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Control of *Varroa destructor* Using Oxalic Acid, Formic Acid and Bayvarol Strip in *Apis mellifera* (Hymenoptera: Apidae) Colonies

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Abstract.- The effectiveness of oxalic acid (3.2%), formic acid (65%) and flumethrin strip (Bayvarol) for the control of *Varroa destructor* (Anderson and Trueman) mite in broodless honeybee (*Apis mellifera lingustica* Linnaeus) colonies was determined. Three groups of five colonies each were treated with one of these agents, while the fourth group of five colonies was used as control. Mite mortality was examined in debris at 4-day intervals before application of the next treatment. The highest number of fallen mites 177±35.12 (Mean ± SE) occurred with oxalic acid and the difference between the treatments was highly significant. The efficacy and honey yield from the experimental colonies were also highly significantly different from the controls. The highest mean efficacy of 99±1.24 and honey yield 12.08± 0.86 was found in 3.2% oxalic acid treatment. No queens were lost and adult honeybee mortality did not occur in any of the colonies during the experiment.

Key words: Varroa mite, oxalic acid, formic acid, bayvarol, honey yield, *Apis mellifera*.

INTRODUCTION

Honeybees are insects of economic importance. They produce honey, pollen, royal jelly, propolis and wax which are valuable for their use in pharmaceuticals, food products and other industrial products (Wakhal *et al*., 1999). Bee pollination improve the size, shape, color, storage capacity and taste of the fruits (FAO, 2004). An Italian strain of honeybee *Apis mellifera lingustica* was imported from Australia in 1977-78 and established in Pakistan after several attempts (Muzaffar, 1982). 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 (PARC, 2010-11).

Each year many honeybee colonies are affected by ectoparasitic mites (*Tropilaelaps clareae* and *Varroa destructor*). Colony losses affected by these parasites have a devastating impact on the beekeeper, which may have to relocate damaged hives or perhaps even be forced out of business. All *A. mellifera* colonies are treated several times a year with various acaricides to prevent the apparently unlimited growth of mite populations and the death of colonies. The ectoparasitic mite of honeybee *V. destructor* was first described by Oudemans (1904) from Java on *Apis cerana*. In 1962-63, the mite was found on A. mellifera in Hong Kong and the Philippines (Delfinado, 1963) and spread rapidly from there. *Varroa* mites feed on the developing honeybee larvae, pupae and on 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 *V. destructor* mite has been associated with *A. cerana* in subcontinent Pak-India for last thousands of years. *Varroa* mite became a serious pest of *A. mellifera* and damage a large number of bee colonies (Ahmad, 1988). With the introduction of *A. mellifera* in Pakistan *Varroa* mite become a serious pest of the newly introduced honeybee. Mite infestation on honeybees, results in low honey yield increased absconding and swarming. Honey, wax and other products of bee hive in Italy were found to be adulterated with high concentration of acaricides residues (Milani, 1995). Various acaricides that have been used effectively to control mites for many years have now reduced efficacy as a result of mite resistance against many registered acaricides (Loglio and Plebani, 1992).

Many beekeepers use unregistered products

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including chlorobenzilate, phenothiazine, sulphur, amitraz or different pyrethroids for controlling the mite infestation because no approved miticides are available. Uncontrolled use of these agents has led to the development of more resistance, resurgence of the infestation and the risk of residues in the honey which might pose a risk for human consumption and commercial value of honey. Beekeepers are also bearing heavy financial losses as the result of the parasitic mite attack on honeybees. It is therefore essential to find other non-toxic and effective methods to suppress mite populations. Miticides like formic acid and thymol might be able to solve this problem in beekeeping industry, once they have been approved, if applied regularly and according to the recommendations. Introduction of organic miticides 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 mind 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 to determine the efficacy of oxalic acid, formic acid and bavyarol® against *V. destructor* mite.

MATERIALS AND METHODS

The experimental work was carried out in Honeybee Research Institute of National Agricultural Research Centre, Islamabad, Pakistan where a good pollen and honey flow was expected in March–April, 2009. Twenty *A. mellifera lingustica* colonies naturally infested with ectoparasitic mite were used in the experiment during December, 2008. The adult (by alcohol wash technique, De Jong *et al*., 1982) and sealed brood populations (by opening 100 cells of worker brood) of test colonies were assessed for *Varroa* infestation week before treatments were applied. Treatments were applied randomly to all experimental colonies which were requeened with hygienic unrelated queens (a routine bee management practice in Pakistan in the start of spring season) during March-April, 2008. Each honeybee colony was placed on a modified bottom board and mite collection trays (mite excluders) were placed through the back side of the hives, covered by a wire screen or mesh to prevent the bees from coming into contact with the debris without disturbing colonies. The mesh also prevents the mites from returning back to the hive. The rate of ectoparasitic mite infestation and treatment efficacy was calculated by counting total falling mites on mite collection tray during whole experiment. To collect the sample from 150-250 bees/colony of mite's 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 and after treatment to calculate the infestation rate of sealed brood-pupa in bee hives (Burgett and Burikam, 1985).

