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THE EFFECTIVENESS OF SEVERAL COMPOUNDS AGAINST *VARROA DESTRUCTOR* – PRELIMINARY RESEARCH IN PODLASKIE PROVINCE

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ABSTRACT

Bees are natural pollinators, which are able to pollinate most flowers in the world. The serious problem of bee population is an external parasite mite *Varroa destructor* that causes many viral and bacterial diseases of bees like American foulbrood, European foulbrood or black queen cell virus. It is very important to find a safe and effective treatment against *Varroa destructor*. There are several methods to fight the parasite, one often used is to remove capped drone brood. Another method is to use various chemical compounds. The aim of this study was to evaluate the effectiveness of five commonly applied commercial products: flumethrin, coumaphos, amitraz, formic acid and thymol. According to the obtained results, the effectiveness of these compounds was about 90%, 82%, 91%, 57% and 56%, respectively. Fumigation with amitraz turned out to be the quickest and the most effective method to fight against *Varroa*. All treatment were carried out in private apiaries located in north-eastern Poland, in Podlaskie Voivodeship. Every chemical compound was tested on six bee colonies.

Key words: *Varroa destructor*, honeybee, flumethrin, coumaphos, amitraz, formic acid, thymol

INTRODUCTION

Varroa jacobsoni was first identified as a natural parasite mite living on the Eastern honeybees (*Apis cerana*) across Asia [Oudemans 1904, De Guzman and Rinderer 1999, Anderson et al. 2000]. It later changed host to the Western honeybees (*Apis mellifera*) and has become a serious problem in most of the world. The results of the studies presented in [Anderson et al. 2000] report that only two of the eighteen different haplotypes of mites infesting Eastern honey bee become pests of *Apis mellifera* worldwide. Both are not *Varroa jacobsoni*, and they belong to *Varroa destructor*. The invasion of the parasite has an indirect influence on the development of diseases, including the development and spread of viral infections, which contributes to weakening bee colonies. Many viral diseases have been described as related to *Varroa destructor* infestation. This type of infection is the least understood mechanism of the development of the parasite [Nordstrom et al. 1999, Martin 2001, Ellis and Munn 2005, Mondet et al. 2014]. *Varroa destructor* still is a challenge for both beekeepers and scientists involved with beekeeping. Understanding the process of development and propagation of the parasite makes it possible to reduce the dynamic development of the parasite. This is extremely important from the beekeeper's point of view, because gives the possibility of using biological control compounds as well as repellents, traps and pheromones to eliminate or prevent the growth of *Varroa destructor* [Anderson and Sukarsih 1996, Fries and Rosenkranz 1996, Walter and Procter 1999, Anderson 2000, Wilde et al. 2005, Rosenkranz et al. 2010].

Acaricides are chemicals applied by beekeepers to control and fight against varroosis [Bogdanov 2006, Desneux et al. 2007, Lambert et al. 2013, Cizeljet al. 2016]. These compounds, when incorrectly used, might represent a source of contamination of the bee products. Their persistence in beeswax are the most frequently reported [Bogdanov, 2006]. Tau-fluvalinate, flumethrin, coumaphos, bromopropylate and thymol, belonging to pyrethroid-based acaricides, are the most used in the treatment of varroosis [Garcia et al. 1995, Celli et al. 1996, Milani1999, Korta et al. 2002].

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The objective of the presented paper was to evaluate efficacy of selected compounds: flumethrin, coumaphos, amitraz, formic acid and thymol, in the fight against *Varroa destructor*. This type of experiment was the first verification in Podlaskie. There is only a few studies from Poland where tested compounds were described. Bąk et al. [2013] conducted research in Olsztyn (Warmińsko-Mazurskie Voivodeship) and presented similar experiment with flumethrin, amitraz and thymol. Semkiw et al. [2013] tested efficacy of strips with amitraz in Puławy, Lubelskie Province. Chorbiński [2010] described a lot of substances used against *Varroa* e.g. organic acids, amitraz, flumethrin, and thymol. Thus, the presented research was important both to evaluate resistance of *Varroa* for chemical compounds and to applicate these compounds in Podlaskie Province.

