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Effect of Thymol and Formic Acid Against Ectoparasitic Brood Mite *Tropilaelaps clareae* in *Apis mellifera* Colonies

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Abstract.- The study was conducted during March-April, 2007 with two groups each of four honeybee colonies at Honeybee Research Institute, NARC Islamabad. One group was treated with thymol and the other with formic acid. The 4 gm thymol (powdered form) and the formic acid 65% (20ml) were applied 4 times at weekly interval. It was found out that formic acid killed significantly higher number of mites as compared to thymol. The total honey production harvested from colonies treated with different acaricides remained the same.

Key words: Formic acid, honeybees, Laelapidae, mites, thymol.

INTRODUCTION

Of all the insect species, honeybee is perhaps the most beneficial as it produces honey, royal jelly, pollen, propolis and wax etc which are well known to possess great value for their use in pharmaceuticals, food products and other industrial products (Wakhal *et al*., 1999). In Pakistan four honeybee species are found *viz. Apis florea, A. cerana*, *A. dorsata* and *A. mellifera* (Ahmad, 1987). Beekeeping is a profitable business in Pakistan where about 7,000 beekeepers are now rearing exotic species *A. mellifera* in the modern beehives. There are about 300,000 colonies of *A. mellifera* producing 7,500 metric tons honey annually (www.parc.gov.pk.).

Honeybees are also playing an important role in bio-diversity. They are known to increase the yield of insect pollinated crops to the extent of 10 to 20 times more than the cost of honey produced by them. Bee pollination improve the size, shape, color, storage capacity and taste of the fruits (Atwal and Goyal, 2002).

Furthermore, honeybees also improve environment through the valuable pollination of grasses and other wild plants including medicinal plants growing on mountains, landscapes, wastelands, etc. in addition to as a source of food and income. In Pakistan, farmers have little information about importance of bees in pollinating the crops. It is estimated that honey bees account for 80% of all pollination and are responsible for ensuring approximately one third of the food supply. Crops that are dependent upon honey bee pollination or that benefit from honeybees pollination include almonds, alfalfa, apple, cherries, oranges, plums, pears, berries, melons and pumpkins (Hoff, 1995; Ahmad, 1987).

Unfortunately, ectoparasitic mites attack on honeybees, resulting low yield of honey and also cause absconding and swarming. The two mite species *Tropilaelaps clareae* (Laelapidae: Acrina) and *Varroa destructor* (Varroidae: Acrina) are considered to be the cause of continued destruction of *A. mellifera* colonies in Asia (De Jong *et al.*, 1982). Each year many honeybee colonies are damaged or destroyed by mites. Such losses have a devastating impact on the beekeeper, which may have to relocate damaged hives or perhaps even forced out of business. All *A. mellifera* colonies must be treated several times in a year with acaricides to prevent the apparently unlimited growth of mites' populations and the death of colonies.

The mite *T. clareae* is parasitic on bee brood which causes brood malformation, death of the bees and subsequent colony decline or absconding. Development requires about one week and mites are dispersed on bees. *T. clareae* are small mites and not easily seen or collected. This mite attacks both the adult and brood. *T. clareae* was the most

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abundant ectoparasitic on *A. mellifera*. The greatest infestation occurred in February, March and April with a decline from May to August. Poor management of bee colonies, hive microclimate, strength of the colony increased the prevalence of *T. clareae* in bee colonies (Mahavir and Gupta, 1999). *T. clareae* caused 30-70% colony loss of *A. mellifera* with reduced honey production (Woo and Lee, 1997).

The *V. destructor* mite has been associated with *A. cerana* in Pakistan possibly for the last millions of the years. But its incidence on this honeybee has been negligible possibly due to some natural enemies. With the introduction of *A. mellifera* in Pakistan in 1977-78, this mite become a serious pest of this newly introduced honeybee and destroyed a large number of its colonies. Varroa mites feed on the developing honeybee larvae, pupae and 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 thoraxes may be present at the entrance of affected colonies. Colonies heavily infected by *Varroa* produce little or no honey (Ritter, 1981).

