Pakistan J. Zool., vol. 44 (4), pp. 985-989, 2012.

Control of Ectoparasitic Mites in Honeybee (*Apis mellifera* L.) Colonies by Using Thymol and Oxalic Acid

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Abstract.- A study was carried out to determine the effects of thymol (powdered form) with 3.2% oxalic acid (OA) on two ectoparasitic mites, *Varroa destructor* Anderson and Trueman (Acari:Varroidae) and *Tropilaelaps clareae* Delfinado and Baker (Laelapidae: Acrina) populations in honeybee *Apis mellifera linguistica* (Hymenoptera: Apidae) colonies in the fall. Thymol 2, 4 and 6 g with 3.2% OA applied to twenty honeybee colonies thrice on different dates, showed 26, 41, 36% mortality in *T. clareae* and 93, 99 and 94% mortality in *V. destructor*, respectively. The results showed that 3.2% OA with 4g thymol was the best treatment for controlling these mites. No queens were lost, and there was no adult honeybee mortality in any of the colonies during the experiment.

Key words: Honeybee, *Varroa destructor*, *Laelapidae*, mites, thymol, oxalic acid, trickling.

INTRODUCTION

Honey bee colonies are subject to infestation by mites, insects and diseases. Ecto-parasitic mites attack honeybees and cause low yield of honey, absconding and swarming of bee colonies. The two mite species *Tropilaelaps clareae* (Laelapidae: Acrina) and *Varroa destructor* (Varroidae: Acrina) are considered to be the cause of continued destruction of *Apis mellifera* colonies in Asia (De Jong *et al.*, 1982). Each year many honeybee colonies are damaged by mites. *A. mellifera* colonies must, therefore, be treated several times in a year with acaricides to prevent unlimited growth of mites' populations and the death of colonies (Khan *et al.*, 1987).

The mite, *T. clareae*, is a parasite on bee brood causing brood malformation, death of the bees and subsequent colony decline or absconding (Hosamani *et al.*, 2006). Development requires about one week and mites are dispersed on bees. *T. clareae* occurs on 5 species of bee - *Apis cerana*, *A. dorsata*, *A. florea*, *A. laboriosa* and *A. mellifera*. *T. clareae* is known to have a wide distribution in Asia extending eastwards from Iran to Papua New Guinea (Matheson, 1995). The greatest infestation occurred in February, March and April with a decline from May to August (Camphor *et al*., 2005). 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 always has been associated with *A. cerana* in Pakistan. With the introduction of *A. mellifera* in Pakistan in 1977-78, this mite become a serious pest and destroyed a large number of colonies (Ahmad, 1988). *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 infested by *Varroa* produce little or no honey (Ritter, 1981).

The beekeepers have been forced to use unapproved chemicals such as chlorobenzilate, phenothiazine, sulphur, amitraz or different pyrethroids to control the mite infestation. Uncontrolled use of these agents 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.

Keeping in view the importance of safe and non-contaminated control methods to suppress mite

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0030-9923/2012/0004-0985 $ 8.00/0
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populations in beehives and to escape from resistance problem, the present study was aimed at determining the efficacy of thymol and oxalic acid as miticide against *T. clareae* and *V. destructor* mites.

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 naturally infested with the *T. clareae* and *V. destructor* mites. Treatments were given randomly to all experimental colonies which were requeened with hygienic queens (Spivak and Reuter, 1998) prior to the start of the experiment.

About 150 adult workers and sealed brood populations of apiaries were assessed for infestation level before selecting the experimental colonies. To collect the sample (250 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 of capped brood 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 24h period and mites fell on the trays were counted and used as measure for mite population (Devlin, 2001). Finally, twenty 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*., 2011). The hives were placed at a distance of 5 meters. The experiment was started in the month of December (*i.e.,* the peak time of mite population) when mean outer temperature was 3°C. Colonies were divided into 4 groups of 5 colonies each. Colonies of the first group were treated with 2gm finely grinded thymol plus 3.2% OA (T1), the colonies of the second group received 4gm finely grinded thymol plus 3.2% OA (T2), the third group was treated with 6gm finely grinded thymol plus 3.2% OA (T3) and the fourth group served as control (untreated) group (C). All colonies received three treatments with a weekly interval. Thymol GPR^{TM} (BDH Laboratory supplies poole, Bh15 1TD, England. Lot A 8947729) crystals finely grinded were placed in Petri dishes (80mm) on top of the brood frame under the top cover of hives. Oxalic acid was applied in sugar syrup. To obtain 3.2% OA solution, 75 g oxalic acid dehydrate was mixed with 1 liter of sugar water (1:1) (Prandin *et al*., 2001). Treatments were only delivered to frame spaces that contained bees; any empty frames were not treated. All colonies of three groups received 3.2% oxalic acid solution with three doses at seven days interval. The 5 ml mixture was trickled directly on to the adult bees in between two frames using a syringe as recommended (Imdorf *et al*., 1997; Brodsgaard *et al*., 1999). 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 both ectoparasitic mites *V. destructor* and *T. clareae* infestation and treatment efficacy was calculated by counting falling mites on mite collection tray. At the end, all the experimental colonies were given Fluvalinate (Apistan) strip for knockdown. Apistan strips were removed from the colonies after four weeks and dropped dead mites were counted. All the colonies were checked for dead worker bees and queens at the end of treatment application. The efficacy of the all the treatments was calculated by using following formula (Marinelli *et al*., 2004).

