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CONTROL OF ECTOPARASITIC MITE *VARROA DESTRUCTOR* IN HONEYBEE (*APIS MELLIFERA* L.) COLONIES BY USING DIFFERENT CONCENTRATIONS OF OXALIC ACID

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ABSTRACT

The research work was carried out to determine the effects of oxalic acid (OA) on reducing ectoparasitic mite *Varroa destructor* Anderson and Trueman (Acari:Varroidae) populations in honeybee *Apis mellifera linguistica* (Hymenoptera: Apidae) colonies in the fall at Honey bee Research Institute, National Agricultural Research centre, Islamabad. Twenty honeybee colonies were used in this experiment. Colonies were divided into four groups of five colonies each. Oxalic acid was applied in sugar syrup with 4.2, 3. 2 and 2.1% concentrations. The OA with different concentrations was tricked directly on the adult honey bees in between two frames using a syringe applied thrice on different dates at five days interval. Average efficacy of OA with 3.2, 4.2 and 2.1 % was 95, 81 and 46 % respectively. No queens were lost, and there was no adult honeybee mortality in any of the colonies during the experiment. It can be concluded that 3.2% OA concentration are very effectively control varroa mite and can be used without any side effect during broodless condition.

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

INTRODUCTION

Honey bee colonies are subject to infestation by insects, mites and diseases. The ectoparasitic mite *Varroa destructor* (Anderson and Trueman, 2000) is considered as one of the most serious pests of beehives, causing huge losses to honey bees (*Apis mellifera* L) and great economic loss to the beekeeping industry (Abbadi and Nazar, 2003). Parasitism can result in a loss of up to 25% of adult weight, severe deformations of the wings and reduced longevity of worker and drone honey bees (De Jong *et al*. 1982; Kanga and James, 2002). Ectoparasitic mite of honeybee *Varroa destructor* was first described by Oudemans (1904) from Java on *Apis cerana*. In 1962-63, the mite was found on *Apis mellifera* in Hong Kong and Philippines (Delfinado, 1963) and spread rapidly from there. The *Varro*a mite parasitizes only honeybees. Its specialized mouthparts enable the mite to feed on bee brood and adult honeybees. The mite brood development is closely synchronized with bee brood development and ccolonies heavily infected by *Varroa* produce little or no honey (Ritter, 1981). Colonies infested with *Varroa destructor* have significantly reduced worker bee populations and eventually die if left without controlling. The development of infested brood is also affected because emerged bees have a low weight and shorter life span (De Jong *et al*. 1982). The *V. destructor* mite has been associated with *A. cerana* in Sub-continent Pak-India for the last thousands of years. Varroa mite became a serious pest of *A. mellifera* and destroyed a large number of bee colonies (Ahmad, 1988).

Efforts to control varrosis have been focused on the use of synthetic miticides; however these miticides have some disadvantages: The mites develop resistance against miticides, toxic to bees and human and may leave chemical residues in honey (Miozes *et al*. 2000). For the immediate control of varroa natural miticides can be used, which have low toxicity and low environmental impact, because these breakdown and volatilize rapidly. Few natural products have shown effectiveness against varroa i.e. formic acid, oxalic acids, thymol and essential oils are among them (Imdorf *et al*. 1999).

Beekeepers are bearing heavy financial losses due to the Varroa mite attack on honeybees and their resistance against acaricides. So, it is very essential to find other non-toxic and effective methods to suppress mite populations. Oxalic Acid (OA) is a natural constituent of honey and very effective against the *Varroa* mite. Uses of OA for the control of *Varroa* have been increasing in recent years (Charriere and Imdorf, 2002). It is safe to use, has no residue problems, cheap and no case of honeybee toxicity has been reported yet (Mutinelli *et al*. 1997; Rademacher and Harz, 2006).

