Entomology

Control of honeybee ectoparasitic mite, *Tropilaelaps clareae* by using formic acid, fluvalinate injection, and apistan strips in *Apis mellifera* L. colonies

Shah Alam[1]*, Jawad Ali[1], Musaddiq Israr[1], Muhammad Naeem Khan[1], Sheraz Malik[1], Kharis Khan[1] and Komal Tariq[1]

[1] Department of Agriculture, Abdul Wali Khan University, Mardan, KPK, Pakistan

Correspondence Author: Shah Alam

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Abstract

Honeybees carry huge economic importance as they produce a wide variety of important foodstuffs that as honey, Royal jelly, Pollens, and medicinal products like beeswax, Bee venom, and Propolis. The honeybees *Apis mellifera* ectoparasitic mite *Tropilaelaps clareae* is observed which is the main preventive issue in beekeeping, especially for *A. mellifera* in Pakistan. *T. clareae* is a minor mite, that cannot easily see and attack as well as adults and the brood causing 30-70% colony loss of *A. mellifera*. This study was conducted to analyze the efficacy of the acaricides formic acid, Apistan strips, and fluvalinate injections for controlling *T, clareae* infestation in *A. mellifera* colonies. Our result displayed that after treatment with formic acid the number of fall dead mites in between 13-24 in the first week, the number of fall mites in ranged between 3-13 in the second week, and between 0-3 in the third week. The range of dead fallen mites recorded after treatment with Apistan strips was between 4.10 in the first week, 4-9 in the second week, and between 3-7 in the third week. The range for fluvalinate injection treatment was between 4-20 in the first week, 2-10 in the second week, and 2-6 in the third week with mean values of 10.62, 5.37. and 3.5, respectively. The efficacy of formic acid was 95.258%, whereas 93.413% and 91.875% efficacy were recorded from fluvalinate injection and Apistan strips, respectively. Formic acid was highly effective to control mites in honeybee colonies.

Keywords: *Apis mellifera*, formic acid, fluvalinate injections, *Tropilaelaps clareae*, colonies

Introduction

Honeybees carry huge economic importance as they produce a wide variety of important foodstuffs that as honey, and other products also good pollinators of different valuable plants (Ahmad. 1987, Hung *et al*. 2018) [1, 7]. The ecto-parasitic mites, *V. destructor* attack and reduce the bee population all over the world and can cause more damages than any other apiculture disease (Locke *et al*. 2014) [8]. The frequently used acaricides such as pyrethroids, tau-fluvalinate (Apistan), flumethrin (bayvarol) show resistance against *V. destructor* (Martin. 2004) [9]. The ecto-parasitic mites *T. clareae* also affects the *A. mellifera* colonies, the infestation level of *T. clareae* was 45-60 percent (Sharma *et al*. 2003) [17]. For thousands of years, the V. destructor mite has been linked to A. cerana across the subcontinent of Pakistan and India. The Varroa mite has become a significant problem of A. mellifera, destroying many bee colonies (Ahmad. 1988) [2]. Mites are minute organisms belonging to class Arachnida. The size of most of the adult mites ranges from 250 to 750 µm (0.01 to 0.03 inch). However, some of the mites are less than 100 µm (0.01 to 0.03 inch) in adult stage. The mites have two body regions i.e., cephalothorax and opisthosoma or abdomen (Ruppert *et al*. 2004) [14]. The hive of the honeybee is appropriate environment for different mites (Acari), counting the non-parasitic mites, omnivorous, and pollen-feeding species, and parasites (Sammataro *et al*. 2000) [15]. Mites are classified into three groups i.e., parasites, phoretic mites and house guests, that effect the honeybee colonies or originate from the bee hives (De Jong *et al*. 1982b) [5]. The primary pests of honeybee, *A. mellifera* is the ecto-parasitic mites *V. destructor* (Cornman *et al*. 2010) [3].

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Control measures should be used in which parasitic mites population can be suppressed, honey bee health can be protected and honey yield can be increased through some the chemicals like formic acid, fluvalinate injection, and Apistan strips, This study was conducted to analyze the efficacy of the acaricides formic acid, Apistan strips and fluvalinate injections for controlling honey bee ectoparasitic mite *T, clareae* in *A. mellifera* colonies as well as their effects on colony growth and

storage.

