Pakistan J. Agric. Res. Vol. 26 No. 4, 2013

THYMOL AS CONTROL AGENT OF MITES (*VARROA DESTRUCTOR*) ON HONEYBEES (*APIS MELLIFERA*)

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ABSTRACT:- Studies were carried out on thymol as a controlling agent for bee mites. In this study thymol powder (5mg hive^{-1}) was compared with formic acid 65 % and fluvalinate (strip) as standard control measure against *Varroa* mite. The mite mortality rate was recorded in honeybee colonies of *Apis mellifera* during 2011-12 at research farm of Beekeeping and Hill Fruit Pests Research Station, Rawalpindi. Analysis of variance for different treatments indicated (F=3.64, F>P=0.15) significantly different effect among treatments as well as for the control of mite. The average efficacy of thymol was 73.72% as compared to 69.21% by fluvalinate, 72.23% by formic acid and 13.1% in control. The reduction in the effectiveness of fluvalinate may be attributed to the development of resistance against fluvalinate strip.

Key Words: Apis mellifera; Varroa Mites; Control; Thymol; Formic Acid; Fluvalinate; Pakistan.

INTRODUCTION

Mites are minute arachnid of subclass Acari, family Varoidae, class Acrina and are among the most diverse of all the invertebrate groups. It is estimated that 48,200 species of mites have been described (Halliday et al., 2000). Parasitic mites i.e., *Varroa destructor*, attach to the body of the honeybee, act as ecto-parasite, feed on the hemolymph of immature and adult honey bees indicated low yield of honey in *Apis mellifera* and also cause absconding and swarming (Webster and Delaplane, 2001). *V. destructor* is considered the cause of destruction of *A. melli-fera* colonies in Asia (Rashid et al., 2011).

V. destructor, is the world's most devastating pest of Western honey bees, *A. mellifera* responsible for the vast majority of the damage and honey bee colony losses (Anderson and Trueman, 2000). Mites have killed hundred thousands of colonies worldwide, resulting in billions of dollars of economic loss (Welsh, 2012). *Varroa* mites feed on the developing honeybee larvae, pupae and on the adult bees. Heavily infested colonies usually have large numbers of unsealed brood cells. Dead or dying newly emerged bees with malformed wings, legs, abdomen and thorax present at the entrance of affected colonies and heavily infected colonies produce little or no honey (Ritter, 1981).

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Varroa mites weaken and ultimately kill colonies by out-reproducing their host. Typically, bee population is on peak in late spring/mid summer with a steady decline in population occurring in mid-late summer as a result of the attack of mite (Keith, 2001). *Varroa* mites can transmit multiple viruses to their hosts and that these viruses, not the *Varroa* mites themselves, may cause the majority of the damage that bees experience while hosting the mites (De Jong, 1997).

The mite *V. destructor,* a parasite of *A. mellifera* has to be controlled by regular use of acaricides to maintain honeybee colonies. About 140 hard chemicals products were used in many countries and the mites had showed resistance against coumaphos, amitraz, fluvalinate and flumethrin (Skerl et al., 2011). These hard chemicals have the disadvantages of leaving resistance in honeybee and wax being a hazard to handle, having some level of toxicity to bees and humans and also because mites quickly develop resistance to these active ingredients. On the other hand the use of organic compounds such as thymol, oxalic acid and formic acid have been tested to control *Varroa* infestation in honeybee colonies and have been found better as compared to chemicals (Elzen et al., 2004). Residual problems also occurred by chemicals, research on using of natural products as organic compound (thymol) has been intensified for the control of *Varoa* mite (Akyl et al., 2008). Thymol is a volatile monoterpenoid, a natural constituent of thyme *(Thymus vulgaris*) and is toxic to *Varroa* mites (Lindberg et al.,2000). The efficacy of thymol has been tested in previous studies and recommended that the product is well known effective compound against the *Varroa*, in place of chemical treatments (Gregore and Plannic,2001).

