| ORIGINAL SCIENTIFIC ARTICLE |

Effectiveness of the innovative remedy ApiBonum *ib* on honeybee colony vitality

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Abstract

Varroosis is a severe disease affecting honeybees and their brood, caused by the parasitic mite *Varroa destructor*. This disease poses the greatest threat to modern beekeeping and is considered a primary factor in the global decline of honeybee colonies. Due to its harmful effects, the beekeeping research sector is actively seeking new, efficient solutions for improved disease control. This study evaluates the acaricidal properties of the innovative remedy ApiBonum *ib* and its effects on honeybee colony vitality. Conducted under experimental field conditions, this study demonstrated a significant decline in parasitic mite populations across all treated honeybee colonies. Additionally, ApiBonum *ib* positively influenced colony strength. As a natural remedy, ApiBonum *ib* poses no harm to honeybees, reduces the risk of mite resistance, and minimises residue accumulation in hive products. Its potential as a honeybee colony hygiene enhancer and immunomodulator suggests that ApiBonum *ib* could be a valuable tool for sustainable beekeeping.

Key words: *honeybee colony; ApiBonum ib; good beekeeping practices; Varroa destructor*

Introduction

The honeybee (*Apis mellifera* L.) is a eusocial insect best known for its role in pollination and maintaining plant biodiversity (Papa et al., 2022). Due to its important role in pollination, the production of honey and other apian products, the honeybee has become a key animal in the agricultural economy, giving it a special place in people's lives (Vidal-Naquet et al., 2014). Given the critical role of honeybees in natural ecosystems, there are growing concerns about the increasing losses and weakening of honeybee colonies (Genersch, 2010; Asllat, 2025).

In recent decades, there has been a decline in bee species across regions and continents, largely due to exposure to multiple stressors (Gregorc et al., 2022; Majoroš et al., 2022). Research has shown that the combined effects of several adverse factors contribute to the weakening and subsequent decrease in honeybee colony numbers. These factors include pesticides, diseases, habitat loss and fragmentation, a reduction in the diversity and availability of flowering plants, and an increase in monoculture farming practices in agriculture (Goulson et al., 2015; Gregorc et al., 2022). As climate change intensifies, it will significantly impact both the population and biodiversity of bees in the future, similar

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to the effects already seen on other beneficial insects. Predicting the influence of various factors on honeybee populations is challenging because these factors do not operate in isolation; they interact with one another, and with the *Varroa destructor* obligate parasite (Traynor et al., 2020).

The global picture shows that the main causes of honeybee colony losses are strong invasions by the ectoparasitic mite *V. destructor* combined with other negative abiotic and biotic factors. *V. destructor* is a parasite that can infect various developmental stages of honeybees, making it the most dangerous pest of honeybee colonies worldwide (Hristov et al., 2020). In the mid-20th century, varroosis spread across Europe, presenting beekeeping with a major challenge (Le Conte et al., 2020; Eliash and Mikheyev, 2020). Since the damage caused by the *V. destructor* mite is crucial in weakening and reducing the number of honeybee colonies, it is essential to carry out regular infestation level estimations and antiparasitic treatments. The fight against varroosis has been based on three pillars in recent decades, and these have proven to be crucial for sustainable management: biotechnological and physical methods, chemical treatments, and the breeding of varroa-resistant honeybees (Traynor et al., 2020). There has been significant progress in the knowledge of these methods and their implementation following local and global conditions, possibilities, and national regulations (Bubnič et al., 2021). To avoid the ongoing and long-term use of synthetic acaricides, exploring alternative acaricides is advisable. The excessive use of synthetic acaricides can lead to the development of resistance in parasites, and it can also result in the accumulation of active ingredients and their metabolites in honeybee products, creating residues that pose a risk to consumers. Alternatives to synthetic acaricides include natural substances like essential oils and organic acids, which also have acaricidal properties. These alternatives have gained significant attention over the years because they are more environmentally friendly, effective at reducing mite populations, and safe for honeybees (Narciso et al., 2024).