Material and Methods

An experimental study on honey bees *A. mellifera* parasitic mites was conducted in Honeybee Research Institute (HBRI), of National Agriculture Research Center Islamabad. The naturally infected with *T. clareae.* Randomly selection of experimental colonies for the treatments.

About 140 adult workers and sealed brood populations of apiaries were assessed for infestation level before selecting the experimental colonies. Randomly select bees sample by infest mites (100 bees/colony) use of Alcohol Wash Technique (De Jong *et al*. 1982a) [4]. Mite infestation of capped brood was evaluated by opening 50 cells of sealed brood before treatment, whereas for the assessment of dead *T. clareae* fallen mite, mite collection trays having white Formica sheet with screen mesh were placed at the bottom of each bee colony. The trays were left for 24 hrs. and dead mites fallen on the trays were counted and used as a measure for mite population. Finally, eight queen right honeybee colonies in Langstroth hives were used that had been standardized (100 mites) for bee frame+ brood + debris infestation levels (Rashid *et al*. 2011b) [13]. The hives were placed at a distance of 3 meters. The experiment was started in July 2019 which is the peak time of the mite population due to the dearth period (Nonavailability of nectar and pollen from flowers). Colonies were divided into 4 groups of 3 colonies each. Colonies of the first group were treated with 10 ml Formic acid (65%) (T_1), the colonies of the second group received Apistan strip 1 strip per colony (T_2), the third group was treated with Fluvalinate injection mixing 1 injection in 1.5-liter water (T_3) and the fourth group served as control (untreated) group (T_4). All colonies received three treatments at a weekly interval. The fallen dead mites of *T. clareae* on mite trays were counted to record their mortality rate. Treatments were only delivered to frame spaces that contained bees; any empty frames were not treated. Each honeybee colony was equipped with a modified bottom board. Mite collection trays (mite excluders) were placed through the backside of the hive covered by a wire screen to prevent the bees from encountering the debris. The rate of ectoparasitic mites *T. clareae* infestation and treatment efficacy was calculated by counting falling mites on a mite collection tray. All the colonies were checked for dead worker bees and queens at the end of the treatment application. The efficacy of all the treatments was calculated by using the following formula.

$$\text{Efficacy} = \frac{\text{Number of mites fallen for each treatment}}{\text{Total number of fallen mites} + \text{Mites fallen in control}} \times 100$$

Results and Discussion

Chemical compounds that occur naturally and are present in honey can be used to control parasitic mites. Few of them including formic acid, fluvalinate strips, and fluvalinate injection have shown the potential effectiveness against these mites, which have no negative effect on the development of colonies. Our results showed that the total number of fallen dead mites after treatment with formic acid ranged between 13-24 in the first week with a mean value of 17.5. The formic acid range between 3-13 in the second week with a mean value of 8.25, Formic acid range between 0-3 in the third week with a mean value of 1.875. The range of dead fallen mites recorded after treatment of Apistan strips was between 4.10 in the first week with the mean value of 7.25, Apistan strips range between 4-9 in the second week with a means value of 6.5, Apistan strips range between 3-7 in the third week with a means value of 4.625. The range for fluvalinate injection treatment was between 4-20 in the first week with a mean value of 10.625, The range between 2-10 in the second week with a means value of 5.375, The range between 2-6 in the third week with a mean value of 3.5, Table 1).

The total maximum number of 221 fallen dead *T. clareae* on the bottom board in mite collection tray was recorded in formic acid (T_1) which was followed by fluvalinate injection (147) and Apistan strip (156), respectively. The efficacy in colonies treated with formic acid was 95.258% while for fluvalinate injection was 93.413 % and for Apistan strip was 91.875% respectively (Table 2). Most of the research work has demonstrated that good efficacy indices have been obtained using at least three applications of liquid formic acid per colony (van Veen *et al*. 1998) which is also confirmed by our experimental study where we used three doses of formic acid.

