Pakistan J. Zool., vol. 50(3), pp 857-861, 2018. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2018.50.3.857.861

Comparative Efficacy of Five Commercial Synthetic Acaricides against *Varroa destructor* (Anderson and Trueman) in *Apis mellifera* L. Colonies

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Article Information
Received 20 October 2017
Revised 02 December 2017
Accepted 22 December 2017
Available online 10 April 2018

Authors' Contribution
MAB designed and conducted the study. MAA and ZAQ helped in review and proof reading. ABMR helped in data collection and analysis. MA helped in statistical analysis. RM and MA helped in preparation of manuscript.

Key words
***Apis mellifera*, *Varroa destructor*, Acaricides, Ectoparasitic, Mite, Perizin, Apistan.**

ABSTRACT

The field experiment was conducted at apiary of University of Sargodha. The coordinates of apiary are 32°13´15.7″N, 72 °68´41.8″E. The apiary had 24 Langstroth standard colonies of *Apis mellifera* naturally infested with *Varroa destructor.* Ectoparasitic mite, *V. destructor* is considered the most important parasite of *A. mellifera* L. that badly affects the development and performance of bees. Main objective of our study was to assess the effectiveness of five synthetic acaricides (Bayvarol®, Apivar®, Apistan® Apitol® and Perizin®) against *V. destructor* in infested colonies of *A. mellifera* in Langstroth standard hives. Four hives were selected for each acaricide and one group of four hives was left untreated as control. As seen from results, all acaricides significantly reduced the infestation levels of varroa mite on adult honeybees and worker brood and the efficacy of Perizin® and Apistan® was high (91.3%±0.81 and 81.6%±1.2, respectively). Lowest mite mortality (52.3%±1.2) was recorded in Apitol® treatment. Overall, the data indicated that acaricides like Perizin® and Apistan® were highly effective against *V. destructor* and can be recommended for better management of this destructive pest.

INTRODUCTION

Honey bees, *Apis mellifera* L. are considered the wings of agriculture due to their important role in crop pollination and hive products (*e.g.* honey, propolis, royal jelly, wax, bee venom and pollen) (Tiwari *et al*., 2014). However, honey bees are susceptible to many insect pests, birds, mites and diseases inducing severe damages (Yousef *et al*., 2014).

Nowadays the ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000) is one of the most important parasite of *A. mellifera* that effects the colony development and performance of the bees (Bowen-Welker and Gunn, 2001). *V. destructor* is considered as the most serious problem of beekeeping industry worldwide (Guzman-Novoa *et al*., 2010). After the introduction of *A. mellifera* into Pakistan, *V. destructor* emerged as a serious threat to the beekeeping industry by infesting a large number of honeybee colonies (Ahmad, 1988; Bakar *et al*., 2017). *V. destructor* feeds on the body fluids of larvae, pupae, and adult bees. This mite has been attributed to the recent widespread of colony collapse disorder (CCD) as a disease vector (Anderson *et al*., 2008).

Varroa mites weaken the immune system of bees and make it more susceptible to viral and bacterial infections (Yang and Cox-Foster, 2005). The parasite causes deformities and weakness of the young workers. Heavy infestation causes death before the emergence of young bees and causes the appearance of mutilated bees (Boecking and Genersch, 2008). The feeding of the mite on honeybees activates the replication process in the infested bees; mites are also vectors of this virus by transmitting them from and to both adult bees and pupae (Ball and Allen, 1988).

A. mellifera and colony death normally occurs within 3–5 years of initial infestation (Korpela *et al*., 1993). The mite acts as a vector for viruses that may cause problems such as bees growing with defective wings and high bee mortality rate (Rosenkranz *et al*., 2010). Without any doubt, most of the colonies of *A. mellifera* in temperate climates will be damaged or even collapse within a few years if no control or inappropriate control methods are used.

Generally, for controlling this ectoparasitc mite *V. destructor* different synthetic acaricides are being used

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0030-9923/2018/0003-0857 $ 9.00/0

worldwide such as amitraz, coumaphos, flumethrin and fluvalianate (Floris *et al*., 2001; Eguaras *et al*., 2003).

Varroa mite has become a major concern of beekeepers since the discovery of the first cases of infestation in the eastern areas of Algeria in 1982 in hives of honey bee *A. mellifera intermissa,* and since that time many acaricides were used against it (Adjlane and Doumandji, 2011). Due to external factors such as climate and/or the application methods, the effectiveness of these products was fluctuating.

The aim of this study was to assess the effectiveness of different synthetic acaricides for controlling *V. destructor* in honey bee colonies.