ApiBonum *ib* is a product designed to improve the hygiene of honeybee colonies. It contains a proprietary blend of plant extracts and formic acid. According to the manufacturer's claims and instructions, ApiBonum *ib* helps bees combat parasites and harmful microorganisms by promoting enhanced hygienic behaviour. It is suitable for use year round, regardless of weather, temperature, or biological conditions, and its composition makes it suitable for organic beekeeping. It is available as long-acting insoles that are easy to apply. The recommended treatment duration is 30 days.

In this study, the acaricidal properties of the innovative remedy ApiBonum *ib* were investigated in honeybee colonies of different strengths. ApiBonum *ib* was applied directly on frames, and its anti-varroa effect was monitored by inspecting the plates on the hive bottom boards and counting the fallen mites over 42 days. Before the treatment, the strength of each honeybee colony was determined, and in parallel, a control group of honeybee colonies treated with the authorised and approved veterinary medicinal product Checkmite+ for use in beekeeping was monitored. This study aimed to assess the acaricidal efficacy of the innovative agent ApiBonum *ib* for maintaining hive hygiene and its impact on the vitality of honeybee colonies.

Materials and Methods

Location of the experimental apiary

The experiment was conducted at a stationary apiary in Jezerišće, northwestern Croatia (46°11'51.0"N, 15°48'17.8"E), near the Slovenian border. The apiary consists of 70 honeybee colonies in Langstroth-Root (LR) hives. The region's primary nectar sources are acacia (*Robinia pseudoacacia*) and chestnut (*Castanea sativa*). A total of 30 honeybee colonies naturally infested with *V. destructor* were selected and divided into three groups: experimental (A) – 10 honeybee colonies treated with ApiBonum *ib*, positive control (B) – 10 honeybee colonies treated with CheckMite+, and negative control (C) – 10 untreated honeybee colonies. Treatments were applied according to the manufacturer's guidelines, and mite infestation was monitored using sticky plates placed on the hive bottom.

Estimation of the strength of honeybee colonies

The strength of honeybee colonies was assessed using a visual method that relies on a formula that takes into account both the number of frames filled with adult bees and the percentage of comb surfaces covered with sealed brood (Delaplane et al., 2013; Guzman-Novoa et al., 2024). Honeybee colony strength was assessed through visual inspections at the beginning and end of the study, with an increase in worker honeybee numbers indicating improved vitality. The collected data were analysed to compare the acaricidal efficiency of ApiBonum *ib* with control treatments. Honeybee colonies of similar strengths were included in the experiment. The assessment of hive honeybee populations was conducted during two inspections: one before treatment began and another after its completion. Both evaluations of strength were conducted by the same person to ensure consistency. Colonies' strength was graded according to TOMLJANOVIĆ et al. (2012).

Application of ApiBonum *ib* / CheckMite+ in hives and efficacy monitoring

Before starting the treatment of honeybee colonies, samples of adult bees were taken from the experimental hives. The sample was taken directly into a 120 mL plastic cup, covered, and marked (Figure 1). Adult bee samples were stored in a freezer at a temperature of -20°C until the analysis in the laboratory. The second sample of adult bees was taken at the end of the experiment's first phase, i.e., on the 35th day after the beginning of treatments. Honeybee colonies (30) were divided into three groups: one experimental (A) and two controls (B and C). The innovative hygiene remedy, ApiBonum *ib,* was applied in each of ten experimental hives in group A. ApiBonum *ib* comes in the form of plastic inserts that were placed directly on the honeycomb frames (Figure 2). The two control groups each consisted of 10 honeybee colonies, where the first control group was the positive control (group B) with the application of the registered and approved medicine CheckMite+ (Figure 3). CheckMite+ contains the active compound coumaphos on a plastic strip that is placed in the brood chamber of the hives between two honeycomb frames. The second control group was the negative control (group C) that was not treated.

