Original Article

VARROACIDAL EFFICIENCY OF TREATMENT WITH AMITRAZ IN HONEY BEE COLONIES WITH BROOD

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Abstract

Field trials were conducted to evaluate the effectiveness of amitraz fumigation against *Varroa destructor* in honey bee colonies with brood. Within this project the following aspects were taken into consideration: strength of colony, the number of treatments, time intervals between treatments and way of its performance. Honey bee colonies with brood were fumigated four times with one tablet of Apiwarol® per each treatment every four, six, eight and ten days. The tablets with amitraz were burned in the electrical device Wakont or directly in hives. In case of amitraz fumigation with Wakont even four treatments reduced infestations of *V. destructor* to a limited extent, on average from 40 to 61% of mite populations. A similar effectiveness among the treatments has been ascertained regardless of intervals between them. The efficacy of amitraz combustion in hives was slightly higher and statistically significant only after four treatments. Moreover, beyond brood area, the population of worker bees turned out to determine treatment efficacy with amitraz in this form and modes of administration. In honey bee colonies with brood, even four amitraz fumigations do not decrease the level *V. destructor* infestation to the extent that it is safe for wintered bees.

Keywords: amitraz fumigation, control, honey bee, *Varroa destructor*

INTRODUCTION

The epizootic situation of a *Varroa destructor* parasite infestation endangers the population of honey bee colonies. In summer, high and even critical level of the parasite invasion is detected in many national apiaries (Pohorecka et al., 2013, 2014). These data reflect that the action undertaken by beekeepers towards fighting Varroa mites are insufficient. For more than thirty years treatment based on amitraz (formulation Apiwarol® and Biowar 500®) have been used by most beekeepers (Pohorecka et al., 2014), which seriously risks the contamination of apiculture products (Kiljanek et al., 2017; Pohorecka et al., 2017, 2018) and the possibility to select more resistant strains of *V. destructor* (Pohorecka & Bober, 2007, 2008; Maggi et al., 2010; Kamler et al., 2016). Low field effectiveness of the administered drug are frequently caused by incorrectly performed treatment procedures. Apiwarol® come in tablets and each contains 12.5 mg of amitraz. Immediately after ignition, the tablet glows and amitraz is release into the air. According to the manufacturer's recommendations, Apiwarol® should be used in colonies with the small amounts of brood, twice during spring and two to three times in autumn, however this is rarely implemented in apiaries. The control of *V. destructor* infestation is very often conducted in the summer months during intensive brood rearing, but unfortunately there is no preferred approach so beekeepers apply Apiwarol® to colonies in any way, often based on recommendations prepared for colonies with no brood. Thus, honey bee colonies are only fumigated with amitraz from one to three times per summer.

In the absence of recommendations and additional data on amitraz efficacy in colonies with brood, beekeepers are unable to correctly combat the threats arising from *V. destructor*

infestation. Therefore, the effectiveness of treatments with amitraz conducted in summer must be assessed to ensure appropriate protection of honey bee colonies.

MATERIAL AND METHODS

Honey bee colonies

Field studies were conducted in 2014 and 2015, in apiaries of the Research Institute of Horticulture at the Apiculture Division in Pulawy and in the apiary of the National Veterinary Research Institute. The experiment was carried out on *Apis mellifera carnica* and *Apis mellifera caucasica* honey bee colonies maintained in Wielkopolski hives (frame size: 360 mm x 260 mm) equipped with deep mesh-covered bottom boards.

Before the start of the trial, colonies were monitored to estimate the bee population. This was done by counting the number of combs with both sides covered by bees. The amount of brood (uncapped and capped brood) was determined by the measuring of vertical and horizontal axes of brood combs were on both their sides. Tables of Brood Area Measurements from the Polish Industry Standard BN-81/9148-01 were used to calculate the area (dm^2).

In July 2015, queens were caged to obtain some colonies without a brood but with a high population of adults. All honey bee colonies were naturally infected with *V. destructor*. For two weeks before treatments the dead Varroa mites from each colony were counted on the bottom boards. The level infestation was determined on the basis of average daily fall of the parasite. Honey bee colonies were subsequently divided into homogeneous experimental and control groups of ten hives each.