Varroa mites can be effectively controlled by using OA dripping method (Nanetti and Stradi, 1997). However, reports from Germany, Switzerland and Italy indicate a concentration related bee tolerance problem. High concentrations (4.2%) are extremely effective, but may influence varroa development in colonies in the spring after winter treatment and at the same time lower concentrations (2.1%), using the same amount of liquid has no adverse effect on colony development. It has not been clear, however, if it is the concentration of the solution applied that is critical for high efficacy or if

similar mite control may be achieved using the same total amount of OA, but in a lower concentration. We have recorded the efficacy on mites honey bee colonies using three concentrations 4.2, 3.2 and 2.1% of OA and applying the same total amount of the active ingredient in three groups and leaving fourth group as control.

MATERIALS AND METHODS

The research work was carried out in Honeybee Research Institute of National Agricultural Research Centre, Islamabad, Pakistan on *Apis mellifera* colonies naturally infected with ectoparasitic mites. Treatments were given randomly to all experimental colonies which were requeened with hygienic queens. Modified bottom boards and hygienic queens were used in all colonies during the experiments. The mite collection trays (mite excluders) were kept under bottom board for assessing the population of mites. The rate of ectoparasitic mites infestation and treatment efficacy was estimated by counting falling mites on mite collection tray and by counting the dead mites in the sealed worker and drone brood before and after treatment. Treatments in all replication was applied complete randomize design.

About 50 adult and sealed brood populations of Honeybee Research Institute 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). 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 (Devlin, 2001). Finally, twenty queen right honeybee colonies in Langstroth hives were used that had been standardized for bee frame + brood + debris infestation levels. The colonies were placed in HBRI premises in December 2008 with mean outside temperature of 3^0 C. Each honeybee colony was equipped with a modified bottom board and a mite collection tray (mite excluder) which was placed through the back side of the hive, covered by a wire screen to prevent the bees from coming into contact with the debris without disturbing colony. The rate of ectoparasitic mite infestation and treatment efficacy was estimated by counting falling mites on mite collection tray. The honeybee colonies of each group were placed at appropriate distance of 5 meters. Colony strength (number of combs covered with bees, brood areas, and amount of food) were equal. Colonies were divided into 4 groups of 5 colonies each.

Group 1 (T1) = 4.2% OA
Group 2 (T2) = 3.2% OA
Group 3 (T3) = 2.1% OA
Group 4 (T4) = Control (untreated)

Oxalic acid was applied in sugar syrup. To obtain 4.2, 3.2 and 2.1 % OA solution, 100, 75 and 50 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. One group was tested with 4.2 % oxalic acid solution. The second group received 3.2% oxalic acid solution while the third group was treated with 2.1 % oxalic acid solution and fourth served as control group (C). 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; Brødsgaard *et al.*1999). All groups received Oxalic acid solution with three doses at five days interval. 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 OA treatments was calculated by using following formula (Marinelli *et al*. 2004).

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

Honey harvesting: Honey was harvested after experiment with the help of 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.

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

RESULTS AND DISCUSSION

Presently, synthetic acaricides are regularly used for the control of *Varroa destructor*, however, due to the persistent nature they accumulate in honey and wax (Bogdanov *et al*. 2002). Acaricide resistant mites have also appeared in many countries (Elzen *et al*. 1999).These problems have initiated the development of non toxic substances i.e. organic acids and essential oils. As an alternative control strategy winter treatment is very important because most of Varroa which are likely to appear in the next year population are destroyed in this way. These are the mites that survived the autumn season treatment. For the winter treatment Oxalic acid offers a promising opportunity.

The mite mortality was different for oxalic acid concentrations in all colonies with 3.2 % concentration yielding the highest mite mortality. The results are summarized in Fig 1. There was significant difference in all the groups (One Way ANOVA, $F_{(3, 19)} = 715.48$, $P < 0.05$).