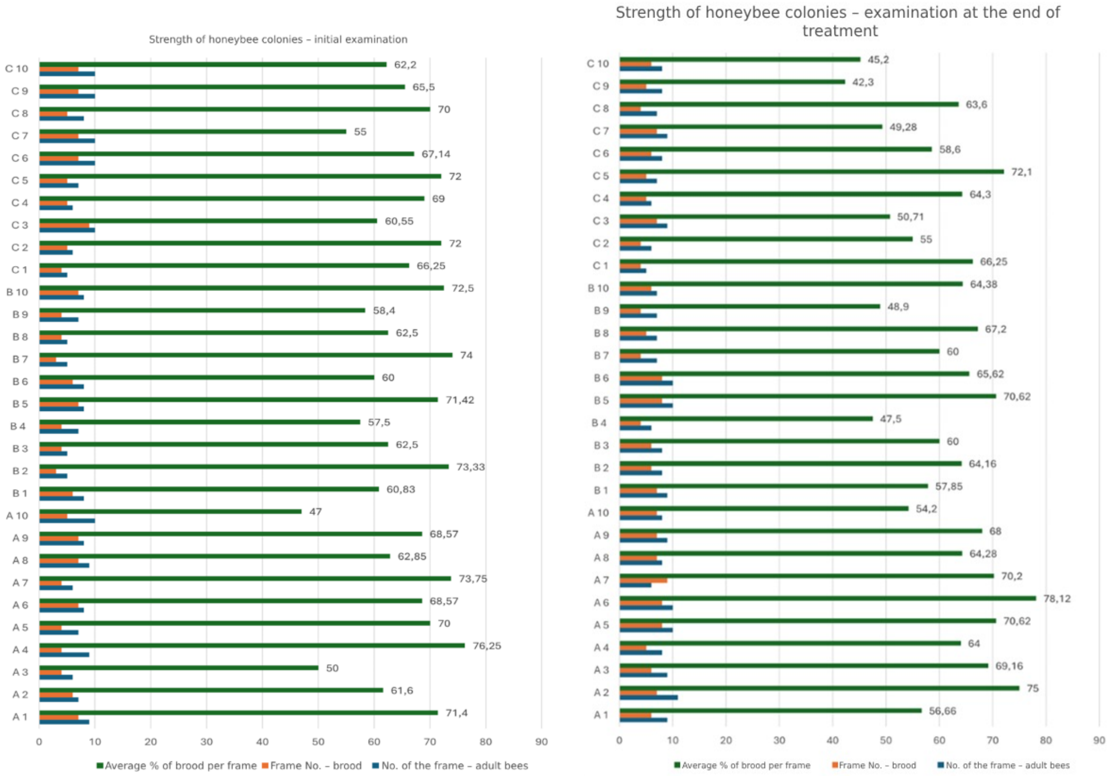
Figure 1. Samples of adult bees.

Figure 2. ApiBonum *ib* applied in the hive.

Figure 3. Application of CheckMite+ in the hive.

Figure 4. *V. destructor* mite detection.

Figure 5. Presentation of the estimated strength of honeybee colonies for experimental and control groups at the beginning (a) and end of the experiment (b) (experimental group A – ApiBonum *ib*; positive control group B – CheckMite+; negative control group C – control without treatment)



The fall of the *V. destructor* mite was observed (Figure 4) and recorded for 30 days, at which time all applied remedies or medicinal products were removed from the hives, and then the mite fall was further recorded for an additional 12 days, for a total of 42 days. After each inspection, we wiped the plates of the hive bottom board clean with a sponge and returned them to the hive.

Laboratory determination of mite infestation levels was performed according to the World Organisation for Animal Health manual (*Manual for Diagnostic Tests and Vaccines for Terrestrial Animals 2021,* Chapter 3.2.7) protocols that describe the quantification method and the morphological identification of adults and/or developmental forms of *V. destructor* (WOAH, 2021).