The study was therefore conducted to evaluate the efficacy of environmentally friendly thymol and formic acid as compared to synthetic acaricides fluvalinate (Apistan) strip against *V. destructor*. This will implement the safe and non-contaminated methods of suppressing mite populations in bee hives, increase honey yield and avoid resistance problem.

MATERIALS AND METHOD

The study was conducted on *A. mellifera* colonies at Bee Keeping and Hill Fruit Pests Research Station, Rawalpindi during 2011-12. The experiment was started in December (the peak time of mite population) when mean outer temperature was 3^{0}C. Assessment of 75 bee hives was conducted out for infestation of mites. Sixteen *A. mellifera* bee colonies naturally infested with the *Varroa* mite were selected for this purpose. The data were collected in the morning before the activity of the bees was started. The population of mites was observed by placing sticky sheets of white paper on the bottom board of the bee hive. A sample of 100 bee / colony was taken for recording mite infestation by using alcohol wash technique. The Langstroth hives were used for this purpose.

The colonies were divided in to four groups of four colonies each, first three groups of four colonies received formic acid 5ml $hive^{-1}$, fluvalinate and thymol while the fourth one was without any treatment (control). The

Table 1. Average mite population (fallen mites) in different treatment

S.No.	Treatment	1st week	2nd week	3rd week	4th week	Average mite population
1.	Thymol	74	81	71	52	70
2.	Fluvalinate	72	77	59	52	65
3.	Formic acid	71	75	56	47	63
4.	Control	54	55	42	35	47

treatments were applied at weekly interval.

The pre-treatment data were taken in each case. Four treatments of formic acid received formic acid 65%. The formic acid was applied in the bottom board of hive in the form of cotton balls impregnated with 5 ml of the acid. One fluvalinate (Apistan) strip per colony was hung between the brood frames for four week. Powdered thymol (EYER, Comp. Chemical Reagent, China) @ 5g $hive^{-1}$ applied as dusting over frames in the hive for four week.

Mite population was counted after weekly interval. Morta-lity was recorded from the debris, by counting the numbers of dead mite, from the card boards placed at the bottom board of the hives. The efficacy of the treatments was calculated by using the following modified for-mula of Marinelli et al. (2004).

$$\text{Efficacy} = \text{No. of dead mites fallen} \times 100 / \text{Total no. of fallen mites}$$

The data were taken on weekly basis and were subjected to statistical analysis (Steel et al., 1997). The recorded data was examined for variance by STATISTIX version 8.1 and LSD at 5% level of confidence used to relate the means of treatments.

RESULTS AND DISCUSSION

Average mite population (fallen mites) in different treatments indicated that during the first week after application, the average number of mite population from thymol, fluvalinate, formic acid and control treatment was 74,72,71 and 54 respectively (Table 1).

Regarding mortality of the mite (Table 2), the overall maximum (73%) mortality was recorded from thymol followed by formic acid (72%), fluvalinate (69%), and control (13.17%). The result indicated that treatments have significantly different (F=3.64, F>P=0.15) effects for the control of mites (Table 3). The mortality of mites through thymol was significantly higher than control. The results showed that control of mites by using the

Table 2. Effect of different treatments for mite control

S.No.	Treatment	Population reduction (%)
1.	Thymol	73 [a]
2.	Fluvalinate	69 [b]
3.	Formic acid	72 [ab]
4.	Control	13 [c]

Means followed by same letter do not differ significantly (P > 0.05 LSD =3.16, CV= 24.47)

Table 3. Analysis of variance by thymol and other acaricides

Source	DF	SS	MS	F value	Pr > F
Repeat	3	75.95	25.31	-	-
Treatment	1	128.72	128.72	3.64	0.152
Error	3	106.00	35.33	-	-
Total	7	310.68	-	-	-

formic acid and fluvalinate were almost equal to the thymol and not significantly differ from each other. Present results depicted that thymol is the most effective with slightly higher percentage of control as compared to formic acid (FA) and fluvalinate against *Varroa* mite. The overall results obtained were in accordance with Sharma et al. (1983) and Rashid et al. (2012) who suggested that formic acid and thymol were quite effective for the control of ectoparasite *V. destructor*.

