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Evaluation of Some Synthetic Acaricides Against *Varroa Destructor* (Acari: Varroidae) in Turkey: an Indication of Resistance or Misuse of Fumigant Amitraz?

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Abstract

The parasitic mite, Varroa destructor is one of the most important agents for substantial losses in honeybee colonies throughout the world. Several acaricides consisting of synthetic and organic compounds are being used to combat mite. This study was conducted to determine the efficacies of three synthetic acaricides in naturally infested honeybee colonies at consecutive two autumn seasons. Acaricides were commercial preparations of coumaphos (liquid and plastic strips), amitraz (fumigation and plastic strip) and flumethrin (plastic and wooden strips) as a treatment group consisting of eight hives per drug. A control group was kept in both seasons. All drugs were applied as prescribed to the homogenised Varroa-infested honeybee colonies at consecutive two years. The evaluation of efficacies was based on the collected mite percentage obtained with powdered sugar method and it was calculated through Henderson-Tilton's formula. Dropped mites onto the pollen drawers were also evaluated statistically and drugs were compared to each other. Results showed us the effective drugs (up to 90%) were amitraz plastic strip, flumethrin wooden strip and coumaphos plastic strip with 98.5%, 96.5%, and 93.2% averages, respectively according to formula if evaluated within two autumn seasons. Fumigation of amitraz is not sufficient if compared to others and the control group. This data is discussed for suspicion of the possible resistance of mites or misuse of the product with this kind of application.

Keywords: Acaricide, chemical, honeybee, Turkey, Varroa destructor

Introduction

Honeybee *Apis mellifera* L. is the most critical insect that has benefited humanity for medicinal and nutritional purposes for thousands of years. It has significant economic value in agriculture not only for honey production but also they play a vital role in crop pollination.[1] Many insect pests and microbes may attack honeybees and cause considerable yield losses. An ectoparasite, *Varroa destructor* is posing a significant threat to the beekeeping industry throughout the world, also in Turkey, for the last four decades.[2] *V. destructor* is causing severe complications in beekeeping all over the world and can easily be observed in adult bees, broods and also in hive debris. Pupa cannot develop into an adult form or if developed and emerge, bees with deformed wings/legs/abdomens in the case of heavy infestation. Untreated honey bee colonies which are infested with *V. destructor* may perish within two years.[3] *V. destructor* has been proved an important vector for different viral and fungal pathogens spread among honeybees like acute bee paralysis virus and deformed wing virus.[4] The infected bees also have a reduced foraging ability to collect nectar and pollinate crops.[5] The usage of synthetic acaricides to mites is legal and common in Turkey if appropriately used. The misuse of synthetic acaricides may cause severe problems in bees such as bee toxicity; may increase the probability of disease-resistance and may leave residues on bee products.[6] Natural plant products/organic

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acids can be the desired alternative or supplementary to synthetic acaricides with low mammalian toxicity and negligible environmental effects.[7]

Some synthetic chemicals licensed on honeybees like coumaphos, flumethrin, amitraz, tau-fluvalinate are widely used in the world. Coumaphos is a neurotoxic organophosphate that inhibits acetylcholinesterase, thus interfering with nerve signalling and function, while amitraz is a formamidine, octopaminergic agonist. A synthetic pyrethroid, flumethrin, acts by inhibiting gated sodium channels in the nervous system of the mite and being widely used as an acaricide also to Varroosis in the field.[8]

Despite the often efficient Varroa control promoted by synthetic acaricides, numerous side effects may be observed due to misuses. Another negative consequence of the indiscriminate use of acaricides to control Varroa infestation is the repeated selection of mites that are resistant to each of these compounds.[9-11] It has also been demonstrated that combined exposure to pesticides may synergise, resulting in the compounds being even more toxic to honey bees than when administered individually.[12,13]

The present study was designed to find out the efficacies of commercial synthetic Varroacidal drugs in Turkey market and compare them to themselves. Possible long-term effects of the drugs were also evaluated.

