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

M. Rashid, E. S. Wagchoure, A. U. Mohsin<sup>\*</sup>, S. Raja and G. Sarwar

Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan \*Department of Entomology, PMAS University of Arid Agriculture, Pakistan Corresponding Author: E-mail: rashid\_ento1@yahoo.com

### 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 Varroa 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  $3^{\circ}$  C. Each honevbee 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).

Efficacy =  $\underline{No. of mites fallen for each treatment} X 100$ Total number of fallen mites

**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.

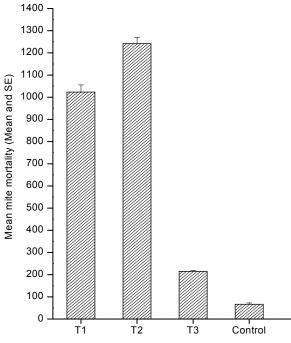


Fig.1 Mean number of mites found dead in colonies after using different concentrations of oxalic acid

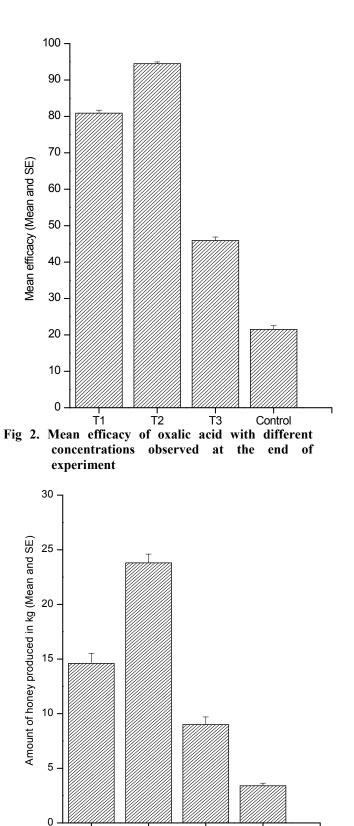


Fig 3. Mean amount of honey produced from colonies treated with different concentrations of oxalic acid

тз

Control

T2

Τ1

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 vield 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.

### REFERENCES

- Ahmad, R. (1988) Honeybee parasitic mites and their control in Pakistan, Prog. farming. 8: 34-36.
- Al-Abbadi, A. and I. K. Nazer (2003) Control of Varrova mite (Varrova destructor) on honey bees by Aromatic oils and plant materials. Agri. Mari. Sci. 8 (1): 15-20.
- Anderson, D. L. and J. W. H. Trueman (2000) *Varroa jacobsoni* (Acari: Varroidae) is more than one species. Exp. Appl. Acarol. 24:165-189.
- Bernardini, M. and M. Gardi (2001) Influence of acaricide treatments for varroa control on the

quality of honey and beeswax. Apitalia. 28(7-8): 21-24.