MATERIALS AND METHODS

The field experiment was conducted at apiary of University of Sargodha. The coordinates of apiary are 32º13´15.7″N, 72 º68´41.8″E. The apiary had 24 Langstroth standard colonies of *A. mellifera* naturally infested with *V. destructor*. Before starting the trial, mite infestation level was monitored to obtain six experimental groups for homogeneity as possible. For testing acaricides, treated and untreated (control) hives were randomly selected in the apiary in order to avoid any bias due to position of the hive in the apiary. Five synthetic acaricides were selected to evaluate their efficacy against *varroa* mites. These acaricides are: Apistan® (fluvalinate), Apitol® (cymiazole), Apivar® (amitraz), Bayvarol® (flumethrin) and Perizin® (coumaphos).

Four colonies were selected for treatments and one remained untreated as control with four replicates. Two strips of each treatment with each having a different amount of active ingredient being impregnated on it was used per hive, one strip was used for every five frames of bees. The strip was hung between the frames by separating slightly so that both sides of the strip came into contact with the bees. All strips remained in the hive for four weeks. The sticky boards were used here to capture falling mites during treatment, in effect, served the same function as screen bottom boards, as mites (if alive) trapped on the boards were prevented from reattaching to host bees.

A white sticky paper sheet was placed on the bottom line of each box covered with wire mesh. Data was recorded once before treatment application in order to estimate the initial population of *varroa* mites in each colony and then at weekly interval. Percent reduction in mites population were calculated by the equation of Henderson and Tilton (1955).

$$\% \text{ Reduction} = \frac{100 \times 1 - (\text{Ta} \times \text{Cb})}{(\text{Tb} \times \text{Ca})}$$

Where, Tb is % infestation of mites before treatment, Ta is % infestation of mites after treatment, Ca is % infestation of mites in control after treatment and Cb is % infestation of mites in control before treatment.

Data analysis

Percent reduction data was analyzed using two way ANOVA to check the significance of treatments and time interval. Means were separated by Tukey HSD all pairwise comparison test. All the analysis was performed in Minitab 16.1 software.

RESULTS AND DISCUSSION

Analysis of variance for percent reduction in fallen mites against different chemicals at their recommended doses after different time interval is showed in Table I. The results showed that treatment (F=546.71, P<0.001) and time interval (F=636.60, P<0.001) showed significant variation in percent reduction of *Varroa*. Furthermore, the interaction of treatment and time interval (F=21.47, P<0.001) was also observed highly significant.

Table I.- Analysis of variance for percent reduction of varroa mite.

Source	DF	SS	MS	F	P
Treatment	4	7398.8	1849.71	546.71	P < 0.001
Time	3	6461.5	2153.82	636.60	P < 0.001
Treatment*Time	12	871.7	72.64	21.47	P < 0.001
Error	40	135.3	3.38		
Total	59	14867.3			

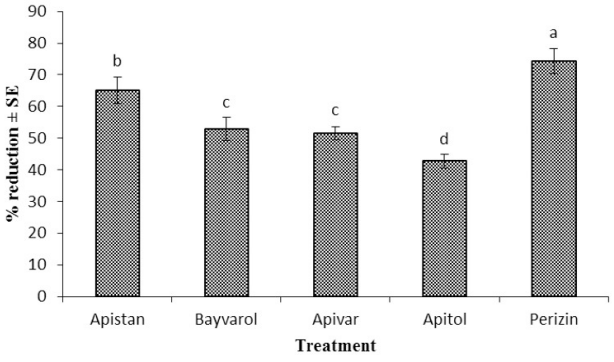

Fig. 1. Percent reduction (Mean ± SE) of fallen varroa mites after application of different chemicals.

Perizin® showed greater reduction (74.3%) of mites and was significantly different from other treatments. The second most effective chemical was Apistan® showing 65.16% reduction of mites. Similarly, the percent reduction

of Bayvrol® and Apivar® was observed 52.9% and 51.5%, respectively. The least affected chemical was Apitol® which showed 42.7% reduction of mites only (Fig. 1).

In present study percent reduction of mites increased with the passage of time. After one week post treatment interval, the percent reduction of mites was 42.67% and reached up to 71% during the 4th week of post treatment interval (Fig. 2).

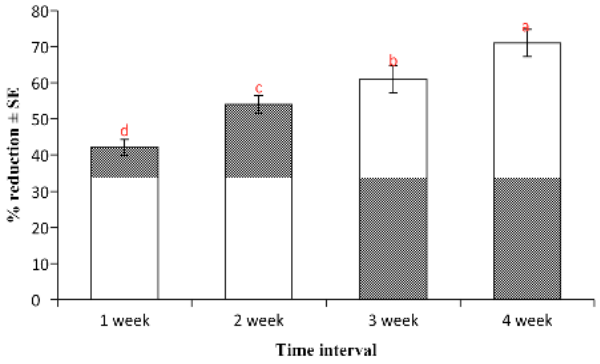

Fig. 2. Percent reduction (Mean ± SE) of varroa mites at different time interval.