Material and Methods

This experiment was conducted during consecutive two autumns, 2012 and 2013 at Bursa - Turkey under field conditions. The colonies of *Apis mellifera* which are settled in the wooden Langstroth hives were used. The bottom boards had drawers enabling to monitor the numbers of dead *Varroa destructor* specimens. The strength of the colonies (with 6-7 frames with bees and less brood or broodless) was assessed before the study in order to establish a homogenous experimental and control group. Natural mite falls were checked by counting them in pollen-drawers at one-week duration. Thus, groups were created with similar mite burdens and bee/brood population.

Six commercial products consist of three synthetic acaricides; coumaphos (liquid-32 mg/ml and plastic strip-13.6 gr/strip), amitraz (fumigation-265 mg/cardboard and plastic strip-500 mg/strip) and flumethrin (plastic strip-3.6 mg/strip and wooden strip-3.6 mg/strip) were applied at recommended applications/doses as treatment group consisting eight hives per drug. A control group consisting of the same number of hives was kept in both seasons. Totally 56 Langstroth-type hives with 6-7 combs, without super and large-size pollen drawers were used, which were highly infested with *V. destructor.*

Approximately 200 adult worker bees from outer frames of each hive were collected into special jars containing icing (powdered) sugar to determine the rate of Varroa infectivity before and after treatment. Aliquoted adult bees and mites were counted as described in Dietemann et al.[14] Additionally, the bottoms of the drawers were cleaned before the trial and were covered with white paper to count dead mites that dropped into the drawer after each drug application.

In each season, the dead mites that had dropped into the pollen drawers were counted on days 1, 3, 5, 7, 14, 21, 28, and 35. Mites on bees were counted on day 35 after treatment. Strips were removed on recommended time when the treatment period finished.

The efficacies of drugs were measured with the Henderson-Tilton formula[15] and significance between the drugs was determined via Tukey's multiple comparison tests defined at the level of 0.05. The formula was:

$$\text{Corrected \%} = \left(1 - \frac{\text{n in Co before treatment} \times \text{n in T after treatment}}{\text{n in Co after treatment} \times \text{n in T before treatment}}\right) \times 100$$

Where: n = mite population, T = treated, Co = control

The mite mortality data was recorded on 1, 7, 14, 21, 28 and 35th days of post-treatment by counting the fallen mites from the bottom drawers of the hives.

Results

The present work was conducted in the South-east Marmara Region conditions; the province of Bursa to test the efficacy of commonly used anti-Varroosis chemical products. These efficacy results are summarized in Table 1. If evaluated to average percentages, the most efficient product was amitraz plastic strip (98.5%), following with flumethrin wooden strip (96.5%), coumaphos plastic strip (93.2%), flumethrin plastic strip (88.3%), coumaphos liquid (76.8%) and amitraz fumigation (30.0%). The efficacies of coumaphos liquid and amitraz fumigation in the second season were markedly lesser than those of the other drugs. Except then those two lesser efficient drugs, other drugs have demonstrated almost a high effect in both autumn seasons. Amitraz plastic strip reached 99.0% efficacy during the second autumn season.

During the experiment, dropping mites on drawers were counted for 35 days. The mean numbers of mites dropped in each treatment in each season were shown in Table 2. Means in all treated colonies were decreased in the second season if compared to the first autumn season, interestingly. Amitraz plastic strip treatment caused the highest mean number of mites to drop throughout the seasons, followed by flumethrin plastic strip, coumaphos liquid, flumethrin wooden strip, coumaphos plastic strip and amitraz fumi-

Table 1. % Efficacy of chemical acaricides by the Henderson-Tilton formula for five weeks period

Season	Coumaphos (liquid)	Coumaphos (plastic strip)	Amitraz (fumigation)	Amitraz (plastic strip)	Flumethrin (plastic strip)	Flumethrin (wooden strip)
1st autumn	91.2a	93.3a	37.7b	98.1a	89.7a	96.1a
2nd autumn	62.5a	93.1b	22.3c	99.0b	86.9b	97.0b
Average	**76.8a**	**93.2a**	**30.0b**	**98.5a**	**88.3a**	**96.5a**

a, b, c values with different letters in each category are significantly different.