Flumethrin is representative of pyrethroids and is frequently applied for the control of mites because of the ease of application [Haarman et al. 2002, Pettis et al. 2004, Rortais et al. 2005]. Coumaphos belongs to organophosphate insecticides. Due to its lipophilic nature, this compound is frequent contaminant of beeswax and may accumulate in all bee products [Cizeljet al. 2016]. Coumaphos has strong effect on the *Varroa destructor* mites but simultaneously has also negative effect on the population of honeybee colonies. When used in large doses, it interferes with the learning process of the bees and affects their memory in a negative way [Haarmannet al. 2002, Pettis et al. 2004, Rortaiset al. 2005].Amitraz because of low toxicity to mammals isamong many other purposes best known as insecticide against mite- or tick-infestation e.g. of dogs [Haarmannet al. 2002, Pettis et al. 2004, Rortaiset al. 2005]. From many years, the organic acids (formic acid, lactic acid or oxalic acid) are successfully used for the control of *Varroa destructor*. The formic acid gives the best results among all organic acids. This acid is a natural ingredient of honey so that it does not contaminate honey or any other hive products for human consumption [Eguaras et al. 2002]. Essential oils containing thymol in the composition exhibit broad biological activity against parasites. Thymol belongs to monoterpenoids, which are present e.g. in the flowers [Gregorc and Planinc 2005].

All the treatments conducted in the experiment were made in bee colonies during the broodless period.

MATERIAL AND METHODS

The experiment was performed in three apiaries located in north-eastern Poland, in Podlaskie Voivodeship. The apiaries were situated at a distance of about 35 km southeast of the city of Bialystok, in the same pedoclimatic area (Fig. 1). The distance between sites was about 20 km. Studies have been conducted from October to December (weeks 40rd to 50th) of the year 2014.

Vitality of honeybees is dependent on the climate in which they live. In Podlaskie are specific breeding conditions for honeybees e.g. long and cold spring, short summer and long autumn. The long spring period is very difficult for beekeepers to manage colonies strength, too big nest can cause hypothermia breed, too small contribute to negative effect like swarming. Longer breeding period in autumn contribute to bigger amount *Varroa* mites. In Podlaskie are short terms between consecutive florescence which have negative impact on the length bees life.

Honeybee colonies (*Apis mellifera*), naturally infested with *Varroa destructor* hosted in standard eleven frame German Normal hive bodies (size 223 mm x 370 mm), were used. The hive bodies were made of polyurethane, while the bottoms of wood. The hives were equipped with removable bottom boards allowing the trapping of falling mites on a sticky surface. Wire screens prevented the contact of the bees with the debris. The amount of capped brood in each colony was about 2–2.5 combs.

Fig. 1 Geographic location of three apiaries near Białystok (north-eastern Poland), within which the field trials were conducted

Rys.1 Lokalizacja trzech pasiek niedaleko Białegostoku (północno-wschodnia Polska), w których przeprowadzane były badania

Thirty colonies, used for the experiment were divided into five groups each consisted of six colonies. In each group, two of the six hives were the control group. Five different commercial products were used in the control of *Varroa* mites. In the first group, at the location A, flumethrin was applied. In the second group, at the location B, coumaphos was conducted. In groups 3–5, settled in location C, amitraz, formic acid, and thymol were employ, respectively. Differences in the distribution of hives re-

sulted from different duration of the treatment and aimed at eliminating the phenomenon of the movement of mites between hives. During the period from 10 May until 30 June, the capped drone brood was regularly removed in all group, because of the intensive building of the brood combs.

All colonies used in these studies were strong with regard to bee population, had been previously treated for *V. destructor* and were apparently free of other bee diseases.

The following commercial compounds were selected for the experiment:

- flumethrin 3.6 mg/strip (Bayvarol, Bayer Animal Health),
- coumaphos 32 mg/ml (Perizin, Bayvarol, Bayer Animal Health),
- amitraz 12.5 mg/tablet fumigation (Apiwarol, Biowet Puławy Poland),
- formic acid (Sigma Aldrich),
- thymol 12.5 g/50g gel (Apiguard, Laleham Healthcare, Hampshire, UK).

The manufacturer instructions were followed in the use of all the products.

Flumethrin was impregnated in plastic strips. Four strips were inserted in the brood chamber of each hive. The acaricide was released from the strips in the process of evaporation. Strips were left for five weeks and then removed from the treated hives.

Coumaphos was applied in water solution and effective concentration was 3.2% (1 ml emulsion of Perizin was dissolved in 50 ml distilled water). The solution was made just before application. The emulsion so formed was dripped evenly along the occupied seams of bees, using an applicator sold for the purpose.

Amitraz was applied in a form of fumigation tablet. A lighted tablet was inserted into the wire holder and putted inside the closed hive. After 20 minutes of fumigation the hive was opened.

Formic acid was used in 85% concentration. Fifty milliliters of acid in the plastic box with the cotton wool and perforated walls were placed on the top bars of the frames of each brood nest which allow to control evaporating process.