It is thus concluded that thymol is a promising candidate controlling *Varroa* mites. it has many advantages easy to use, safe for beekeepers, it also causes no honeybee toxicity / no loss of queen or brood / adult bee mortality, Furthermore it can also be concluded from this study that , thymol and formic acid proved as effective against mites control, therefore they can be used safely without any side effects in controlling *Varroa* mites.

LITERATURE CITED

Anderson, D.L., and J.W.H. Trueman. 2000. *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Experimental Appl. Acarol. 24: 165-189.

Akyl, E., L. Hali, and Yeninar. 2008. Controlling *Varoa destructor* (Acari: Varroidae) in honeybees *Apis mellifera* (Hymenoptera: Apidae) colonies by using Tymovar and Beevital. J.Anim.Sci. 7: 237-242.

De Jong, D. 1997. Mites: Varroa and other parasites of brood. In Honey Bee Pests, Predators, and Diseases. 3rd edn. A.I. Root Company. USA, p.278-327.

Elzen, P.J., D. Westervolt, and L. Raymond. 2004. Formic acid treatment for control of *Varroa destructor* (Mesostigmata: Varroidae) and safety too *Apis mellifera* (Hymenoptera: Apidae) under Southern United States conditions. J. Econ. Entomol. 97(5):1509-1512.

Gregorc, A. and Planinc, I. 2001. Acaridical effect of oxalic acid in honeybee (*Apis mellifera*) colonies. Apidologie, 32: 333-340.

Halliday, R.B., B. M. O'Connor, and A. S. Baker. 2000. Global Diversity of Mites. In: Peter H. R. and Tania W. (eds.) Nature and Human Society: The Quest for a Sustainable World: Proc. of the 1997 Forum on Biodiversity. National Academies, p. 192-212.

Keith, S. D. 2001. *Varroa destructor*: Revolution in the Making. Bee World, 82 (4): 157-159.

Lindberg, C.M., A.P. Melathopoulos and M.L. Winston. 2000. Laboratory evaluation of miti-

cides to control *Varroa jacobsoni* (Acari: Varroidae), a honeybee Hymenoptera: Apidae) parasite. J. Econ. Ent. 93: 189-198.

Marinelli, E., P. Pulcini, C. Morgia, F. De pace, F. Allegrini and Persanoddo, L. 2004. Prevention of residues in honey. Apiacta 39: 39-43.

Rashid, M., E. S. Wagchoure , S. Raja, G. Sarwar and M. Asim. 2011. Effect of thymol and formic acid against ectoparasitic brood mite *Tropilaelaps clareae* in *Apis mellifera* colonies, Pakistan J.Zool. 43(1): 91-95.

Rashid, M., E. S. Wagchoure, A. U. Mohsin, S. Raja and G. Sarwar. 2012. Control of ectoparasite mite *Varroa destructor* in honeybee (*Apis millifera*) colonies by using different concentrations of oxalic acid. J. Anim. and Pl. Sci. 22(1): 72-76.

Ritter, W. 1981. Varroa disease of honeybee *Apis mellifera*. Bee World, 62:141-153.

Sharma, 0.P., R. Garg, and G.S. Dogra. 1983. Efficacy of formic acid against *Acarapis woodi* (Rennie) Indian Bee J. 45:1-2.

Skerl, M.I.S., M. Nakrst, L. Zvokel and G. Ales. 2011. The acaricidal effect of flumethrin, oxalic acid and amitraz against *Varroa destructor* in honeybee (*Apis mellifera carnica*) colonies, J. Actavet. Brano, p.51-56

Steel, R.G. D., J. H. Torrie, and D. A. Dickey. 1997. Principles and Procedures of Statistical and Biometrical approach. 3rd edn. Mc GrawHill Book Company, New York, p. 182.

Webster, T.C., K.S. Delaplane. 2001. Mites of the Honey Bee. Dadant and Sons, Inc., Hamilton, Illinois. p.28.

Welsh, J. 2012. Mites and Virus Team Up to Wipe out. Beehives Live Science, Retrieved 11 June 2012.