. Eurobit, Timișoara.

Shumkova, R., R. Balkanska (2021): Influence of IMMUNOSTART HERB feeding on the bee colonies development, Bulgarian Journal of Agricultural Science, 27 (5): 896-902.

Stanimirović, Z., U. Glavinić, M. Ristanić, N. Aleksić, N. Jovanović, B. Vejnović, J. Stevanović (2019): Looking for the causes of and solutions to the issue of honey bee colony losses, Acta Veterinaria-Beograd, 69 (1): 1-31

Zaobidna, E. A., K. Żółtowska, E. Łopieńska-Biernat (2017): Expression and activity of lysozyme in *Apis mellifera carnica* brood infested with *Varroa destructor*, Journal of Apicultural Science, 61(2): 253-256.