Thymol was administered in the form of a gel to evaporate. The gel was packed in ready-to-use aluminum tray protected by a foil. Protective film was removed before placing the opened tray face upwards in the top of brood frames, centered over the colony. After 12 days, the whole content was evaporated and the tray was replaced with a new.

Any observations concerning the colony strength estimation and the number of fallen mites *V. destructor* were noted on the protocol submitted in Table 1.*Varroa* mortality was monitored continuously during treatment evaluation board traps, placed on the floorboard of each hive. Traps were placed in colonies 10 days prior to treatment and were replaced every day. Mites were counted every day during the treatment period and colony strength estimation were conducted every eight day. To control effectiveness of all substances, traps were used in each colony 10 days after experiment. All honeycombs used during the application of commercial compounds were removed from the hives in the spring of the next year, in order to avoid honey contamination.

RESULTS AND DISCUSSION

The daily minimum and maximum values of the external temperatures (°C) occurred over the entire experimental period are presented in Figure 2. On the time of the first commercial products application the external temperature was 8°C at night, and 21°C during the day. When the second dose of thymol was applied, the minimum and maximum temperature were –2°C and 3°C, respectively. Extremely low temperature, –8°C, was recorded in the 25th and 30thday of the experiment. At the end of the experiment temperature was around –2°C at night and 2°C during day. Outer temperature had not influence to effectiveness of applied compounds inside the hive – the polyurethane, the material from which the hive bodies were made, has very good thermal stability.

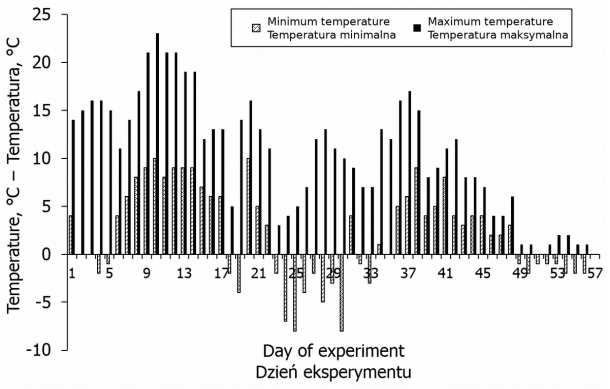

Fig. 2 Daily external temperature (minimum and maximum values) registered in Białystok during the experimental period; temperature measured using a home weather station

Rys. 2 Maksymalne oraz minimalne dobowe wartości temperatur zarejestrowane w Białymstoku podczas trwania eksperymentu; temperatury zanotowano wykorzystując domową stację pogodową

The 10-days pretreatment period displayed a daily natural mite mortality (mean ± S.E.) of 17 ±2 mites which was not significantly different between the five examined groups. During the experimental period the total number of the trapped mites in the group treated with flumethrin ranged from 90 to 353, in the second group treated with coumaphos from 170 to 823, in the third group treated with amitraz from 183 to 1327, in the fourth group treated with formic acid from 25 to 738, and in the last

Table 1. The scheduling of the *Varroa* mite treatment chemicals – version for 3 hives, where X – colony strength estimation

Tabela 1. Harmonogram leczenia Warrozy – wersja dla 3 uli, gdzie X – ocena siły rodziny

		Day of experiment – Dzień eksperymentu					
Coumaphos – Perizin, Amitraz Apiwarol, formic acid	Hive number Numer ula	1–10	11	12–17	18	19–27	28
		Natural fall of mites Naturalny osyp	Treatment – Leczenie	Counting dead mites after treatment Osyp po leczeniu		Natural fall of mites – Naturalny osyp	
	1						
	2						
	3						
	Side effects Efekty uboczne		X		X		X

		Day of experiment – Dzień eksperymentu											
Thymol – Apiguard	Hive number Numer ula	1–10	11	12–17	18	19–22	23	24–29	30	31–35	36	37–44	45
		Natural fall of mites Naturalny osyp	Thymol	Counting dead mites Osyp			Thymol	Counting dead mites Osyp			Natural fall of mites Naturalny osyp		
	1												
	2												
	3												
	Side effects Efekty uboczne		X		X		X		X		X		X

		Day of experiment – Dzień eksperymentu													
Flumetrin – Bayvarol	Hive number Numer ula	1–10	11	12–17	18	19–24	25	26–31	32	33–38	39	40–45	46	47–55	56
		Natural fall of mites Naturalny osyp	Flumethrin	Counting dead mites after treatment – Osyp po leczeniu										Natural fall of mites Naturalny osyp	
	1														
	2														
	3														
	Side effects Efekty uboczne		X		X		X		X		X		X		X

group treated with thymol from 90 to 353. The infestation level of the colonies agreed with the recommendations provided by European Group for Integrated *Varroa destructor* Control [1999] amounting 100–5000 mites per colony. The natural mite mortality collected before and after treatment ranged from 12 to 24and it was not depended from the treatment method.