Honey bee colonies of each group that had been previously standardized one week dated 12-12-2008 before on the basis of five bee frames, one brood frame and at least twenty numbers of mites per colony in debris. Colonies were placed at appropriate distance of 5 meters from each other by using Complete Randomized Design (CRD). Colonies were divided into 4 groups of five colonies each, *viz.*, Group 1 oxalic acid (3.2%), Group 2 formic acid (65%), Group 3 Bayvaro® strip and Group 4 was control.

One group was tested with oxalic acid (OA), which was applied in sugar syrup. To obtain a 3.2% OA solution, 1 kg of OA added to 1 liter of warm water and stirred until the sugar was dissolved. Then, 75 g of OA dihydrate was added to the syrup and the resulting solution was 3.2% OA; (50% sugar, weight/volume) as described (Sammataro *et al*., 2008; Nanetti and Stradi, 1997). Treatments were only applied to frame spaces that contained bees, empty frames were not treated. The 5 ml mixture was trickled directly on to the adult bees in between two frames using a syringe as recommended (Brodsgaard *et al*., 1999; Imdorf *et al*., 1997). The second group received 65% formic acid (FA) which was applied a dose of 20 ml on a cotton cloth placed in the trays under the mesh screen. The third group was treated with Bayvarol® one strip per colony was hung between the brood frames for one week (two strips/colony suggested for 10 frame bee hive but in this experiment bee hive were 5 frame, so used 1 strip/colony) and colonies of fourth group served as control without treatment (C). Groups first and second received three treatments after 4 days interval. Group 1 (OA) received three treatments of 3.2% Oxalic acid with a four day interval. Group 2 (FA) received three treatments 20 ml each, total of 60 ml of 65% formic acid. At the end, after seven days dated 31-12-2008, all the experimental colonies including control group in broodless condition were given 3.2% OA for knockdown.

The mite populations were measured in mite collection tray placed on the bottom boards of each test honeybee's colonies after 24 days period. The efficacy of the treatments was calculated by using following formula (Marinelli *et al*., 2004).

$$\text{Efficacy} = \frac{\text{No. of mites fallen during treatment (per treatment)}}{\text{Total number of fallen mites (per and post treatment)}} \times 100$$

Honey yield

At the end of the experiment honey was harvested (from Acacia crop which started blooming in April and ends in May) with the help of manually operated honey harvester and honey yield of treated colonies were 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.

Statistical analysis

All data recorded during the study were analysed by using computer based software MSTAT C (Freed and Eisensmith, 1986). Analysis of Variance techniques were applied to test the significance of data using least significance difference test (LSD) at 5% probability level (Montgomery, 2001).

RESULTS AND DISCUSSION

There was a trend of fallen number of mites range from 66-242, 56-62, 94-187 and 32-40 in 3.2% OA, 65% FA, Bayvarol® strip and control groups, respectively. There was a trend of fallen mean number of mites increasing, between FA, 56±2.11 to bayvarol® 163±19.97 and highest for OA 177±35.12. The mean number of fallen mites for the control colonies were 35±1.40 (Fig. 1). Our results are in agreement with those of Rashid *et al*. (2012) who concluded that 3.2% OA concentration is very effective in controlling *Varroa* mite and can be used without any side effect during broodless condition. Our results are in accordance with those of Gregorc and Planinc (2001) and Brodsgaard *et al*. (1999) who also showed that OA is very effective against *Varroa* mites. Nanetti *et al*. (2003) considered OA a good method for controlling the mites but noted that it may cause reduction in the brood, conversely Imdorf *et al*. (1997) reported that OA treated hives did not show any significant decrease in brood area, which supports our results, in which the brood showed no lasting loss and the slight damage to eggs and larvae can be tolerated and barely influenced the total population of the bee colonies. No raised bee mortality was found during the application of treatments. Neither loss of queens after any of the treatments as indicated by other authors, nor superseding of the queens was found (Wachendorfer *et al*., 1985). When different treatments were compared a highly significant difference was found for the mean number of fallen mites ($F3, 19=12.9< 0.00$). Although the greatest number of mites fell in the OA treatment (Fig. 1), no significant difference was found between OA and Bayvarol but the results were significantly different for FA than OA and Bayvarol. The control when compared to OA, FA and Bayvarol was significant different (as 5% level of significance). Range of efficacy from 88-95, 58-60, 75-89 and 18-21% in 3.2%, 65% FA, Bayvarol® strip and T4 (control), respectively. Mean efficacy was compared highly significantly different (at 5% level of significance) 91± 1.24, 59±0.32, 80± 2.48 and 20±0.55 in 3.2% OA, FA, Bayvarol® strip and control, respectively as shown in Figure 1 ($F3, 19=482<0.00$).