$$\text{Efficacy} \quad \frac{\text{No. of mites fallen for each treatment}}{\text{Total number of fallen mites}} \quad \text{X } 100$$

The results were analyzed using SPSS statistical program version 14. Comparisons between means were made using the least significant difference at $P < 0.05$ probabilities (SPSS). For statistical data, standard descriptive statistics were performed for each of the quantitative parameters.

Honey harvesting

Honey was harvested with manually operated honey harvester and honey yield of treated colonies 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.

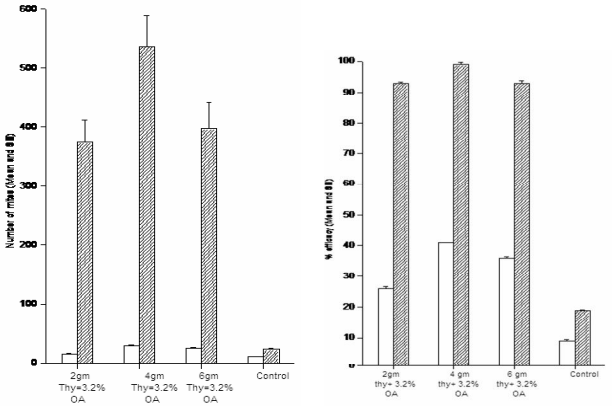
RESULTS AND DISCUSSION

In our results the number of *Tropilaelaps* mites fallen for the T1, T2 , T3 and control ranged between 10-22, 22-38, 18-39 and 8-13, respectively. In the case of *Varroa* the ranges for T1, T2, T3 and C was 186-570, 234-789, 168-612 and 12-35. The mean number of *Tropilaelaps* and *Varroa* fallen for each treatment are shown in Figure 1.

When different treatments were compared for *Tropilaelaps* a highly significant difference was found for the number of fallen mites (one way ANOVA, $F_{3, 59}$= 52.387, $P < 0.00$). A significant difference was found between all the treatments [post-hoc test, $P > 0.01$]. The number of *Varroa* fell for each treatment was also compared and the results obtained were in accordance with the *Tropilaelaps* mites (one way ANOVA, $F_{3, 59}$= 30.670, $P < 0.00$). The highest number of mites fell in T2 and when different treatments were compared by using Post hoc tests it was found that only T2 was significantly different from all the other treatments (post-hoc test, $P < 0.00$), which clearly showed T2 to be the most effective miticide against *Varroa* mites.

In the case of *Tropilaelaps* the range of efficacy in colonies treated with T1, T2, T3 and control was 26-28, 39-43, 35-39 and 8-9%, respectively. The mean value of efficacy for the said treatments also varied between different treatments. The percentages were arcsine square root transformed and when compared were found to be significantly different (One Way ANOVA, $F_{(3, 19)}$ = 220.41, $P < 0.00$) where T2 was found to have the highest efficacy (Fig.1). For the *Varroa* mites the efficacy range for T1 T2, T3 and control was 91-95, 98-99, 93-95 and 18-21%. The results showed a highly significant difference between efficacies (One Way ANOVA, $F_{(3, 19)}$ = 220.41, $P < 0.00$). The T2 again showed the highest efficacy of 99% respectively (Fig. 1).

Overall OA was found to be quiet effective against mites which is in accordance with other studies results (Brodsgaard *et al.*, 1999; Gregorc and Planinc, 2001; Gregorc and Poklular, 2003) showing that OA is very effective against *Varroa*. The results also confirm that the 3.2% OA for normal sized colonies can be used for mite control with good results without any obvious adverse effects on bee colonies over winter (Fries, 2007). 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 (Calderone *et al.*, 1997; Imdorf *et al.*, 1996, 1999). Both Apiguard (thymol) and OA could provide beekeepers with an effective tool for controlling *varroa* mite (Gregorc, 2005).