The evaluation of the efficacy (%) for each applied compounds was calculated from the following equation [Bacandritsos et al. 2007]:

$$V_{Te} = \frac{V_{DT}}{V_N + V_{DT}} \cdot 100\%$$

where: V_{Te} – *Varroa* treatment efficacy, V_{DT} – *Varroa* dead mites after treatment, V_N – natural *Varroa* falling.

All commercial products used during the present experiment have shown high efficiency against the mite of *Varroa destructor*, a summary of their effectiveness is illustrated in Table 2.

In the present studies, flumethrin has been found to be over 90% effective against mites on adults bees in colonies which is consistent with studies reported in [Loucif-Ayadet al. 2010]. Using this compound was very comfortable for the beekeepers. That method required only hanging strips into beehives and removing them after several weeks. The average time spent on the treatment of one colony took about 7 minutes. There were not any negative effects for the colony during and after treatment.

Coumaphos, also used during the experiment, required the longest preparation, but its effectiveness was

about 82% against mites. The prepared solution must be maintained at an appropriate temperature which can be problem for beekeepers having hives at out apiaries (away from home). This makes necessity of solution preparation in advance and placing it in a thermos. The treatment period is highly dependent on weather conditions. *Varroa destructor* may be resistant to coumaphos which was described in [Pettis 2004]. This resistance has not been shown in the present studies because in all treatment colonies another therapeutic product was used every year.

In the reported study, the negative influence of coumaphos on queen and bee workers was not observed. The colonies developed properly, similar observations were noted in [Haarmann et al. 2002]. The research presented in [Chaimanee et al. 2016, Pettis et al. 2004] convinced that coumaphos cause's lower breeding. Coumaphos is a lipophilic substance, and therefore soluble in the wax. The wax contamination can be moved by bees on their bodies and legs to other bee products placed in honeycombs [Bogdanov 2006, Chauzat and Faucon 2007]. It is noteworthy that the use of coumaphos entails wax contamination which persists through commercial recycling [Chauzat and Faucon 2007]. Therefore, after acaricide treatment, the wax should be removed from the hives during spring.

In the described experiment, the fastest, the most effective and the most comfortable method was fumigation by amitraz. The application per one colony took only 5 minutes. Amitraz was very effective against mites, more over 91%, and any side effects has been observed. Amitraz is not stable in honey, and so almost completely degrades into several decomposed metabolites after 3–4 weeks [Korta et al. 2002, Wallner 1999]. After that time, all efforts to detect amitraz residues in honey have been unsuccessful. The obtained results are in agreement with those reported by Semkiw et al. [2013] who, in Poland, observed the decreasing amount of mites after treating, and high efficiency of amitraz. The same authors also show that effectiveness of amitraz depends on temperature and time of use. In the case of large-volume hives and weak colonies it can be related to early binding of the winter swarm, wherefore access of smoke to the bees is much more difficult. The use of fumigation tablets requires windless weather, because the resulting smoke is toxic for human health.

In the present research, formic acid has been found to be above 57% effective against *Varroa destructor*. However, the treatment method required a lot of preparation and precaution. Although formic acid is a natural composite of honey, this organic acid should be used very carefully because showed some negative effect. After treatment, the amount of opened brood (eggs) was decreased which resulted in a serious problem with colony strength on the spring next year. Formic acid can stop the queen with brooding. Strong evaporation of the compound can also cause problems with bees – in front of hives more dead bees were found. Obtained results are similar to those of Skinner et al. [2015] who, in USA, observed the decreasing amount of bees and brood after treating with formic acid. Formic acid should be applied on spring or autumn when temperature outside is near 20 degrees and evaporation process is more effective [Calderone and Nasr 1999, Skinner et al. 2015].

The first application of thymol in the broodright period produced small amount of dead mites (Table 2) probably due to the high level of foretic mites inside the capped brood. During the second application there was an increase on the mite mortality. This finding is in agreement with that described by Gregorc and Planinc [2005] who, after the first thymol application in beehives in Mediterranean area, observed the highest mite mortality during the second application. During the second application the external temperature decreased to -8 °C at night but it had not influence on thymol evaporating, because colonies were very strong, and temperature inside beehives was stable. From the beekeeper's point of view, application of thymol in the form of a gel is a fast and simple method which does not require complicated activities. However, the efficacy of thymol was small and caused only 56% mite mortality.