Treatment of honeybee colonies with oxalic acid

On the 35th day of the experiment, a shock treatment using oxalic acid (OA) was applied to all observed honeybee colonies. To prepare the treatment solution, 35 g of oxalic acid dihydrate was dissolved in 1000 mL sugar syrup. The treatment was administered by dripping approximately 5 mL of the prepared oxalic acid solution through a syringe into the space between frames populated with adult bees from medium-strength honeybee colonies. On the same day, we began monitoring the fall of *V. destructor* mites, and this monitoring continued for seven days following the oxalic acid treatment.

Statistical analysis

Statistical analysis was conducted using software tools. A non-parametric Wilcoxon signed-rank test was used to assess the differences between paired groups (e.g., the first treatment versus the OA treatment for each treatment group). Means, standard deviations, minimums, and maximums were computed for each group, and percentage changes in mite counts were calculated to determine treatment efficacy. Significance was denoted by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The assessment results on the strength of honeybee colonies and the evaluation of the efficiency of various experimental treatments against *V. destructor* mite populations were processed using Microsoft Excel and GraphPad Prism 8.

Figure 6. The number of fallen *V. destructor* mites after honeybee colony treatment (ApiBonum *ib*; CheckMite+; control with no treatment)

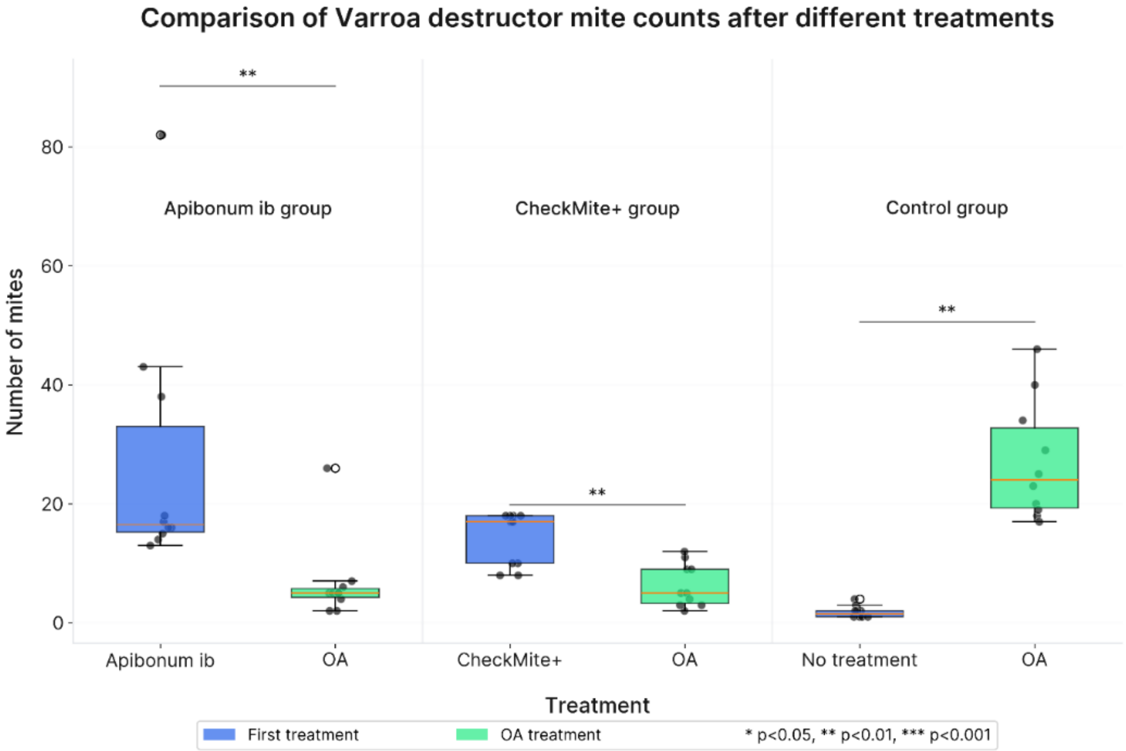
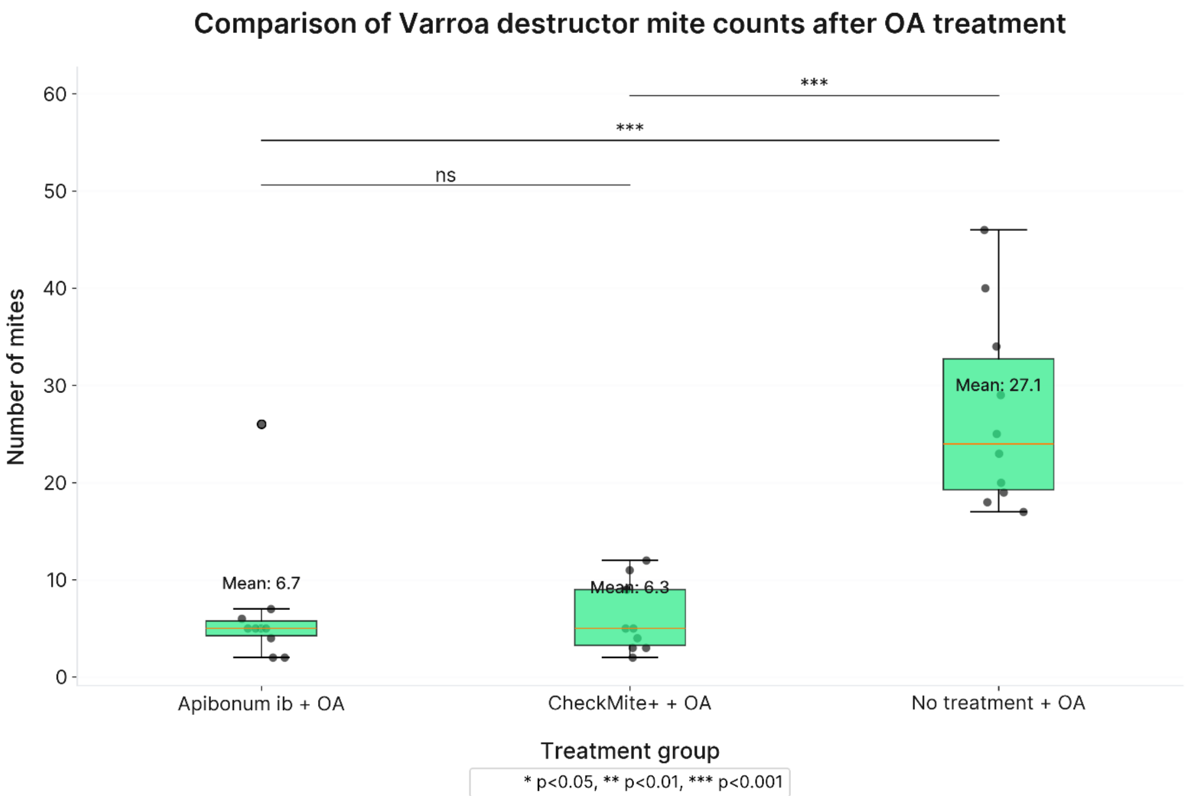


Figure 7. The number of *V. destructor* mites that fell after a single shock treatment with oxalic acid after treatments with ApiBonum ib; CheckMite+; no treatment



Results

Strength of honeybee colonies

The strength of honeybee colonies in both the experimental and control groups is presented individually for each colony included in the study (Figures 5a, 5b). A comparison between the two groups reveals that the experimental colonies (ApiBonum *ib* – group A) exhibited greater strength after treatment, as seen in the final clinical examination of the honeybee colonies compared to their strength in the initial examination.

Laboratory morphological identification

Laboratory quantitative diagnostic results for initial sampling showed a medium invasion level for all tested honeybee colonies, and according to morphological identification, all isolated mites were *V. destructor*. At the end of the experimental treatments but before OA shock treatment (on day 35 from the start of treatment), mites were isolated only in the control group without treatment.