In our results the number of mites fallen for the T1 (4.2 % OA) T2 (3.2 % OA), T3 (2.1 % OA) and T4 (Control) ranged between 931-1123, 1188-1348, 200-225 and 49-80 respectively . Overall oxalic acid was found to be quiet effective against the mites which is in accordance with the results (Mutinelli *et al*.1997; Gregorc and Poklular 2003; Marinelli *et al.*2004; Rademacher and Harz 2006) showing that OA is very effective against *V. destructor*.

Nanetti *et al*. (2003) considered OA a good method for controlling the mites but stated that it may cause reduction in the brood, conversely Imdorf *et al.* (1997) reported that OA did not show any significant decrease on brood area which is in conformation with our results as the brood also showed no lasting loss and the slight damage to eggs and larvae can be tolerated as it hardly influences the total population of the bee colonies. No raised bee mortality was observed during the application of treatments. Neither loss of queens after any of the treatments as other authors have indicated (Wachendorfer *et al*. 1985) nor supersedure of the queens was found.

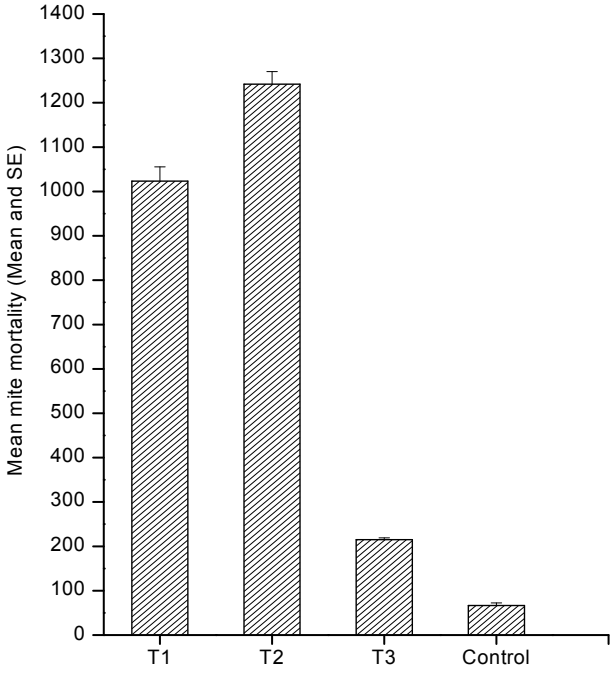

Fig.1 Mean number of mites found dead in colonies after using different concentrations of oxalic acid

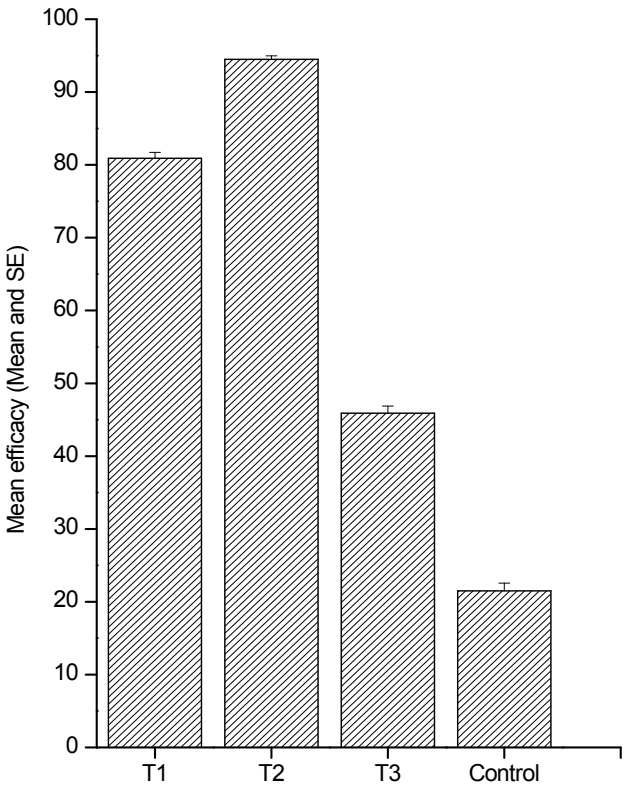

Fig 2. Mean efficacy of oxalic acid with different concentrations observed at the end of experiment