Amitraz treatment and efficacy assay

The varroacidal effectiveness of fumigation with amitraz (Apiwarol®) was assessed in fifty honey bee colonies with uncapped and capped brood and in thirty broodless colonies as the positive control. In the last week of July 2014, forty honey bee colonies with brood, were divided into four groups marked as groups I, II, III and IV (Tab. 1) and fumigated four times with one tablet of Apiwarol® per each treatment every four, six, eight and ten days, respectively. The amitraz tablets smouldered in an electrical Wakont device and the smoke was then introduced through a nozzle to the entrances of hives. In autumn, ten broodless honey bee colonies were also fumigated with Wakont four times with one tablet of Apiwarol® per each treatment every four days (named as group VIII - positive control in Tab. 1).

In the first half of August 2015, ten colonies with uncapped and capped brood (group V in Tab. 1) and ten broodless honey bee colonies (group VII - positive control) were fumigated four times every four days with one tablet of Apiwarol® per each treatment. The smouldering tablets of Apiwarol® were located directly on the bottom board in both groups of the hives. Indirect amitraz-generated smoke was applied with a Wakont to another ten broodless colonies (group VI - positive control). The dates of amitraz administration are given in Tab. 1.

Each treatment was performed in the evening after the end of bee flights. During fumigation the hive entrances were closed. The natural mortality of the parasite was assessed in the ten untreated colonies (negative control). Two days after the last fumigation, a control treatment with two strips of Biowar 500® was applied for six weeks in each colony within groups I-VII to evaluate the efficiency of each treatment with amitraz and to assess the number of mites that survived the treatment with Apiwarol®. The second control formulation of one trickling with 3.5% oxalic acid solution was administrated to the no-brood colonies directly after the removal of the strips in October or November (depending on the starting date of amitraz treatments). The control treatment of colonies within group VIII was executed only by trickling with 3.5% oxalic acid solution on 18 October.

The dead Varroa mites dropped on the bottom boards were counted after each treatment. The efficacy (E%) of amitraz treatments was calculated as: $E\% = 100\ [TA / (TC + TA)]$. TA equals the number of mites fallen on the bottom board of each hive during the amitraz

treatment period, and TC equals the number of mites collected after the control treatments. E% was calculated for treatments one, two, three and four, respectively.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Before the performing of the statistical analysis, data were examined for normality with the Kolmogorov–Smirnov and Lilliefors tests and variance homogeneity with Levene's test as parametric test assumptions. Differences in mean values of varroacidal efficacy of amitraz treatment after each fumigation and area of brood between all treatment groups were determined whether to be significant using one-way analysis of variance (ANOVA) with Welch test. All the statistical analyses were carried out using Statistica 10 StatSoft, and $p < 0.05$ was considered to indicate statistical significance.

RESULTS

The population of honey bee workers in the groups of colonies treated with amitraz in August

Table 1.

Pattern of treatments with amitraz and biological status of honey bee colonies

Period and methods of amitraz administration (for each group n=10 colonies)		Number of combs covered by bees before the first treatment with amitraz (mean±SD)	Area of brood before the first treatment and after the last treatment with amitraz (mean in dm^2±SD)
Group I 31.07-12.08.2014 4 x every 4 days	W	18.9^{b} ±0.3	35.9^{bc} ±6.2 37.7^{bc} ±6.4
Group II 31.07-18.08.2014 4 x every 6 days	W	18.8^{b} ±0.3	41.5^{bc} ±14.8 31.5^{bc} ±7.4
Group III 28.07-21.08.2014 4 x every 8 days	W	18.6^{b} ±0.7	33.4^{bc} ±5.8 6.7^{a} ±1.8
Group IV 29.07-28.08.2014 4 x every 10 days	W	18.4^{b} ±08	33.5^{bc} ±12.3 2.3^{a} ±1.8
Group V 13.08-25.08.2015 4 x every 4 days	BB	18.4^{b} ±0.7	54.8^{c} ±14.8 19.1^{b} ±9.1
Group VI - positive control 13.08-25.08.2015 4 x every 4 days	W	18.6^{b} ±0.5	0
Group VII - positive control 13.08-25.08.2015 4 x every 4 days	BB	18.8^{b} ±0.3	0
Group VIII - positive control 01.10-17.10.2014 4 x every 4 days	W	9.5^{a} ±0.8	0
Group IX - negative control untreated	-	18.9^{b} ±0.3	32.5^{bc} ±5.4