Efficiency of V. destructor mite fall

Figure 6 illustrates the number of *V. destructor* mites to fall after treatments. During the treatment period, the highest number of fallen parasitic mites was observed in group A honeybee colonies where ApiBonum *ib* was applied, while the lowest number occurred in the negative control colonies (group C) that received no treatment.

The effect of oxalic acid treatment

The effectiveness of oxalic acid shock treatment on all experimental and control honeybee colonies is illustrated in Figure 7. Oxalic acid proved to be effective for all honeybee colonies in the study. The results indicate that the highest number of fallen *V. destructor* mites was observed in the control group, which consisted of honeybee colonies that had not received any prior treatment.

Discussion

The ectoparasitic mite *V. destructor* is considered to be the greatest threat to honeybee health (Rosenkranz et al., 2010; Gashout et al., 2020). Since the mite transitioned from its original host the Asian honeybee to the Western honeybee, *V. destructor* has become a globally widespread invasive species (Reams and Rangel, 2022). The rapid spread of the mite highlights the importance of protecting honeybees and implementing effective treatment strategies. The primary goal of veterinary medicine is to advise about control methods (Tlak Gajger et al., 2020, 2021) and address the prevalence of this parasitic mite, as honeybees are a vital species of pollinating insects essential for global food production.

Currently, physical, biological, and chemical methods are employed to combat this parasitic mite; however, none of these approaches ensures complete control of the disease. While chemical methods play a significant role in managing varroosis, the extensive use of chemical acaricides presents at least two major challenges for the beekeeping sector. Firstly, there is a risk of mites developing resistance due to the repeated use of the same chemical acaricides. Secondly, there is a concern regarding chemical residues or their degradation products persisting in honeybee products (Ayan et al., 2019). Resistance is a developed trait that enables mites to tolerate synthetic acaricides, leading to increased survival rates even after effective treatment. As a result, the emergence of resistance to these chemicals poses a direct threat to bee health and the survival of honeybee colonies (Coles and Dryden, 2014; Morfin et al., 2023). Resistance to amitraz was first reported in 1991, followed by flumethrin in 1995, and kumafos in 2001. Since then, this resistance has become globally widespread (Mitton et al., 2022; Bertola and Mutinelli, 2025). Data from the USA and France confirm the concerning trend of developing resistance, showing that the resistance to amitraz among populations of *V. destructor* mites is increasing across apiaries in these countries (Marsky et al., 2024).

Due to the issues associated with synthetic acaricides, there has been a growing emphasis on alternative methods for controlling varroosis. In recent years, the use of natural substances has gained popularity, particularly organic acids like formic and oxalic acids, as well as essential oils and their components, particularly thymol. The main advantages of using natural compounds in beekeeping include their effectiveness against mites and the low risk of residue accumulation in bee products (Gracia et al., 2017). Recent scientific studies have demonstrated the efficacy of various natural substances as acaricides against *Varroa destructor*, a parasitic mite affecting honeybee colonies. A 2021 study evaluated formic acid, oxalic acid, and thymol, finding that oxalic acid at concentrations of 3.2% and 4.2% achieved mite mortality rates of 94.84% and 92.68%, respectively, under broodless conditions (Qadir et al., 2021). Similarly, a 2020 study reported that thymol, applied as dust or in a glycerin solution, resulted in mite control efficacies of 96.6% and 92.4%, respectively (Sabahi et al., 2020). In 2022, carvone and citral were identified as promising compounds, exhibiting significant toxicity to mites while maintaining low toxicity to bees (Sabahi et al., 2022). A 2023 systematic review indicated no observed resistance development in *V. destructor* to oxalic acid over 30 years (Kosch et al., 2024). Furthermore, a 2024 meta-analysis highlighted thymol's higher estimated efficacy compared to oxalic and formic acids, though results varied based on application methods and dosages (Ceccotti et al., 2024). These findings underscore the potential of natural compounds in integrated pest management strategies for controlling *V. destructor* infestations. Successful summer brood interruption for controlling *V. destructor* in the Mediterranean region was demonstrated (Kovačić et al., 2023) as a biotechnical control measure.

