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Control of *Tropilaelaps clareae* Mite by Using Formic Acid and Thymol in Honey Bee *Apis mellifera* L. Colonies

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Abstract.- The proposed research work was conducted in March, 2008 at Honeybee Research Institute of National Agricultural Research Centre, Islamabad on *Apis mellifera lingustica* honeybee colonies infested with the *Tropilaelaps clareae*. Treatments were given by dividing experimental 36 colonies into two groups (Formic acid and Thymol). Formic acid (70%) and Thymol were applied in six treatments including control (To=0.0 ml, T1=5ml, T2=10ml, T3=15ml, T4=20ml and T5= 25ml) and (To=0.0g, T1=5g, T2=10g, T3=15g, T4=20g, and T5=25g) respectively with three replications for five weeks. The treatments were given randomly by using complete randomized design (CRD). The maximum number 782 and 518 of mites collected in mite collection trays treated with 25 ml formic acid and 25 g of thymol. The highest efficacy of formic acid and thymol against *T. clareae* on honeybee was 97 and 95.3%. In formic acid group T5 (25 ml) yielded the maximum honey 22.54 kg/colony. In thymol group, T5 (25g) yielded the maximum honey 20.7 kg/colony. From all the results it was clearly observed that all the treatments of formic acid and thymol are effective against the *T. clareae*. It was observed that during whole experiment no queens were lost and there was no adult honeybee mortality in any of the colonies.

Key words: Formic acid, thymol, *Tropilaelaps clareae*, honey yield.

INTRODUCTION

Of all insect species, honeybee is perhaps the most beneficial as it produces honey. About 200 million pounds of honey is produced commercially each year. But the honeybee makes its greatest contribution by pollinating plants. More than one half of all fruit and vegetable crops, are pollinated by honeybees. In Pakistan four honeybee species are found *viz. Apis florea*, *A. cerana*, *A. dorsata* and *A. mellifera*. There is lack of knowledge among farmers about importance of bees in pollinating the crops. Low honey production and destruction of colonies is mainly due to the attack of parasitic mites. In Asian countries like, China, Pakistan and India, regular destruction of *A. mellifera* colonies is caused by two mite species *i.e*. *Tropilaelaps clareae* and *Varroa destructor* (Ahmad, 1987).

About 80% of all crop pollination is being done by honey bees and thus ensuring approximately one third of the food supply. Crops like almonds, alfalfa, apple, cherries, oranges, plums, pears, berries, melons and pumpkins are mainly dependent upon honey bee pollination (Hoff, 1995).

In Pakistan, beekeeping is a profitable business. It is reported that there are more than 4,000 beekeepers rearing *A. mellifera* in the beehives, about 400,000 colonies of *A. mellifera* has been producing 10,000 MT honey annually and 27000 families are being benefited from beekeeping (Annual Report, PARC 2010-11). Pakistan Agricultural Research Council (PARC) in late 1977-78 introduced the western honeybee *A. mellifera* from Australia among the commercial beekeepers because of least output of *A. cerana* (Waghchour-Camphor and Martin, 2008). Just after the introduction of *A. mellifera* in Pakistan, *V. destructor* mite became a serious pest of this newly introduced *A. mellifera* and attacked over a large number of honeybee colonies (Ahmad, 1988).

Infestation by *Tropilaelaps* is accompanied by irregular brood pattern and death of many bee larvae (up to 50%). Many malformed bees occur, with distorted abdomens, stubby wings and deformed or missing legs. Some of the affected bees crawl at the hive's entrance and especially the presence of fast-running, large, red-brown, elongated mites on the combs are diagnostic for the

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presence of *T. clareae.* An early diagnosis can be made after opening brood cells and finding immature and adult mites therein. The hive (colony) may be treated with various chemicals that cause the mites to drop off combs and bees. In some cases the colony absconds, carrying the mites to a new location (Delfinado and Baker, 1982; Burgett *et al.*, 1983). Hive microclimate and insufficient management of bee colonies are the main factors for multiplication of mites. Approximately, 30-70 % reduction in honey yield was observed due to infestation of *T. clareae* (Woo and Lee, 1997). Apiculturists remained bound to tackle the parasitic mites with acaricides, like coumaphos and fluvalinate, the most regularly remedies used for this purpose. In the last decade resistances against medication of the synthetic acaricides have been increased (Loglio and Plebani, 1992; Elzen *et al*., 1998).