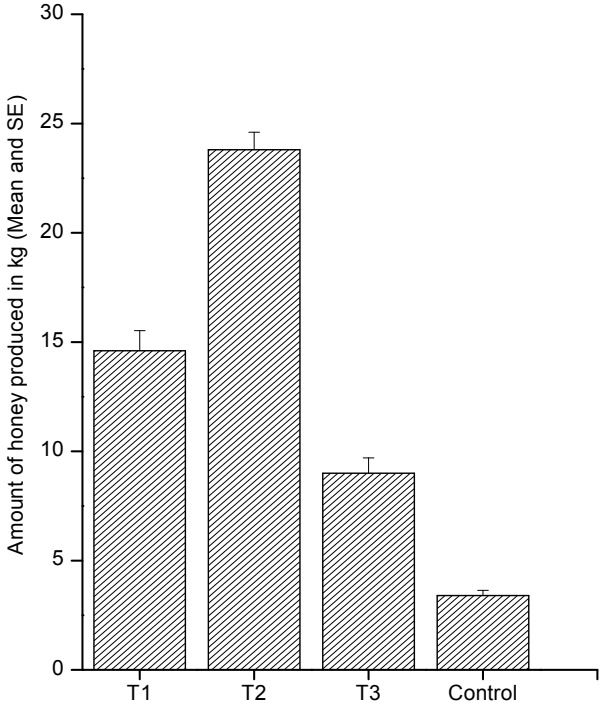

Fig 3. Mean amount of honey produced from colonies treated with different concentrations of oxalic acid

After several trials in different countries regarding testing of different combinations between OA and sucrose concentrations, it was observed that 4.2 % OA is the most effective; nonetheless 3.2 % option gave similar results while 2.1 % OA did not yield sufficient mite mortality (Nanetti *et al*.2003). 3.2% of OA was the best concentration for the control of varroa mites. While 2.1% concentration of OA was least effective for mite control. Our results agreed with (Fries *et al*.2000; Nanetti *et al*.2003) who found that 3.2 % OA was effective for mites control without obvious adverse effect on bee colonies.The range of efficacy in colonies treated with T1, T2, T3 and T4 was 77.91-82.74, 93.67-96.29, 42.11-47.91 and 17.82-23.27 respectively. The mean efficacy value also varied between treatments. The percentages were arcsine square root transformed and when compared were found to be significantly different (Fig 2).

The honey produced from different hives when treated with different concentrations of OA was also weighed at the end of experiment. The mean amount of honey produced in kg from T1, T2, T3 and T4 was 14.6 $\pm$ 0.97, 23.0 $\pm$ 0.8, 9.0 $\pm$ 0.7 and 3.4 $\pm$ 0.24 (Mean$\pm$ SE) respectively(Fig.3) . The honey produced also showed significant results. (One Way ANOVA, $F_{(3, 19)} = 146.9$, $P < 0.05$).

Oxalic acid is a very promising candidate chemical for the control of Varroa mites. It has many advantages as it is easy to use, cheap, safe for beekeepers, and presents low variability between colonies in its final efficacy. It is a natural constituent of honey and many vegetables, and no significant residues have been found in hive products in Europe (Del Nozal *et al*. 2000; Bernardini and Gardi, 2001). The results also clearly demonstrate that the concentration of oxalic acid is critical for high efficacy. The results also confirm earlier results from trials under Swedish and Norwegian conditions (Fries *et al*.2000) that the currently recommended dose of 30 ml 3.2% for normal sized colonies can be used for mite control with good results without any obvious adverse effects on bee colonies over winter.

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