- Bogdanov, S., E. F. Harriere, C. Imodorf, J. D. Kilchnimann and P. Fluri (2002). Determination of residues in honey after treatments with formic acid and oxalic acid under field conditions. Apidologie. 33(4): 399-409.
- Burgett, D. M. and I. Burikam (1985) Number of adult honeybees (hymenoptera: Apidae) occupying a comb: A standard for estimating colony populations, J.Eco. Entomol. 78: 1154-1156.
- Charrière, J. D. and A. Imdorf (2002) Oxalic acid treatment by trickling against *Varroa destructor:* recommendations for use in central Europe and under temperate climate conditions, Bee World. 83(2): 51-60.
- De Jong, D., R. D. Deandrea, and L. S. Gonçalves (1982) A comparative analysis of shaking solutions for the detection of *Varroa jacobsoni* on adult honeybees, Apidologie.13(3): 297-306.
- Delfindo, M. D. (1963) Mites of the honeybee in Southeast-Asia, J.Apicul. Res.2: 13-114.
- Del Nozal, M. J., J. L. Bernal, J. C. Diego, L. A. Gomez, J. M. Ruiz and M. Higes (2000) Determination of oxalate, sulfate and nitrate in honey and honeydew by ion-chromatography, J. Chromat. 881: 629-638.
- Devlin, S. (2001) Comparative Analyses of sampling methods for Varroa mite (*Varroa destructor* Anderson and Trueman) in honey bees (*Apis mellifera* L.), MPM Thesis, Simon Fraser Univ., Burnaby BC, Canada.
- Elzen, P. J., F. A.Eischen, J. R. Baxterg, W. Elez and W. T. Wilson. (1999) Detection of resistance in U.S *Varroa jacobsoni* Oud. (Mesostigmate: Varroidae) to the acaricide Fluvalinate, Apidologie. 30 (1): 13-17.
- Fries, I., S. Helland, S. Korpela, and P. Kristiansen (2000). Bekämpning Av Varroakvalster med oxalsyra.*Bitidningen*, 99:18-21.
- Gregorc, A. and J. Poklular (2003). Rotenone and oxalic acid as alternative acaricidal treatments for *Varroa destructor* in honeybee colonies. Vet. Parasit. 111: 351-360.
- Imdorf, A., J. D. Charrière, and B. Bachofen (1997) Efficiency checking of the *Varroa jacobsoni* control methods by means of oxalic acid, Apiacta.. 32(3): 89-91
- Imdorf, A., R. Bogdanov, I.Ochoa, and N. W. Calderone, (1999). Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie. 30 (2-3): 209-228.
- Kanga, L. H. B. and R. R. James (2002). Varroa control with fungal pathogens may be an option soon. Amer.Bee. J. 142: 519.

- Miozes-Koch, R., Y. Slabezki, H. Efrat, H. Kalev, Y. Kamer and D. A. Yakobson (2000). First detection in Israel of fluvalinate resistance in the Varroa mite using bioassay and biochemical methods. Exp. Appl. Acarol. 24: 35-43.
- Marinelli, E., P. Pulcini, C. Margio, F. De pace, F. Allegrini and L. Persona Oddo (2004). Oxalic acid by Varrox® to *Varroa* control in Central Italy. Apiacta. 39: 39-43.
- Mutinelli, F., A. Baggio, F. Capolongo, R. Piro, L. Prandin, and L. Biasion (1997). A scientific note on oxalic acid by topical application for the control of varroosis. Apidologie. 28(6): 461-462.
- Nanetti, A. and G. Stradi. (1997). Varroasi: trattamento chimico con acido ossalico in sciroppo zucherino, Ape Nostra Amica. 19: 6-14.
- Nanetti, A., R. Buchler, J. D. Charriere, I. Fries, S. Helland, A. Imdorf, S. Korpela, and P. Kristiansen (2003). Oxalic acid treatments for Varroa control (review), Apiacta.38: 81-87.

- Oudemans, A. C. (1904). On a new genus and species of parasitic acari (Varrova) notes. Leyd. Mus.24: 216-222.
- Prandin, L., N. Dainese, B. Girardi, O. Damolin, R. Piro and F. Mutinelli (2001). A scientific note on long-term stability of a home-made oxalic acid water sugar solution for controlling varroosis, Apidologie. 32: 451-452.
- Rademacher, E. and M. Harz (2006). Oxalic acid for the control of varroosis in honey bee colonies a review. Apidologie. 37: 98-120.
- Ritter, W. (1981). Varroa disease of honeybee *Apis mellifera*, Bee world. 62(4): 141-153.
- Wachendörfer, G., J. Fijalkowski, E. Kaiser, D. Seinsche, and J. Siebentritt (1985). Labor- und Feldversuche mit der Illertisser Milbenplatte als neue Anwendungsform der Ameisensäure im Rahmen der Varroatose-Bekämpfung. [Laboratory and field tests with the illertisser milbenplatte (mite plate) a new way of application of formic acid to control varroatosis.], Apidologie. 16: 91-306.