Table 1: Total number of dead fallen mites after treatment with formic acid, apistan strip, and fluvalinate injection in honey bee *Apis mellifera* colonies

Treatments	Colonies no	1 week	2 week	3 week
Formic acid	1	14	9	3
	2	20	11	1
	3	13	7	0
	4	21	8	2
	5	24	13	3
	6	16	9	1
	7	15	3	2
	8	17	6	3
Control		6	3	2
Apistan strips	1	7	7	4
	2	9	9	5
	3	6	6	4
	4	10	8	4
	5	5	4	5
	6	7	5	7
	7	4	7	3
	8	10	6	5
Control		5	4	4
Fluvalinate injection	1	13	10	3
	2	20	5	2
	3	5	4	4
	4	8	6	5
	5	8	3	6
	6	4	9	3
	7	15	2	2
	8	12	4	3
Control		5	3	3

Table 2: Efficacy of formic acid, apistan strip, and fluvalinate injection in *Apis mellifera*

Treatment	Average total number of mites fallen	The average number of mites fallen in control	Total number of mites in colonies + control	Acaricides efficacy %
Formic acid	221	11	232	95.258
Apistan strip	147	13	160	91.875
Fluvalinate injection	156	11	167	93.413

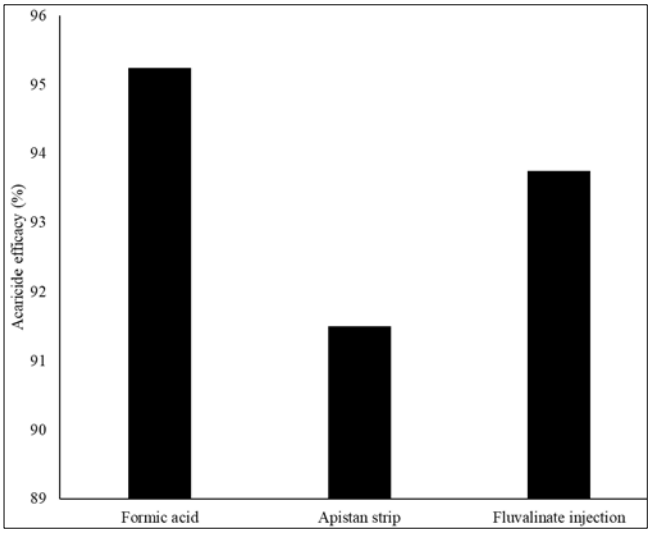

Fig 2: Efficacy of the different Acaricides

In these studies explain that Formica acid fluvalinate injection, and Apistan strips more effective to control ecto parasitic mites. The high fallen mites count in Formic acid application. Formic acid applicators placing in German colonies bee hives at the end bottom board of colonies box (Rashid *et al*. 2011a) [12]. the effect of Formic acid on mites is very high and no side effect on colonies losses (Floris *et al*. 2004) [6]. In our result the lowest fall mites count in Apistan strips treatment colonies as compared to other two chemicals. The effect of Apistan strips is low because of long time of this application introduce, as result the mites may developed a resistance against this acaracides which has also been detected by many researcher (Sammataro *et al*. 2005) [16]. The fallen mites percentage is lower than the Formic acid and high mortality rate as compared to Apistan sptrips.

The Formic acid treatments do not affect adults, offspring, and queens. After treatment the behavior of bees in a colony is normal. No damages in brood but few cases the high doses of Formic acid applied to the bees clustered at the flight. It declared that the doses of acid in small amounts apply in treatment otherwise the high dose effect on bee's health and worker broods. No adult bees mortality was observed during our experimental study. According to the study, 4 doses of formic acid killed 94 percent of the mites, with the most efficient treatment (62 percent of mites destroyed) being 40 ml of 65 percent formic acid (Raffique *et al*. 2012a) [10]. And no losses of the queen during the experiment. In our Experiment we use only German bee hives which is best and easy to mites management as compared to other traditional beehive.

Conclusion

Generally, the chemical Formic acid help beekeeper to control *T. clareae* in a honey bee colony, and it has no side effect the beekeeper is easily used to control both Endoparasite and Ectoparasites Mites in infesting honey bees colonies. Formic acid was a high effective to control mites in honey bees colonies.

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