This research focused on the innovative remedy ApiBonum *ib*, an auxiliary solution for maintaining hive hygiene and combating the *V. destructor* mite. The study was conducted under field conditions, where the reduction of mites on flooring plates was observed. ApiBonum *ib* contains a proprietary formulation of plant extracts and formic acid, allowing it to function as a natural acaricide for extended periods. In honeybee colonies treated with ApiBonum *ib*, there was a significant decrease in mite populations compared to those treated solely with an authorised medicinal product and when compared to the control group of untreated honeybee colonies ($P<0.01$). Comparing the results after OA treatment, significant differences were found between the experimental groups and the control group ($P<0.001$), where Apibonum *ib* and Check Mite+ gave similar results, confirming their efficacy in the first part of the field test.

Treatment with ApiBonum *ib* may positively influence the strength of honeybee colonies. In our observations, five of ten honeybee colonies were assessed to be stronger in the final clinical examination compared to the initial examination. Therefore, we believe that ApiBonum *ib* can serve as a natural stimulant to enhance hygienic behaviour and help strengthen honeybee colonies. ApiBonum *ib* is a safe adjuvant for both honeybees and beekeepers. When applied, it reduces the risk of residues in honey and other honeybee products. A key advantage of remedies made from natural ingredients, such as ApiBonum *ib*, is their ability to prevent the development of resistance in parasitic mites. This is a significant challenge in modern veterinary medicine and the beekeeping sector.

Conclusions

ApiBonum *ib* can serve as a valuable aid in hive hygiene, helping to maintain the health and vitality of honeybee colonies. Its effectiveness is enhanced when combined with biotechnical methods for controlling varroosis. In experimental honeybee colonies treated with ApiBonum *ib*, a significantly higher number of *V. destructor* mites were observed to have fallen compared to control colonies. After the final shock treatment with oxalic acid, a lower mite count was recorded, suggesting a potential positive immunomodulatory effect. This may be attributed to an increase in worker bee hygiene behaviour. This study did not find any statistically significant differences in the strength of honeybee colonies between the control and experimental groups. However, during the final clinical examination of the honeybee colonies, most treated colonies with the ApiBonum *ib* remedy were stronger compared to their initial clinical examination.

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> Učinkovitost primjene inovativnog sredstva ApiBonum *ib* na vitalnost pčelinjih zajednica

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Varooza je bolest pčela i pčelinjeg legla koja predstavlja najveću prijetnju modernom pčelarstvu. Uzročnik varooze je nametnička grinja *V. destructor*. Zbog izrazito štetnog utjecaja na pčele, varooza se smatra glavnim uzrokom propadanja pčelinjih zajednica diljem svijeta. Zbog navedenog, istraživački pčelarski sektor je u stalnoj potrazi za rješenjem koje bi omogućilo što bolju kontrolu bolesti. Cilj je ovog rada bio utvrditi akaricidna svojstva inovativnog sredstva ApiBonum *ib*, kao i njegov utjecaj na vitalnost pčelinjih zajednica. Istraživanjem koje je provedeno u terenskim uvjetima, utvrđeno je opadanje grinja kod svih pčelinjih zajednica tretiranih sredstvom ApiBonum *ib*. Isto tako, utvrđeno je da ApiBonum ib ima pozitivan utjecaj na vitalnost, odnosno jačanje pčelinjih zajednica. Kao prirodno pomoćno sredstvo, ApiBonum *ib* nije štetan za pčele, pri uporabi manja je opasnost od razvoja rezistencije kod nametničkih grinja, kao i pojave rezidua u pčelinjim proizvodima. ApiBonum *ib* može biti korisno sredstvo za održavanje vitalnosti i higijene zajednica te kao imunomodulator koji potiče higijensko ponašanje pčelinjih zajednica.

Ključne riječi: *pčelinja zajednica, ApiBonum ib, dobre pčelarske prakse, Varroa destructor*