Beekeepers are bearing heavy financial losses due to the parasitic mites attack on honeybees. So, it is very essential to find other non-toxic and effective methods to suppress *T. clareae* mite populations. Keeping in view the importance of safe and (for honey) non-contaminated methods to suppress mite populations in beehives to increase honey yield, the present study was aimed at determining the efficacy of formic acid and thymol against *Tropilaelaps clareae*, and to observe the honey production of colonies treated with formic acid and thymol.

MATERIALS AND METHODS

This work was conducted in March 2008 at Honeybee Research Institute of National Agricultural Research Centre, Islamabad on *Apis mellifera lingustica* honey bee colonies infested with the *T. clareae*. About 150 colonies with adult workers and sealed brood bee were assessed for infestation before selecting the experimental colonies. In deep bottom board of beehives, the mite capturing trays were placed for counting fallen mites. Infestation levels of *T. clareae* and treatment effectiveness were determined by counting falling mites on mite capturing trays for 24 h. To determine the mite infestation level on adult honeybees, the sample of 250 bees/colony was collected by using alcohol wash technique (De Jong *et al.*, 1982). The mite infestation was evaluated by opening 100 cells of sealed brood before treatment (Burgett and Burikam, 1985) and counting the mite population level in debris mite collection trays kept for 24h in the bottom board of bee hive (Devlin, 2001). Finally, thirty six queen right honeybee colonies in Langstroth hives were used on equal mite infestation levels with at least ten bee frames.

Control of mites in honey bee colonies using the formic acid or thymol

Thirty six Langstroth honeybee colonies were divided into two groups (Formic acid and Thymol). The treatments were given randomly by complete randomized design (CRD). A modified bottom board for placing mite collection trays covered by a wire gauge to avoid the bees contact with debris was provided to each experimental honeybee colony without disturbing colonies through the rear door of the hive. The fallen dead mites into trays were counted to record their mortality rate. A distance of 5 meters was kept between the honeybee colonies of each group. Experimental colonies were divided into 2 groups having 18 colonies each. One group (F.A) received six treatments of formic acid including control (To=0.0 ml, T1=5ml, T2=10ml, T3=15ml, T4=20ml and T5=25ml) with three colonies for each treatments at weekly interval for five weeks. Second group (T) was tested with six treatments of finely ground Thymol including control (To=0.0g, T1=5g, T2=10g, T3=15g, T4=20g, and T5=25g) in three replications at weekly interval for five weeks. The formic acid was applied by pouring on a cotton cloth placed in the deep bottom board of honeybee colonies. The Petri dishes of thymol crystals were placed on the top of brood frames under the top cover of hives.

Treatment effectiveness

To assess the efficacy, the mite population on adult honey bee and in mite collection trays of all bee colonies were examined from week 0 to week 5 at weekly intervals. To compare the mite infestation between the group's geometric means were calculated. To calculate the efficacy, the mean of mites per colony in the treatment groups (Nt) will be compared with the mean of mites per colony in the control group (Nc) at each time point. To follow a

normal distribution more closely and not to overbalance extreme values geometric means were calculated as recommended by guidelines on assessment of parasitic drugs (Woyke, 1985). The treatment percent efficacy was calculated by using following formula:

$$\text{Efficacy (\%)} = \frac{N\,c - N\,t}{N\,c} \times 100$$

Honey yield

Honey was harvested after experiment with the help of manually operated honey harvester and honey yield of treated colonies was compared with control. It was observed that during whole experiment no queens were lost and there was no adult honeybee mortality in any of the colonies.

Statistical analysis

All the data collected were analyzed for analysis of variance (ANOVA) using MSTAT-C software. Least significant difference (LSD) test at 5% probability level was used to compare the means (Steel *et al*., 1997).

RESULTS AND DISCUSSION

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).