From our results we found OA to be the most effective and FA to be least effective miticide against *Varroa* mites which contrasts with Sharma *et al*. (1983), who suggested that FA was quite effective for the control of endoparasitic and ectoparasitic mites and Rashid *et al*. (2011) who calculated FA is most effective for the control of *T. clareae* in spring treatment. Here perhaps our result differs due to mite and season variation. Jelinski

(1993) calculated that the mean effectiveness of medication was 95.6%. Bayvarol® strip were harmless for bees. There were used 4 strips for a treatment. They were placed between combs of honey bee colony. But in this experiment we used only one strip/colony.

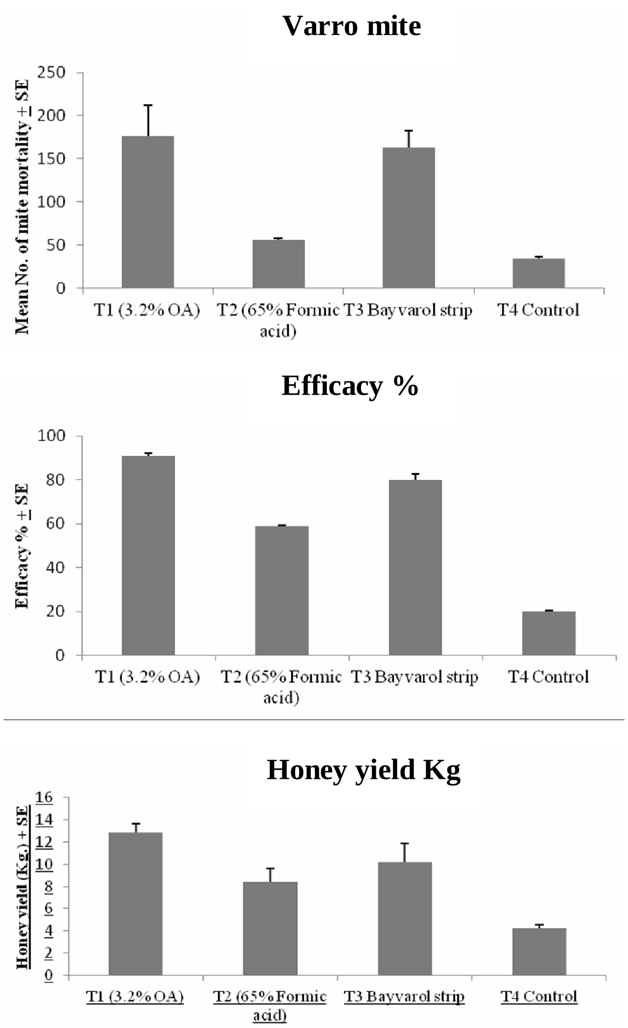

Fig. 1. Number of fallen mites count, efficacy (%) of three treatments of different acaricides against *Varroa* mite and honey yield (kg) with various treatments in May, 2009

The honey harvested at the end of experiment was also weighed and the mean amount of honey from different treatments in kg is presented in Figure 1. The mean honey yield (kg.) compared between treatments were 12.08±0.86, 8.4±1.12, 10.2±1.66 and 4.2±0.37 in 3.2% OA, 65% FA, Bayvarol® strip and Control respectively and significant differences was observed (F3, 19=12.9<0.00) at 5% level of significance.

Most of the beekeepers in the developing countries use unapproved miticides not according to the label instructions to control the mites (Pichai *et al*., 2008). Such misuse may cause the development of resistance, and the risk of residues in honey which might be dangerous for human consumption. The miticides tested here provide the beekeepers with a legal and safe solution to fight against *Varroa* mites. All experimental applications are completed 2-3 months before the active season of honey flow in December, 2008. Treatment with these products could help to increase the production of high quality honey. Since, the treatments were applied in winter which is offseason for any type of honey and later when the honey flow period started in April-May we did not predict the residues in honey so they were not determined in treated colonies.

OA is a very promising candidate chemical for the control of *Varroa* mites. It has many advantages: it is easy to use, cheap, safe for beekeepers, and presents low variability between colonies in its final efficacy. It also causes low or no honeybee toxicity and there is no record of queen loss or brood /adult bee mortality. It is a natural constituent of honey and many vegetables, and no significant residues have been found in hive products in Europe (Bernardini and Gardi 2001; Del Nozal *et al*., 2000). Each individual country can apply for national approval of OA or products containing it to control *Varroa* in honey bee colonies (Rademacher and Imdorf, 2004).

At completion of experiment; mite data was collected from brood and adult bees but no mite was found there. Both OA as well as Bayvarol® strip can be used safely for effectively reducing the damage caused by *Varroa* mites in bee colonies.

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