W - tablet with amitraz burned in Wakont
BB - tablet with amitraz burned on bottom board of hives
a,b,c - means with different small letters in the same column are significantly different ($p<0.05$)

Table 2.

Number of *V. destructor* mites fallen on bottom board during amitraz treatments of honey bee colonies with and without brood

Biological status of honey bee colonies	Period and manner of honey bee colonies treatment (for each group of colonies n=10)		Number of dead Varroa mites after each treatment with amitraz (mean±SD)					Number of dead Varroa mites after control treatments
			after first	after second	after third	after fourth	In total, after four treatments	
With brood	Group I 31.07-12.08.2014 4 x every 4 days	W	221.9 ±104.3	261.4 ±151.3	229 ±135.7	214.1 ±142.0	926.4 ±467.6	1183.4 ±758.7
	Group II 31.07-18.08.2014 4 x every 6 days	W	291.6 ±309.4	290.2 ±407.1	271.8 ±217.6	230.9 ±215.9	1084.5 ±1118.9	872.8 ±522.7
	Group III 28.07-21.08.2014 4 x every 8 days	W	441.9 ±240.9	410.9 ±218.8	446.4 ±237.6	633.7 ±309.1	1932.9 ±714.9	2248.6 ±1250.8
	Group IV 29.07-28.08.2014 4 x every 10 days	W	377 ±292.2	537.4 ±329.3	562.1 ±332.1	1016.9 ±354.2	2493.4 ±1171.5	1827.1 ±1568.2
	Group V 13.08-25.08.2015 4 x every 4 days	BB	579.3 ±779.7	673.5 ±663.0	1073.5 ±1072.3	871.7 ±600.5	3198 ±2806.8	1050 ±928.0
Without brood (positive control)	Group VI 13.08-25.08.2015 4 x every 4 days	W	357.1 ±398.0	82.1 ±96.8	44.8 ±94.3	5.8 ±5.5	489.8 ±443.9	31.0 ±17.0
	Group VII 13.08-25.08.2015 4 x every 4 days	BB	741.1 ±896.1	92.6 ±61.5	20.8 ±19.7	3.6 ±7.4	858.1 ±919.1	35.1 ±36.6
	Group VIII 01.10-17.10.2014 4 x every 4 days	W	658.6 ±425.9	10 ±12.5	1.8 ±1.9	0	670.4 ±438.6	9.4 ±5.9

W - amitraz tablet burned in Wakont
BB - amitraz tablet burned on bottom board of hives
a,b,c - means with different small letters in the same column are significantly different ($p < 0.05$)

(groups I-VII) and group of untreated colonies (negative control) were similar (Tab. 1). In each of them, on average the eighteen combs were covered by bees. Colonies treated in October (group VIII - positive control) had considerably less adult insects. Before the start of treatment, in the groups of colonies raising brood (groups I-V and IX), the capped brood surfaces of were similar with an area from 32.5 to 54.8 dm^2. The *V. destructor* infestation level was high in most colonies from all groups. Groups of colonies with brood had an average number of up to 4320 Varroa mites. In groups without brood because queens were placed in cages, 520 to 893 parasites were found. In colonies with queens which seasonally stopped laying eggs, the average number of parasites was 679 mites per colony. In the control colonies (untreated), the average number of *V. destructor* was 2550 mites per colony.

Colonies with brood which were amitraz-fumigated four times every four and six days (group I, and II) similar numbers of mites destroyed after each treatment. However, the colonies treated four times every eight and ten days had a much higher number of dead parasites was

Table 3.