Effect of formic acid

Fallen mites

Maximum number 782 of mites collected in mite capturing trays in T5 followed by T4, T3, T2 and T1 where the total number mite captured were 550, 378, 315 and 255, respectively where as the corresponding level of mites fallen in case of T0 was minimum and only a total number of 99 mites were collected in five weeks of study (Fig. 1A). Higher doses of the formic acid results in increased number of fallen mites. These results are line with the findings of Gregorc and Planinc (2001) who obtained 99% *T. clareae* mite mortality by the application of 20 ml formic acid. Rashid *et al*. (2011) found out that formic acid killed significantly higher number of mites as compared to thymol and control group. Our results are 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 in conformation with Harold *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 and the most effective treatment (62% of mites killed) was with 40 ml of 65% formic acid (Greatti *et al.*, 1993).

Honeybees

The formic acid treatments were tolerated well by all the colonies since there was no loss of queens. The behavior of the bees after treatment was normal. No severe damage to the bee brood could be found but it could be seen in certain cases when high doses of formic acid was applied and many bees clustered at the flight entrance. It seems that they wanted to escape from the unusual smell of the acid. No mortality occurred during experiment. These observation are in line with those of Elzen *et al*. (2000) who found no queen loss and no supersedure of the queens. Rashid *et al*. (2011) reported that no bee mortality was observed when applied 20 ml formic acid for four weeks. Mutinelli *et al.* (1996) reported low or no mortality in all tests of formic acid, lactic acid or Apilife-VAR®. El-Shaarawy (1999) claimed that honey yield increased after colonies treated with Apiguard® or formic acid.

T. clareae *on adult bees*

The efficacy of the different formic acid treatments against *T. clareae* on adult bees was calculated based means per treatment, compared to the mean of untreated group. In this study, the highest efficacy against *T. clareae* on adult bees was demonstrated for the treatment with T5 *i.e.* 97% followed by the T4, T3, T2 and T1, which showed the efficacies as 81%, 74%, 63% and 54%, respectively (Fig. 2A). The other proof regarding the efficacy of the treatments in same trend is the

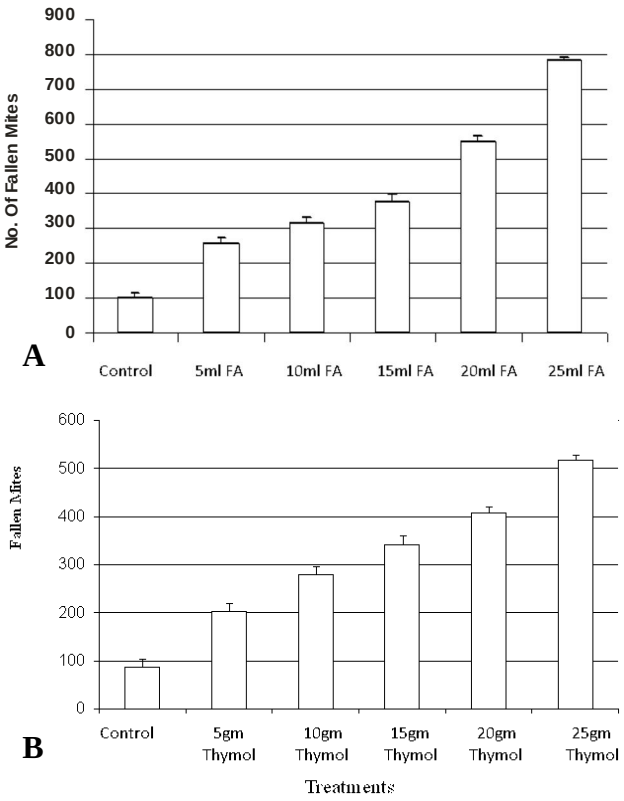

Fig. 1. Average number of fallen *Tropilaelaps* mites in mite capturing trays by administering five applications of different concentrations of formic acid (A), and thymol (B) for five weeks.

fallen of dead mites in mite capturing trays. These results are in agreement with the findings of Pichai *et al*. (2008) who observed the high efficacy of formic acid against *T. mercedesae* and *T. clareae* in the European honeybees. The decreasing percentage of *T. mercedesae* in each colony in the first, second, third and fourth week after applying the treatments of 5, 10, 15 and 20 ml of formic acid averaged 86.1, 51.6, 94.2 and 100 % elimination of *T. clareae* the respectively. The high effectiveness showed by formic acid in this study and low toxicity for bees and men jointly with its low residual capacity in the honey (Imordf *et al.*, 1995) makes this compound a good alternative of parathyroid (apistan, fluvalinate) for mite management in the hives for good honey yield. Rashid *et al*. (2011) also found that efficacy of 20 ml formic acid against *T. clareae* was higher as compared to 4g thymol and untreated group.