Table 2. Mean number of dead mites on pollen drawers on the 1st, 3rd, 7th, 14th, 21st, 28th and 35th days (mean ± Standard Error Mean)

Drugs	1st autumn	2nd autumn	Both seasons
Coumaphos (liquid)	58.95 ± 15.95a	40.12 ± 5.75a	**49.53 ± 10.85a**
Coumaphos (plastic strip)	69.78 ± 13.65a	14.50 ± 1.60b	**42.14 ± 7.62a**
Amitraz (fumigation)	17.87 ± 5.81b	14.01 ± 2.27b	**15.94 ± 4.04b**
Amitraz (plastic strip)	107.83 ± 37.48a	28.46 ± 3.95a	**68.14 ± 20.71a**
Flumethrin (plastic strip)	91.75 ± 21.93a	30.62 ± 3.74a	**61.18 ± 12.83a**
Flumethrin (wooden strip)	66.6 ± 24.47a	21.37 ± 5.45a	**43.98 ± 14.96a**
Control	**49.95 ± 11.87b**	**18.57 ± 2.61b**	**34.26 ± 14.48b**

a, b values with different letters in each category are significantly different.

gation. Unexpectedly, a mean number of dead mites in control group is higher than the amitraz fumigation group. The differences between the control group and the drugs were significant in the first season except for amitraz fumigation. Besides, in the second autumn season, the differences between the control group and the drugs were significant for coumaphos liquid, amitraz plastic strip and flumethrin plastic strip, but not significant for coumaphos plastic strip, amitraz fumigation and flumethrin wooden strip ($p>0.05$). Finally, there were no observable side-effects or abnormal bee deaths during any of the trials in the treated or control colonies.

Discussion

There is always a potential hazard of the usage of synthetic acaricides like building-up residue in bee products and/or mite resistance. However, Varroa mites are widely being controlled by using synthetic acaricides which were applied in formulated different forms like plastic/wooden strips, liquid or soaked cardboard. It has been noted that the efficacy of some drugs (coumaphos liquid, amitraz fumigation and flumethrin plastic strip) can be variable in different seasons.

Our results obtained for plastic strips of amitraz, coumaphos and flumethrin either in first and second autumn agreed with other published works as 90.6%, 82.8% and 99.9%, respectively.[16-18] We detected a drop in the efficacy of coumaphos liquid in the second autumn, probably due to the lower external temperatures or internal (in-hive) conditions like colony population. A similar observation has also been reported by Semkiw et al.[17] and Leza et al.[19] with the studies of amitraz plastic strip.

Although the results of the assessments of amitraz strips in our experiment are high/not variable, some researchers have gained variable results as 83.8% on average (78.8 – 87.3%) in Italy and maximum 60.1% efficacy in Portugal.[20,21]

Amitraz fumigation is still being used in Turkey since the 1980's. According to its short-time effect, its efficacy can be different in the whole year's Varroa combat management. There is limited data on the efficacy of amitraz fumigation applications on honeybees in Turkey such as by Kumova[22] conducted in an autumn period and reached 91.1% efficacy. In contrast to our study, although, low efficacy of amitraz fumigation seems like a possible mite resistance, we think that using this fumigation is not related to resistance. Amitraz fumigation is also not suitable for a long time period (during autumn) Varroacide. It can be used as a short time and fast mite determiner. To ensure this theory, further investigations into the specific resistance of the mites should be needed.

Chemical acaricides with plastic strips possess some advantages, such as the simplicity of application and the low economic cost. However, the main disadvantages of these products are their limited efficacy after continuous use due to the development of resistance,[9,10,21] as well as the residues in bee products. Due to the prescribed use, there were no side-effects on adults/broods or abnormal bee deaths during treatment.

All drugs except amitraz fumigation were proved proper methods to control Varroa mites. Our results showed that the effective drugs (up to 90%) were amitraz plastic strip, flumethrin wooden strip and coumaphos plastic strip with 98.5%, 96.5%, and 93.2% averages, respectively according to formula if evaluated within two autumn seasons. According to formulated data, efficacies of high affected drugs are almost stable in both autumn seasons, except for coumaphos liquid one. That decrease can be explained by the result of some environmental variables like climate, brood population and colony strength. Although usage of chemical acaricides is tending to be reduced due to its possible side-effects, if they are appropriately used and rotated, they are adequately effective, especially in long-term autumn treatment.

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