Average varroacidal efficacy of amitraz treatments of honey bee colonies with and without brood

Biological status of honey bee colonies	Period and manner treatment of honey bee colonies (for each group of colonies n = 10)		Percentage of Varroa mites dead after another treatments with amitraz (mean and range)			
			after one	after two	after three	after four
With brood	Group I 31.07-12.08.2014 4 x every 4 days	W	10.8[a] 1.7-18.5	22.7[a] 3.2-31.7	33.2[ab] 3.5-43.9	43.3[a] 4.7-60.7
	Group II 31.07-18.08.2014 4 x every 6 days	W	13.1[a] 6.6-18.6	25.5[a] 15.5-42.5	39.5[ab] 22.1-56.6	50.3[a] 30.7-69.0
	Group III 28.07-21.08.2014 4 x every 8 days	W	10.7[a] 4.9-22.7	20.7[a] 11.5-32.9	31.9[a] 15.2-43.5	46.9[a] 28.1-59.6
	Group IV 29.07-28.08.2014 4 x every 10 days	W	8.1[a] 0.2-14.7	20.8[a] 3.8-31.7	33.8[ab] 15.8-45.8	61.2[ab] 42.3-83.3
	Group V 13.08-25.08.2015 4 x every 4 days	BB	11.4[a] 0.8-24.0	27.3[a] 13.2-48.9	49.4[b] 39.3-63.9	74.5[bc] 54.0-92.0
Without brood (positive control)	Group VI 13.08-25.08.2015 4 x every 4 days	W	51.1[b] 16.5-93.3	70.7[b] 37.3-96.2	79.7[c] 37.3-98.3	83.3[c] 54.5-98.3
	Group VII 13.08-25.08.2015 4 x every 4 days	BB	55.0[b] 4.8-93.3	80.9[bc] 20.1-99.5	88.2[c] 48.3-99.7	88.7[c] 48.3-99.7
	Group VIII 01.10-17.10.2014 4 x every 4 days	W	96.9[c] 95.2-98.8	98.2[c] 97.1-99.0	98.5[c] 97.1-99.2	98.5[c] 97.1-99.2

W - amitraz tablet burned in Wakont
BB - amitraz tablet burned on bottom board of hives
a,b,c - means with different small letters in same column are significantly different ($p < 0.05$)

after the fourth fumigation. In the groups of broodless colonies (VI-VIII), most mites had died after the first amitraz treatment (Tab. 2). In the untreated group (negative control), on average ninety-one mites per colony had died within one month as a result of natural mortality.

The efficacy of amitraz in the eradication of *V. destructor* achieved after each treatment is presented in Tab. 3. The share of Varroa mites fallen on bottom boards after each treatment differ significantly mainly between the groups of colonies with and without brood ($p = 0.00$). The proportion of dead parasites after another fourth treatments, regardless of intervals between them, did not differ significantly in the colonies with brood Wakont - fumigated (groups I-IV) averaging between 8 - 13%; 21 - 25%; 32 - 39% and 43 - 61% of the total mites population, respectively. Four operations of amitraz being directly burned in hives release the honey bee colonies with brood from significantly higher rate of parasites (74%) in comparison to the four

Wakont fumigations (43 – 61%). Both methods of burning Apiwarol® in three groups of broodless colonies was significantly more effective in comparison to the therapeutic outcomes of the colonies with brood. The highest efficacy (96.5% after one and 98.5% after fourth treatments) was found in the group of broodless colonies fumigated in October. In both groups of colonies with no brood treated in August, a similar percentage of mites had died after another round of treatments. The average efficacy of one, two, three and four treatments in the group fumigated with Wakont amounted to 51, 71, 80 and 83%, respectively. The *V. destructor* population decreased on average by 55%, 81%, 88% and 89% in relation to the total number of mites found in the colonies fumigated by direct combustion of Apiwarol® in hives.

