

## Efficacy of varroacidal food additive appliance during summer treatment of honeybee colonies (*Apis mellifera*)

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### ABSTRACT

The aim of this field study was to evaluate the varroacidal efficacy of the product Hive Clean<sup>®</sup> during summer treatment of honeybee colonies (*Apis mellifera*). Treatment efficacy was assessed by counting the number of *Varroa destructor* mites killed after multiple applications of Hive Clean<sup>®</sup>, according to the instructions provided by the producer. Hive Clean<sup>®</sup> was applied consecutively, with two varroacides with different active ingredients as shock treatment. The treatment with Hive Clean<sup>®</sup> resulted in a median mite drop of 91.51% (89.25-93.26%). Total mite mortality was always significantly higher than the natural mite fall observed during the pre-treatment period. According to the results obtained, Hive Clean<sup>®</sup>, as a food additive with acaricidal effect, could be considered an appropriate alternative for varroosis control and also suitable for use in combination with authorized veterinary medicine products, in organic and conventional apiaries under mild climate conditions.

**Key words:** *Apis mellifera*; *Varroa destructor*; Hive Clean<sup>®</sup>; varroacidal efficacy

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### Introduction

Pathogens, pests and other adverse environmental factors, whether individually or simultaneously, have been implicated in high annual losses of honeybee colonies (*Apis mellifera*) (GOULSON et al., 2015). Potentially, the major contributing factor in the dissipated health of honeybee colonies is the obligate ectoparasitic mite *Varroa destructor* (MARTIN et al., 2012; NAZZI et al., 2012; ZIEGELMANN et al., 2013). As the *V. destructor* mite feeds on the haemolymph of adults and developing stages of bees, while also facilitating the transmission of certain viruses (CHEN et al., 2004; DI PRISCO et al., 2011; RYABOV et al., 2014; NAZZI and

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LE CONTE, 2016), this seems to have a significant negative effect on the host immune response (YANG and COX-FOSTER, 2005). New research shows that this parasite damages host adult bees by consuming their fat body (RAMSEY et al., 2018). The *V. destructor* mite population in honeybee colonies requires regular control, as the high rate of parasitic mites and poor colony management leads to its collapse.

There are numerous active ingredients available incorporated into different formulations and authorized as veterinary medicinal products (VMP) for use in beekeeping, including a range of acaricidal substances, different methods of application, and techniques for combating *V. destructor* mites. A variety of synthetic acaricides have been widely used in recent decades with variable effects, often in response to poor beekeeping practices, such as: multiple consecutive and repeated use of the same product, improper time and manner of varroacidal product application, sub- or over dosage, too short or too long treatment time, too few active ingredients in the same treatment, etc., which has led to increased tolerance to most of them. Since *V. destructor* mites have developed resistance to the most widely used synthetic active ingredients of acaricides (SAMMATARO et al., 2005; MAGGI et al., 2010), and in order to avoid the accumulation of chemicals residues in hive products (LODESANI et al. 2008; MULLIN et al. 2010), beekeepers are extremely interested in alternative treatments with substances of natural origin with varroacidal effects (i.e. soft acaricides), such as organic acids, plant extracts, essential oils and their components (GOSWAMI et al., 2014; RUFFINENGO et al., 2014; STANIMIROVIĆ et al., 2017; TLAK GAJGER et al., 2013). Eco friendly, natural products have certain advantages after repeated use, such as the lower risk of honey and beeswax contamination, or the small possibility of inducing *V. destructor* mite resistance. However, their efficacy may be inconsistent and more variable compared to synthetic acaricide formulations (ROSENKRANZ et al., 2010).

Hive Clean® is a natural mite control product, distributed and marketed as a food additive for honeybee colonies. According to the product label, it is a mixture of oxalic, formic and citric acids, extracts of propolis, essential oils, sucrose and water. Research has been conducted on the efficacy of this varroacidal product for controlling mites during autumn treatments (AKYOL and YENINAR, 2008; HOWIS and NOWAKOVSKI, 2009). The objective of this study was to evaluate and compare the varroacidal efficacy and mite mortality dynamic during summer treatment of honeybee colonies with Hive Clean®, when used consecutively with shock treatment using an authorized VMP for varroosis control (coumaphos, CheckMite+®). Additionally, the commonly used amitraz (Varidol®) was used for winter treatment in broodless honeybee colonies where it was believed to establish the final parasitic mite drop.