Number of mites fallen **% Efficacy**

Fig. 1. The mean number of mites fallen for *Tropilaelaps clareae* (plain) and *Varroa destructor* (dense) bars for different treatments. OA, oxalic acid; Thy, thymol.

Chemical resistance, the variable efficacies of current miticides, the adverse effects of treatments on bees and the risk of hive and hive product contamination create a need for alternate treatment methods. From the range of available organic compounds occurring naturally we selected a combination of thymol and OA. 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.*, 1995). Lindberg *et al*. (2000) and Ali *et al*. (2002) recently

evaluated several essential oils and related compounds including thymol, benzyl acetate and methyl salicylate as treatments for mites. The results indicated that the compounds they tested may not be highly effective under all conditions, but they suggest that they could be useful component on an integrated pest management approach.

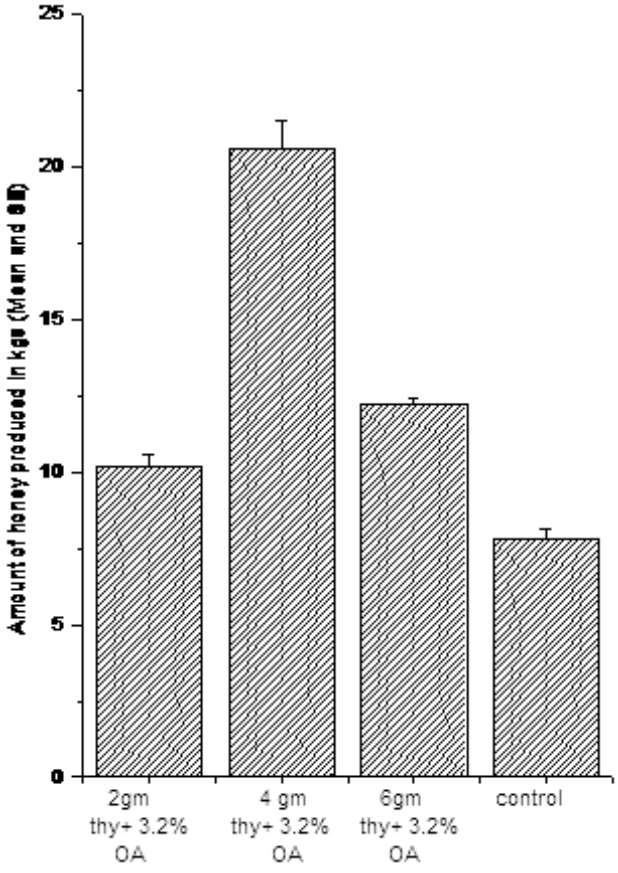


Fig 2. The mean amount of honey produced from colonies treated with different treatments. OA, Oxalic acid; Thy, Thymol.

The usefulness of OA as mite control agent has been known since the end of 20th century (Popov *et al*., 1989). The commercial availability and low risk of hive product contamination render OA an attractive chemical for mites. The trickling method we used in our experiment for applying OA is simple, quick inexpensive and effective. Charriere and Imdorf (2002) evaluated OA and lactic acid as an alternate mite treatment. It has been observed from our pervious experimental studies that 3.2% OA could be effectively used for controlling the honeybee mites (Rashid *et al*., 2012). In the present trial combination of 3.2% OA with different quantities of thymol was tried against honeybee mite's *i.e. Tropilaelaps clareae* and *Varroa destructor* which is in accordance with observations of Fries (2007), who showed that trickling 30 ml of a 3.2% OA solution is significantly more effective (92.2%) than trickling 60 ml of 1.6% OA solution (68.3%). He clearly demonstrated that concentration of OA was critical for high efficacy than the total amount of OA applied to the colony.

The mean amount of honey produced by different groups is shown in Figure 2 where significantly more amount of honey was obtained from the colonies treated with T2 [One Way ANOVA, $F_{(3, 19)} = 49.71$, $P < 0.00$).

It can be concluded from this study that since both thymol and OA are effective against mites they can be safely used together without any side effects in controlling both mites.

ACKNOWLEDGEMENT

We highly acknowledge Agricultural Linkage Programme supported by Agricultural Research Endowment Fund for providing us funding for this study.

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(Received 1 August 2011, revised 8 December 2011)