The beekeepers have been forced to use unapproved products consisting of chlorobenzilate, phenothiazine, sulphur, amitraz or different parathyroid to control the mite infestation as no approved miticides for use in bees has been available to date. Uncontrolled use of these agents has led to the development of resistance, resurgence of the infestation and the risk of residues in the honey which might pose a risk for human consumption. The miticides like formic acid and thymol might be able to solve this problem in beekeeping industry, once they have been approved, if they are applied regularly and according to the recommendations. Introduction of these scientifically approved miticides would be beneficial to beekeepers and could enhance the production and export of high quality honey (Pichai *et al*., 2008).

Keeping in view the importance of safe and non-contaminated methods to suppress mite populations in beehives to increase honey yield and to escape from resistance problem, the present study was aimed with the objectives to (i) To determine the efficacy of formic acid and thymol against *Tropilaelaps clareae*, (ii) To observe the honey production of colonies treated with formic acid and thymol.

MATERIALS AND METHODS

The present study was conducted at Honeybee Research Institute of National Agricultural Research Centre, Islamabad on *Apis mellifera linguistica* honey bee colonies infested with the *T. clareae.* Treatments were given randomly to all experimental colonies which were requeened with hygienic queens prior to the start of the experiment.

About 50 adult and sealed brood populations of apiaries were assessed for infestation before selecting the experimental colonies. To collect the sample (150 bees/colony) of mite infestations the alcohol wash technique was used (De Jong *et al*., 1982). To get an accurate adult bee count the colonies were inspected at sunrise before the bees started foraging. The mite infestation was evaluated by opening 100 cells of sealed brood before treatment (Burgett and Burikam, 1985) while for the assessment of mite population in debris mite collection trays placed at the bottom of the bee colony. The trays were left for 24hrs period and mites fell on the trays were counted and used as 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. The hives were placed at a distance of 5 meters. The experiment was started in the month of March (*i.e*. the peak time of mite population) when mean outer temperature was 24°C and bees were bringing Brassica pollen and nectar which was in the amount only for the survival of bees not for producing honey for extraction purpose. Colonies was divided into 2 groups of 4 colonies each. One group (T) was tested with thymol, finely grinded and the second group (F. A) received formic acid. Group T received four treatments (4g) with a weekly interval, testing a total amount of 16g thymol crystals placed in Petri dishes (80mm) on top of the brood frame under the top cover of hives. Group F. A received 4 treatments of formic acid (20ml each) applied on card board

placed in the mite collection trays placed in the deep bottom board of the hive. Total 80 ml was applied at weekly interval. At the end, all the experimental colonies were given Apistan strips. Each honeybee colony was equipped with a modified bottom board. Mite collection trays (mite excluders) were placed through the back side of the hive covered by a wire screen to prevent the bees from coming into contact with the debris. The rate of *T. clareae* infestation and treatment efficacy was estimated by counting falling mites on mite collection tray.

The mite fall were counted on the mite collection trays on day 7, 14, 21 and 28. Mite mortality was examined weekly in debris collected in mite collection trays placed under the screen (Table I). In order to evaluate total mite population an Apistan strip was applied to the colonies. Apistan strip was removed from the colonies after 30 days and dropped dead mites were counted. Treatment efficacy was calculated for each colony as follows:

$$E = \frac{V\,D7 + V\,D14 + V\,D21 + V\,D28}{VT} \times 100$$

where E is effectiveness, V D+n is mites collected per week, and VT is total mites collected.

Honey harvesting

Honey was harvested (from Acacia crop which started blooming in April and ends in May) after experiment with the help of manually operated honey harvester and honey yield of treated colonies (Thymol and Formic acid) was compared. Honey production was measured by taking the weight of each hive body used for honey collection before and after the honey extraction process. The weight difference was considered as the amount of harvestable honey.

RESULTS AND DISCUSSION

We used SPSS statistical programme version fourteen to analyze our data. Comparisons between means were made using the least significant difference (LSD) at 0.05 probabilities.