## Materials and methods

*Location and field trial design.* The test apiary was located in the continental part of Croatia (45° 55' 36" N, 16° 32' 42" E) which has a mild climate. The field experiment was conducted on thirty honeybee colonies (*A. mellifera*) populated in a standard Langstrot Root (LR) hive type containing ten combs in each brood and honey compartments. Prior to the experimental period all colonies were equalized in respect to brood size, the comb area coverage with adult bees and the amount of stored honey and pollen (DELAPLANE et al., 2013). During equalization, all honeybee colonies were visually inspected for adult bees and brood pathology, and then were divided into

three experimental groups (A, B, C), each of 10 hives (Table 1). In early June, metal sheets with oily paper were placed on the floor of each test hive in order to record the natural mite mortality prior to treatment (30 days, from June 1 to June 30). Above the sheets wire screens were installed to prevent contact of the bees with debris, and to prevent ants from removing dead mites.

Table 1. Field treatments design

Experimental group	Term of honeybee colonies treatments					
	1.6.-30.6.	2.7.	9.7.	16.7.	1.8.-14.9.	01.12.
A	<i>V. destructor</i> mite mortality prior to treatment	Hive Clean formulation				Amitraz
B		Hive Clean formulation			Coumaphos	Amitraz
C		Coumaphos				Amitraz

*Drugs and treatments.* Treatments were conducted during the summer season, immediately after the main pasture honey harvesting, as follows: in groups A and B, the recommended dose of one plastic stick of 15 mL of Hive Clean® (BeeVital, Food & Beverage Handels GesmbH, Seeham, Austria) was sprinkled between the frames, on the bees, three times at 7 day intervals (T1 - first application on day 1, T2 - second application on day 8, T3 - third application on day 15). In group C, Check Mite+® (Bayer, Germany) was applied, i.e. two beehive pest control strips were inserted between frames with combs in the brood chamber for a 45 day period. The August follow-up treatment with Check Mite+® was applied in group B only as described above. In the winter treatment period, Varidol® (TolnAgro, Hungary) was used once in all three experimental groups (A, B, C) by fumigation, to determine the residual amount of mites. The treatment conditions are presented in Table 1.

Table 2. Calculations of treatment efficacy for acaricides applied on *V. destructor* infesting honeybee colonies

Experimental group	Treatment type	% in total <i>V. destructor</i> mite fall				
		Median	Min.	Max.	Lower quartile	Upper quartile
A (n = 10)	Hive Clean®	91.51	89.25	93.26	90.71	93.26
	Varidol®	8.49	6.74	10.75	7.96	10.75
B (n = 10)	Hive Clean®	88.75	84.32	91.46	87.07	91.46
	CheckMite+®	9.56	7.18	13.31	7.91	11.03
	Varidol®	1.69	0.66	2.93	1.38	2.34
C (n = 10)	CheckMite+®	91.70	89.56	94.36	90.55	93.31
	Varidol®	8.30	5.63	10.40	6.68	9.21

During the treatment period, fallen mites stuck to the oily paper were sampled and transported to the laboratory every two days. After each sampling, the oily paper was replaced with a new sheet. Fallen *V. destructor* mites were counted in the pre-treatment and treatment periods, and the sum of those results, calculated after the final treatment, was considered the total mite drop. The proportion of mites falling after each treatment of the total fallen mites was estimated (%). The

percentages of mites killed by the experimental treatments for Group B were estimated according to the recommendations of the European Medicines Agency (ANONYM., 2008).

*Statistical analysis.* The data analyses were performed by ANOVA using the software package STATISTICA v. 13.3 (StatSoft, Inc, USA). The results were presented as the median, interval of variation and interquartile differences. Statistical differences between treatments were tested using the Mann-Whitney U and Kruskal-Wallis tests.

## Results

During the treatment period, there were no reports of any toxic or other negative effects on the vitality of the adult honeybee population or brood development at the apiary level. During the 30-day pre-treatment period, the average daily mite drop in the 30 honeybee colonies was 1.76 ( $\pm 1.20$ ), and these values did not differ significantly ( $P>0.05$ ) between the experimental groups (Fig. 1). During the treatment period, the observed total mite mortality that resulted was significantly higher ( $P<0.0005$ ,  $t = 5.663$ ) than natural mite drop (Table 2).