It has been demonstrated that good efficacy indices are 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 five doses of formic acid.

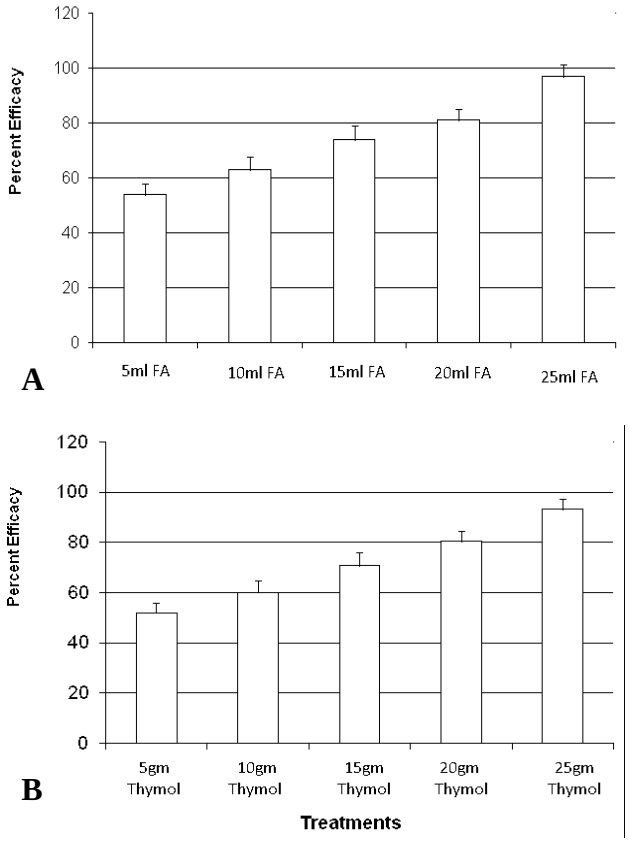

Fig. 2. Average percent efficacy of different concentrations formic acid (A) and thymol (B) against *T. clareae* on adult honeybees.

Effect of thymol

Fallen mites

Maximum number of mites (518) were collected in mite capturing trays in T5 followed by T4, T3, T2 and T1 where the average total number of mite captured were 407, 342, 280 and 202, respectively. The corresponding level of mites fallen in case of T0 was minimum and only a total number of 86 mites were collected in five weeks of study (Fig.1B). The results are clearly in agreement that if

we go on applying higher doses of the thymol the number of fallen mites increases due to the killing effect of Thymol. These results are line with the findings of Gregore and Planinc (2001) and Rashid *et al*. (2011) who obtained 60 to 99% mite mortality by applying 20g thymol and collected maximum number of *T. clareae* and recorded minimum number of mites fallen in case of control hives where no application of thymol was done. Bollhalder (1998) and Calderone (1999) reported that thymol was very effective for the control of bee mites and has no side effects on honeybees.

some researchers have recorded adverse effects on bees after treating them with essential oils or their components. Lensky *et al.* (1996) found that 30% thymol was harmful to the bees depending on dose and ambient temperature. Chiesa (1991) and Gal *et al.* (1992) have also reported adverse effects of thymol on bee colonies during summer. Mattila and Otis (1999) reported that honey production was reduced 30% by Apiguard® treatment.

Honeybees

The thymol treatments were tolerated well by all the colonies. There was no loss of queens. The behavior of the bees after treatment was normal. No severe damage to the bee brood could be found. No increase in bee mortality rate could be found during the test. These observation are in line with the observations of the Elzen *et al*. (2000) who found no queen loss and nor supersedure of the queens after the application of thymol for mite control on honeybees. Rashid *et al*. (2011) reported that no bee mortality was observed when 20g thymol was applied for four weeks.