DISCUSSION

Although we expected a lower amitraz efficiency in colonies with brood, we were surprised by the results achieved in the field studies. It turned out that even after four amitraz Wakont treatments (one more than is recommended for the autumn period), the number of mites declined approximately 50%. After any single treatment regardless of the length of time between subsequent fumigation the honey bee colonies were released on average from 10.1 to 15.0% parasites. Only the last intervention within a group of colonies treated every ten days resulted in the deaths of 27.4% parasites. Because the fourth treatment in the mentioned above colonies was performed at the end of August, we believe that a higher efficacy of amitraz fumigation caused the decreased brood area. However, in honey bee colonies fumigated four times every ten days the level of infestation was the highest possibly due to the mite proliferation during the extended period of full treatment (30 days). At the four-day intervals between treatments, comparable results of the efficacy were already obtained after twelve days. The total level of Varroa mite infestation in colonies treated this way was twice as low (Tab. 2). The results of the fight against *V. destructor* in honey bee colonies fumigated four times every four days are particularly worrying, as parasites removed from colonies accounted only around 44% of their total population. This manner should hypothetically cause the destruction of most mites. This scheme includes four treatments with period of duration of the capped brood which is present in colonies during the first fumigation, whereas a further three treatments are scheduled at intervals shorter than the shortest period (four days) of the Varroa female phoretic phase (Rosenkranz, Aumeier & Ziegelmann, 2010). Therefore, all mites which leave the cells together with emerging bees should stay on the surface of bee bodies, thereby being exposed to amitraz over the consecutive treatments.

In colonies with brood, the application of amitraz through direct combustion in hives or indirect combustion in a Wakont had a greater influence on treatment efficacy. However, during direct Apiwarol® combustion, efficacy statistically increased by over 30% was only after the implementation of four treatments.

In the broodless colonies, the percentage of dead mites was considerably higher in comparison to the groups with brood regardless of the application manner of amitraz fume. Nevertheless, the most varroacidal activity of amitraz was observed in colonies without brood and treated in October, which reflects how the strength of colonies (population of worker bees) influences the effects of amitraz fumigation. In this group of broodless colonies on average 97% mites were killed already after the first treatment. Such a rate represented close to over half of the percentage of mites fallen in broodless colonies after the first treatment in August. All these data lead to the conclusion that both the brood and adult population have a significant impact on the effectiveness of bee colony protection with amitraz. Bąk, Wilde, & Siuda (2013) also assessed summer Apiwarol® treatment and obtained better results with an average efficiency of was 94.1% for three-time fumigation.

Increasing amitraz fumigation efficiency

through more treatments is not recommended as the amitraz may negatively affect bees. Amitraz treatment has been proven to affect honey bee cuticle proteolytic enzymes. The natural cuticle inhibitors of acidic, neutral, and alkaline proteases were suppressed through such treatments, corresponding with reduced antifungal and antibacterial activity (Strachecka et al., 2012). Amitraz in Apivar® form caused a notable reduction in the quantities of protein, carbohydrates and lipids in the hemolymph and body tissues of adult worker bees (Loucif-Ayad et al., 2010). Amitraz has been shown to increase the frequency of cell death in larval midgut cells (Gregorc & Bowen, 2000; Gregorc & Ellis, 2011). The exposure of honey bees to amitraz and its metabolite substantially changes honey bee cardiac functioning and decreases the survival of bees infected with viruses (O'Neal et al., 2017). Developmental rate decrease and bee survival decrease in individuals treated with amitraz was found by Dai et al. (2018).

Recent studies by Kiljanek et al. (2017), Pohorecka et al. (2017, 2018) indicate a potential problem related to amitraz residues in honey bees and their products, and the relatively low effectiveness of measures performed in colonies with brood could be due to the development of *V. destructor* resistant strains. The efficacy of amitraz treatments executed in broodless colonies and results of the evaluation varroacidal activity of other veterinary preparations containing amitraz (Semkiw, Skubida, & Pohorecka, 2013; Węgrzynowicz et al., 2017) proves the opposite point of view.

In summary, the treatments with amitraz fumigation performed in honey bee colonies in the period of intensive brood rearing are significantly less efficient in comparison to the treatment outcomes of broodless colonies. The ultimate effectiveness of amitraz fumigation in strong colonies with brood will depend on the number of treatments and to a lesser degree on the method of amitraz combustion. In these cases, the other additional treatments must be implemented in hive management for the proper protection of colonies against *V. destructor* infestation.

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