Similar results were described by [Bąk et al. 2013], who tested Bayvarol, Apiwarol, formic acid and another substances.

The most important condition for the effectiveness of the treatment against *Varroa destructor* is an appropriate time period when queens stop breading and there is a small amount of caped brood. Caps protect mites against contact with the active agent. The application of acaricides during the honey production is not be in accordance with the manufacturer's recommendations and contributes to contamination of the bee products [Bogdanov 2006, Desneux et al. 2007].

Drone brood removed from colonies can be used to produce homogenate. The drone brood homogenate is a natural mixture drone larvae pressed together with honey. Such prepared mixture, however, has low durability and should be used quickly. Freshly prepared homogenate is a source of easily digestible protein, macro- and microelements [Isidorov et al. 2016].

The use of biological fight against *Varroa destructor* mites contributed to the reduction of their population and improved the efficiency of applied compounds. Removing drone brood is a serious problem, because it should be done regularly, when the brood is capped but during the season this is hard to do. Removed drone brood can be a source of diseases like American foulbrood, European foulbrood or black queen cell virus, so should

Table 2. Total number of dead mites during the treatment; data expressed as mean ± the standard deviation calculated in the package STAT 30

Tabela 2. Całkowity osyp pasożytów w trakcie trwania eksperymentu; dane wyrażone jako średnia ± odchylenie standardowe obliczone w programie STAT 30

	Substance – Preparat				
	Flumethrin Flumetryna	Coumaphos Kumafos	Amitraz Amitraza	Formic acid Kwas mrówkowy	Thymol Tymol
Total amount of dead mites after treatment Całkowita liczba roztoczy po leczeniu	2693 ±17	2455 ±19	2724 ±18	1678 ±20	1711 ±23
Treatment efficacy Skuteczność substancji	90%	82%	91%	56%	57%

be destroyed as soon as possible. In fact, doing so may be a real problem for beekeepers.

CONCLUSIONS

The effectiveness of the five selected substances in the fight against *Varroa destructor* was compared in the article. As a result of the experiment, there was not found any compound, which was 100 percent successful in the fight against the mites. However, all the compounds used allow for sufficient overcome these mites. Fumigation method turned out to be the quickest and the most effective. Amitraz give possibility to perform treatment in all apiary in short time. Drone brood removal is biological method to reduce population and the development of *Varroa* mite during the season.

Acaricides used improperly can contaminate bee products like honey, wax, royal jelly or propolis, therefore the use of commercial agents should be done at the appropriate times. The colony strength, hive type as well as weather conditions have high influence on these compounds action. The usage of hives made from polyurethane and wintering colonies on one body allow to achieve good treatment effects with application of selected products.

Experiment described in the presented paper has preliminary research character. In the future it will be important to repeat similar test on higher group of bee colonies and with different substances like oxalic acid or lactic acid to comparison. Application two different substances one by one in the same hive could be a good method for increasing efficacy against *Varroa*.

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SKUTECZNOŚĆ WYBRANYCH ZWIĄZKÓW W WALCE Z *VARROA DESTRUCTOR* – WSTĘPNE BADANIA W WOJEWÓDZTWIE PODLASKIM

STRESZCZENIE

Pszczoły należą do naturalnych zapylaczy, które zapylają większość kwiatów na świecie. Poważnym problemem populacji pszczół jest zewnętrzny pasożyt – roztocze *Varroa destructor*, który przyczynia się do wielu wirusowych i bakteryjnych chorób pszczół, takich jak zgnilec amerykański, zgnilec europejski czy wirus czarnych mateczników. Niezwykle istotne jest znalezienie bezpiecznego i skutecznego leku przeciwko *Varroa destructor*. Istnieje kilka metod walki z pasożytem, jednym z nich jest usuwanie czerwiu trutowego. Inną metodą jest użycie różnych związków chemicznych. Celem tego badania była ocena skuteczności pięciu powszechnie stosowanych produktów handlowych: flumetryny, kumafosu, amitrazu, kwasu mrówkowego i tymolu. Zgodnie z uzyskanymi wynikami, skuteczność tych związków wynosiła odpowiednio około 90%, 82%, 91%, 57% i 56%. Metoda fumigacyjna z użyciem amitrazy okazała się najszybszą, najwygodniejszą oraz najskuteczniejszą. Wszystkie zabiegi przeprowadzono w prywatnej pasiece zlokalizowanej na terenie północno-wschodniej Polski na Podlasiu. Wszystkie związki testowano na grupie 6 rodzin pszczelich.

Słowa kluczowe: *Varroa destructor*, pszczoła miodna, flumetryna, kumafos, amitraza, kwas mrówkowy, tymol