T. clareae *on adult bees*

The efficacy of different thymol treatments against *T. clareae* on adult bees was calculated. The highest efficacy against *T. clareae* on adult bees was demonstrated for treatment with T5 *i.e.* 95.3% followed by the T4, T3, T2 and T1, which showed the efficacies of 86.5%, 79.3%, 68.2% and 56%, respectively (Fig. 2B). The other proof is the fallen dead mites in mite capturing trays. These results are in agreement with the findings of Pichai *et al*. (2008) who observed high efficacy of thymol against *T. mercedesae* and *T. clareae* in *Apis mellifera*. The decreasing percentage of *T. mercedesae* in each colony in the first, second, third and fourth week after applying the treatments of 5, 10, 15, 20 and 25g of thymol averaged 86.1, 51.6, 94.2 and 100% elimination of *T. clareae*, respectively. The high effectiveness of thymol and low toxicity for bees and men jointly with its low residual capacity in the honey (Imordf *et al.*, 1995) makes this compound a good alternative of parathyroid (Apistan) for mite management in the hives for good honey yield. Rashid *et al*. (2011) also found that efficacy of thymol against *T. clareae* was higher as compared to untreated group.

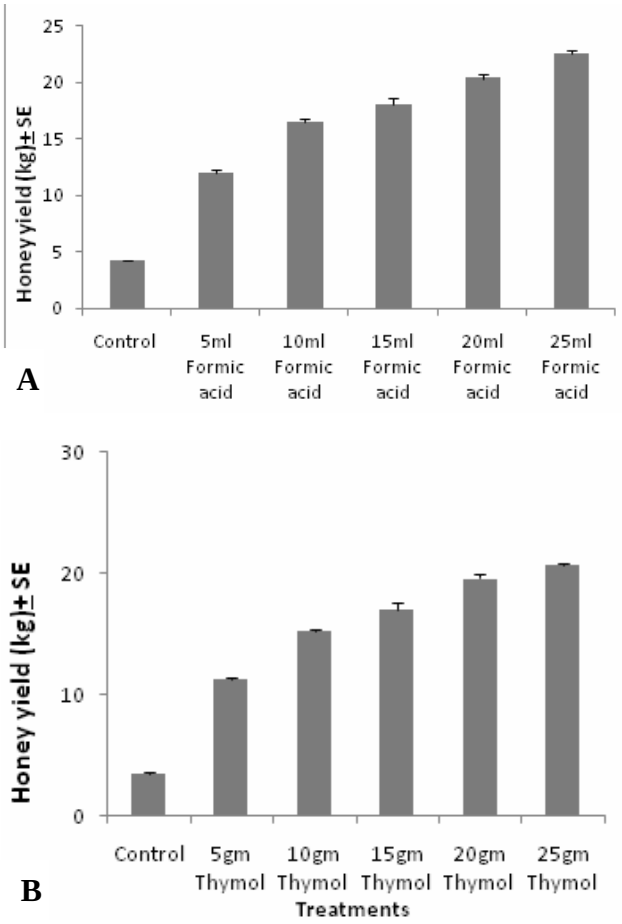

Fig. 3. Honey harvested (mean per colony) from formic acid treated (A) and thymol-treated colonies (B).

Honey yield

Formic acid treated colonies

After five weeks of study, the honey was

harvested from all the experimental colonies treated with formic acid. In T0 only 4.2 kg of honey per colony was obtained which shows the increased mite infestation on bees causing low honey yield; whereas T5 yielded 22.54 kg honey/colony followed by the T4, T3, T2 and T1 which yielded honey 20.34, 18, 16.5and 12 kg honey/colony, respectively (Fig. 3A). Rashid *et al*. (2011) found out that formic acid treated colonies produced more honey compared to the thymol-treated and control groups.

Thymol treated colonies

After five weeks of study, the honey was harvested from all the colonies treated with thymol. In T0 only 3.5 kg of honey per colony was obtained which shows the increased mite infestation on bees causing lower honey yield; whereas T5 yielded the 20.7 kg honey/colony followed by the T4, T3, T2 and T1which yielded 19.6, 17, 15.3 and 11.3 kg honey/colony, respectively (Fig. 3B). Rashid *et al*. (2011) found out that thymol treated colonies produced more honey compared to untreated colonies.

It can be concluded from the experiments 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. So it was concluded that formic acid and thymol could provide beekeepers an effective solution to fight *T. clareae* mite in bee colonies.

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