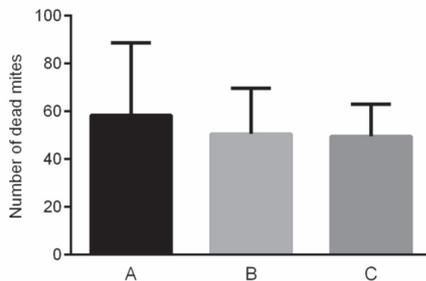


Fig. 1. Natural *V. destructor* mite drop in the pre-treatment period. Mean  $\pm$  SD

In experimental group A, the treatment with Hive Clean<sup>®</sup> resulted in a median mite drop of 91.51% (89.25-93.26%) of the total mite mortality during the overall treatment period. The winter treatment with Varidol<sup>®</sup> in broodless honeybee colonies induced the fall of the remaining 8.49% parasitic mites (6.74-10.75%). In experimental group B, the summer treatment with Hive Clean<sup>®</sup> resulted in a median mite drop of 88.75% (84.3 -91.46%), while the shock treatment with CheckMite+<sup>®</sup> induced the death of 9.56% (7.18-3.31%) mites, and finally the winter treatment with Varidol<sup>®</sup> caused the death of 1.69% (0.66-2.93%) *V. destructor* mites. In experimental group C, the summer treatment with CheckMite+<sup>®</sup> caused median mite mortality of 91.70% (89.56-94.36%), whilst the subsequent winter treatment of broodless colonies with Varidol<sup>®</sup> caused the death of the remaining 8.30% (5.63-10.40%) *V. destructor* mites.

Mite mortality during the main summer treatment with Hive Clean<sup>®</sup> in experimental groups A and B increased in the following order: T1<T2<T3, with the exception of hives 6 and 7 in group B (Fig. 2).

According to the results of the Mann-Whitney U test, the varroacidal efficacy of Hive Clean<sup>®</sup> during the summer treatment significantly ( $P<0.05$ ,  $t = 3.173$ ) differed between experimental groups A ( $91.37 \pm 0.36$ ) and B ( $88.80 \pm 0.72$ ) (Fig. 3). In experimental group B, the treatment efficacy of Hive Clean<sup>®</sup> was 88.75%.

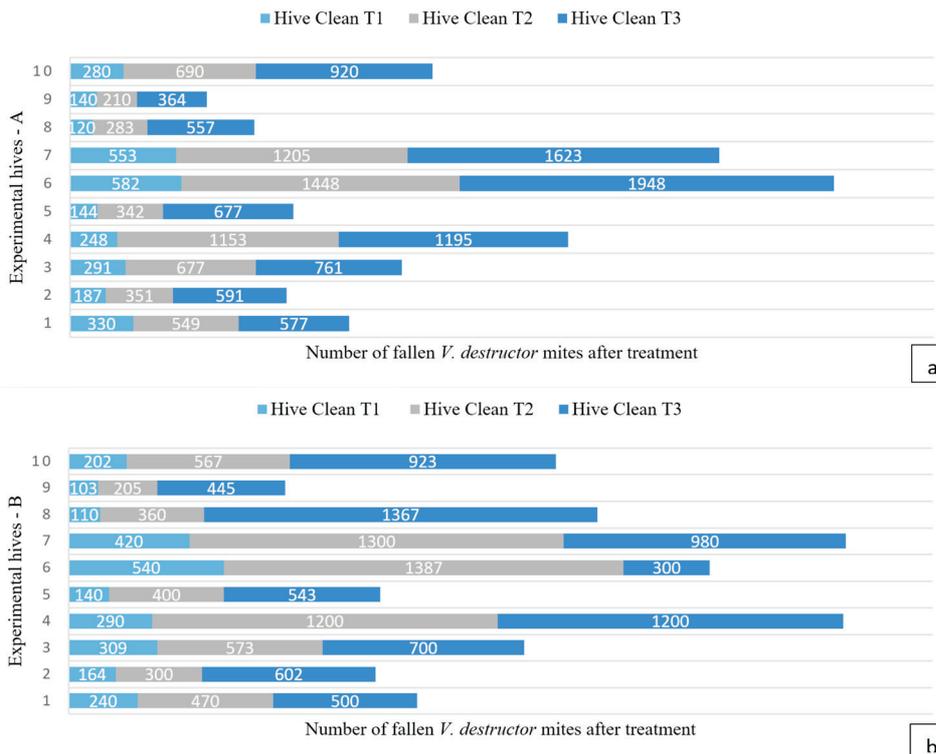


Fig. 2. a, b. *V. destructor* mite mortality during treatment of honeybee colonies (experimental hives A and B) with Hive Clean® (T1 - first application, T2 - second application, T3 - third application)