The results showed that with the passage of time the number of fallen mites increased in all chemicals but the efficacy in percent reduction was greater in Perizin® compared to other chemicals. The percent reduction of mites shown by Perizin® was 56.67% after the 1st week of application and increased up to 91.3% after the 4th week. So, a 37.95% difference was observed in the reduction percentage from Perizin® during 1st to 4th week—The Apistan® also showed better result after Perizin® showing 81.66% reduction percentage of mites after the 4th week. The lowest percent reduction of fallen mites was observed (32.34%) in Apitol® after 1st week of treatment application and reached to 52.33% after 4th week only. Similarly, the percent reduction of mite was 37.67% after 1st week in case of Bayvarol® and reached to 68.66% after the 4th week. Apivar® showed 42.33% reduction of mites after 1st week of application and increased up to 61% during last week of the experiment. The percent reduction was variable among all the treatment and a statistically significant difference was observed (Table II).

As seen from results, Perizin® and Apistan® showed highest toxicity (91.34%, 81.6%), as followed by Bayvarol® (68.67%) and Apivar® (61.0) %. The least effective acaricide against *V. destructor* was Apitol which showed only 52.3% mortality. The reduced effectiveness of this compound can be due to the extensive and consistent use of this chemical which can show resistance against *Varroa* mite. Our findings are in accordance to Ritter (1981) who obtained 95.7% control of *Varroa* by using Perizin®. The second most effective acaricide was Apistan® in which 81.6% mite fall after application. This observation was also reported by Pileckas and Klimas (2011) in Lithuania. Apivar® exhibited excellent efficacy (76.5%) against *V. destructor* following a short fall treatment (22 days) in a temperate climate (Al Naggar *et al*., 2015). Low effectiveness of Bayvarol® showed by the present experiment agreed with the results obtained by Milani and Barbattin (1989) who obtained effectiveness ranged between 84% and 100%. Jelinski (1993) reported only 59.6% mortality of mites after application of Bayvarol®. The results were also in accordance with Rashid *et al*. (2012) showing a 75-89 % efficacy against *Varroa* mite by using Bayvarol® (flumethrin) strips in Islamabad, Pakistan.

Table II.- Percent reduction (Means±SE) in fallen mites against chemicals after different post treatment interval.

Treatment	1 week	2 week	3 week	4 week
Apistan®	44.33±2.02^{b}	61.33±0.88^{b}	73.34±0.89^{b}	81.66±1.2^{b}
Bayvarol®	37.67±0.88^{cd}	45.67±1.2^{d}	59.66±0.88^{c}	68.67±0.89^{c}
Apivar®	42.33±0.89^{bc}	52.34±0.87^{c}	50±0.57^{d}	61±0.57^{d}
Apitol®	32.34±0.89^{d}	43±1.15^{d}	43.34±1.45^{e}	52.33±1.2^{e}
Perizin	56.67±1.2^{a}	67.67±0.88^{a}	81.66±0.89^{a}	91.34±0.81^{a}

Loucif-Ayad *et al*. (2010) reported in trials conducted in eastern Algeria, efficacy of 89% for Bayvarol® and 85% for Apivar®. Alloui *et al*. (2002) have found 99.1% efficacy using flumethrin soaked stripes on November in Algeria, at the end of six weeks. Trials about another commercial product which contains flumethrin were performed in Turkey and detected 87.7% and 100.0% efficacies in broodless period in fall season (Akkaya and Vurusaner 1996, 1997). In Slovenia, Apivar and Apitol was the least effective chemicals against varroamites. The variations in percent mortality of mites by using acaricides might be due to different climatic conditions and geographic locations, particularly temperature and hive management systems (Trouiller and Watkins, 2001).

The test carried out in Ghazvin, Iran against *V. destructor* showed a good effectiveness, of Apivar® and Bayvarol® (Shahrouzi, 2009). Similarly, average efficacy of amitraz calculated for two years amounted to 90.6% and 94.6% mortality, respectively (Semkiw *et al*., 2013).

CONCLUSION

It can be concluded that all the synthetic chemicals used in this experiment are very effective in reducing the damage and controlling honeybee ecto-parasitic

mite *V. destructor* in honeybee *A. mellifera* colonies without showing any harm to bees. Perizin® (coumaphos) and Apistan® (fluvalinate) strips are highly effective in lowering mite populations with also being advantageous because of being cheap, safe and easy to handle. While, both Bayvarol® (flumethrin) strip as well as Apivar® (amitraz) showing promising results can be included in an integrated mite control program. Beekeepers should use the recommended dosage of chemicals and follow proper application methods to avoid developmental resistance in mites so that proper effectiveness of a chemical can be obtained.

ACKNOWLEDGMENTS

We acknowledge Mr. Qurban, Senior Scientific Assistant and Mr. Umar Daraz, Bee Attendant for collecting mite collection trays.

Statement of conflict of interest

Authors have declared no conflict of interest.

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