A range of organic compounds that occur naturally and are present in honey can be used to control parasitic mites. Few of them including formic acid and thymol have shown potential effectiveness against these mites, which have no negative effect on the development of colonies (Melathopoulos and Gates, 2003; Floris *et al.*, 2004). The results obtained are shown in Tables II, III. Significant number of mites presented in both groups were found (One Way ANOVA, $F_{(1, 31)} = 8.140$, $P < 0.005$) showing colonies with different levels of infestation. Thymol is the main constituent of several commercially available medicinal products and numbers of studies have demonstrated its efficacy at controlling mite infestations in honey bee colonies, but with variable results (Calderon *et al.*, 1997; Imdorf *et al.*, 1996; 1999). In our results the number of mites fallen for the thymol ranged between 37-105 with a mean value of 69.31±4.85 (Mean±SE), while the range for formic acid treatment was between 33 to 142 with the mean number of 94.12±7.21(Mean±SE) respectively (Tables II, III) which is not in agreement with Imdorf *et al.* (1995) who demonstrated that thymol had the highest varroacidal activity at concentrations well tolerated by the bees but is confirmed by Harolds *et al.* (1989) who found that mites were best controlled by placing formic acid plates at the bottom board of the colonies and after four treatments at four days intervals 94% of the mites were killed.

The range of efficacy in colonies treated with thymol was 60.50 to 62.15 while for formic acid the range was 77.59 to 82.87. The mean value of efficacy for thymol and formic acid was 61.49 and 79.52 (Tables II, III). The percentages were arcsine square root transformed and when compared between thymol and formic acid they were found to be significantly different (One Way ANOVA, $F_{(1, 7)} = 199.67$, $P<0.005$). Much research has demonstrated that good efficacy indices have been obtained using at least three applications of liquid formic acid per colony (Fries, 1989; Mutenelli *et a1.*, 1994; Eguaras *et al.*, 1996; Van Veen *et al.*, 1998) which is also confirmed by our experiment where we used four doses of formic acid.

The honey produced from different hives when treated with acaricides was also weighed at the end of experiment. The mean amount of honey produced in kg from thymol and formic acid treated colonies were 14.33±5.93, 11.81±16.5 (Mean±SE),

Table I.- The number of mites collected from colonies of *Apis mellifera* when treated with thymol and formic acid.

Colonies	Thymol treatment				Formic acid treatment			
	Week 1	Week 2	Week 3	Week 4	Week 1	Week 2	Week 3	Week 4
1	50	75	90	75	75	89	125	33
2	53	72	98	37	71	81	110	53
3	61	82	105	69	97	111	129	82
4	47	69	80	46	100	121	142	87

Table II.- Acaricides efficacy of thymol in *Apis mellifera* colonies.

Colonies	Mites killed by thymol	Mites killed by apistan	Total number of mites/colony	Acaricides efficacy (%)	Honey production (kg)
1	290	180	470	61.70	13.12
2	260	162	422	61.61	15.09
3	317	193	510	62.15	14.01
4	244	156	398	60.50	15.11
Mean	69.43	43.18	112.50	61.49	14.33

Table III.- Acaricides efficacy of formic acid in *Apis mellifera* colonies

Colonies	Mites killed by formic acid	Mites killed by apistan	Total number of mites/colony	Acaricides efficacy (%)	Honey production (kg)
1	322	93	415	77.59	11.56
2	315	85	400	78.59	11.89
3	419	111	530	79.05	12.55
4	450	93	543	82.87	11.24
Mean	94.12	23.87	118.00	79.52	11.81

respectively (Tables II, III). The honey produced was also compared but the results were non significant (One Way ANOVA, $F_{(1, 7)} = 0.31$, $P > 0.005$).

It can be concluded from the experiment that since formic acid is also effective against *Acarapis woodi* (Sharma *et al*., 1983), it can be used safely without any side effects in controlling both endo and ectoparasitic mites infesting honey bee colonies.

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