The results of Kruskal-Wallis test revealed that the efficiency of Varidol® between the three experimental groups was significantly different ( $P < 0.0001$ ,  $H = 19.52$ ). The Mann-Whitney U test showed the significantly higher efficacy of Varidol® in experimental group A compared to group B ( $P < 0.0005$ ,  $t = 4.563$ ), but the differences in the efficacy between experimental groups A and C were not significant ( $P > 0.05$ ). The efficacy of Varidol® was also significantly different between experimental groups B and C ( $P < 0.0001$ ,  $t = 11.488$ ).

Treatment with CheckMite+® in experimental group B induced a median drop of 9.56% mites, while in honeybee colonies from group C, when applied as the first summer treatment, it caused a median mite drop of 91.70% (Fig. 3). There was a significant difference in the varroacidal effect ( $P < 0.0001$ ,  $t = 82.74$ ) between groups B ( $9.51 \pm 1.95$ ) and group C ( $91.93 \pm 1.64$ ).

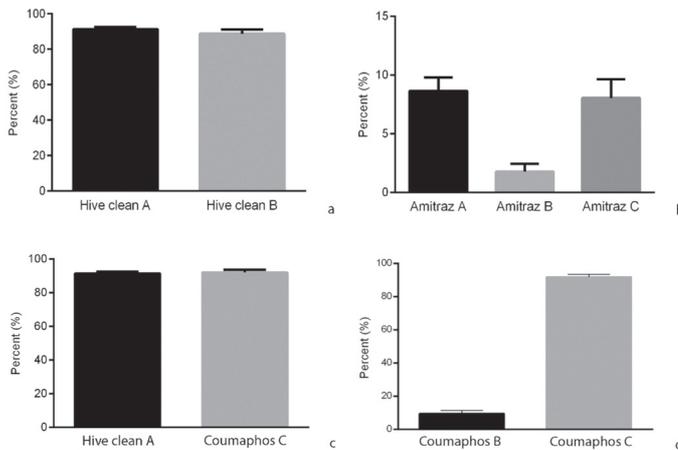


Fig. 3. a, b, c, d. Treatment efficacy of varroacides with different active ingredients a - Varroacidal efficacy (%) of Hive Clean formulation in experimental groups A and B; b -Varroacidal efficacy (%) of Amitraz during broodless treatment on residual mites in all experimental groups (A, B, C); c - Varroacidal efficacy (%) of Hive Clean formulation in experimental group A and Coumaphos in experimental group C, d - Efficacy (%) of Coumaphos in experimental group B on residual mites after primary treatment with Hive Clean formulation and in experimental group C where it was applied as the first treatment. Mean  $\pm$  SD

## Discussion

According to the Varroa Control Program, which is part of the Croatian National Regulation on animal protection measures against infectious and parasitic diseases and on related financing (ANONYM., 2015; ANONYM., 2018), beekeepers nationwide are required to implement one obligatory treatment of honeybee colonies against *V. destructor* mites using an authorized VMP. Varroosis control is recommended in the period from 1<sup>st</sup> July to 31<sup>st</sup> August, with the schedule dependent on the geographical, climatic and pasture conditions in different regions.

Almost all available VMPs, as reflected in manufacturer's instructions, are most effective during the autumn treatments. Nevertheless, there are certain areas with main pastures and the last honey harvest from stationary apiaries in mid-June, and therefore honeybee colonies require treatment much earlier (during the summer) to prevent varroosis damage and to decrease the extent of the extra risk of winter losses.

Most authorized VMPs are acaricides, only present on the local market for periods of three or more active beekeeping seasons. Accordingly, beekeepers are aware of the risks of mite resistance development, and of contamination of beeswax and honey. Also, special requirements linked with mite control in ecological beekeeping are leading to the necessity for testing new product formulations and food additives with acaricidal efficiency to improve *V. destructor* mite infestation techniques. Hive Clean<sup>®</sup> is an entirely organic natural mite control product and, as such, is suitable

for both ecological and conventional beekeeping, and is also appropriate for use in combination with authorized VMPs.

The use of natural compound product formulations, in some situations of need, offers an alternative strategy for control of varroosis. However, to obtain reliable results, these acaricidal products must be evaluated under temperate climate conditions where the evaporation rate of active ingredients should be stable to achieve good efficacy and to avoid negative impacts on the honeybee colony. The results of this study showed that Hive Clean<sup>®</sup> was clearly effective, similar to the reports by HOWIS and NOWAKOWSKI (2009), and IMDORF et al. (2003). In contrast, its effectiveness under tropical conditions after treatment of Africanized hybrid bees, presented a limited therapeutical impact of 19.7% (RODRIGUEZ-DEHAIBES et al., 2017).

The results of this study show that the Hive Clean<sup>®</sup> formulation has an average efficiency of 91.25% after split summer treatments. This varroacidal efficiency is higher than previous tests of its main individual components, applied under different field conditions: oxalic acid (TOUFAILIA et al., 2015); formic acid (PIETROPAOLI and FORMATO, 2017), or various essential oils (ALLAM et al., 2003; GOSWAMI et al., 2014). The number of fallen *V. destructor* mites after three Hive Clean<sup>®</sup> split treatments (Fig. 2) is particularly interesting. This could be the consequence of the treatment time, when colonies in the continental part of Croatia mainly have got pretty much honeybee brood, or due to the fact that the acaricide applied contains a combination of several organic acids, and because of the residual activity of the split treatment. Formic acid is known to be effective against both the phoretic and reproductive phases of *V. destructor* mites (ROSENKRANZ et al., 2010).

In this field study, the acaricidal effect of the food additive Hive Clean<sup>®</sup> was very similar to the effect observed after the treatment of honeybee colonies with the authorized VMP CheckMite+<sup>®</sup> (91.96%). During the winter treatment, Varidol<sup>®</sup> showed the lowest varroacidal efficacy in experimental group B (Fig. 3), which could be explained by the precedent impact of the Hive Clean<sup>®</sup> formulation and CheckMite+<sup>®</sup> treatment.

Hive Clean<sup>®</sup> as a food additive with acaricidal effect might thus be considered to be an appropriate alternative for varroosis control for organic and conventional apiaries under mild climate conditions. Additionally, it is possible to alternate Hive Clean<sup>®</sup> with other synthetic acaricides in rotation programmes in order to decelerate the resistance of *V. destructor* mites to pesticides used repeatedly, or to reduce the impact on increasing beeswax contamination. All treatments must be performed in accordance and in combination with other specific regulations ordered by the national authorities.

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**TLAK GAJGER, I., P. SUŠEC: Varroacidna učinkovitost primjene dodatka hrani tijekom ljetnog tretmana zajednica medonosne pčele (*Apis mellifera*). Vet. arhiv 89, 87-96, 2019.**

**SAŽETAK**

Cilj ovog istraživanja bio je utvrditi učinkovitost Hive Clean® proizvoda tijekom ljetnog tretmana zajednica medonosne pčele (*Apis mellifera*) protiv varooze, u poljskim uvjetima. Učinkovitost tretmana je utvrđena brojenjem otpalih grinja *Varroa destructor* nakon višekratne uporabe Hive Clean® proizvoda prema uputama proizvođača, kao i nakon kombinirane uzastopne primjene dva varroacida s različitim aktivnim tvarima. Nakon provedenog tretmana s Hive Clean® proizvodom prosječna učinkovitost pada grinja iznosila je 91,51 % (89,25-93,26 %). Ukupan broj otpalih grinja je u svim tretiranim zajednicama medonosne pčele bio značajno veći u odnosu na prirodni pad grinja utvrđen tijekom razdoblja prije tretmana. Sukladno dobivenim rezultatima provedenog istraživanja učinkovitosti Hive Clean® proizvoda, kao dodatka hrani s akaricidnim učinkom, može ga se smatrati prikladnim za kontroliranje varooze. Također, prihvatljiv je za uporabu u kombinaciji s odobrenom veterinarsko medicinskim proizvodima za kontroliranje varooze, i to u pčelinjacima s ekološkim i konvencionalnim načinom pčelarenja.

**Ključne riječi:** *Apis mellifera*; *Varroa destructor*; Hive Clean®; varroacidna učinkovitost

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