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Efficacy of Screen Bottom Board Tray with and without Soft Chemicals for Controlling *Varroa destructor* in Honeybee Colonies

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Authors' Contributions
RM was the main investigatator, he designed the study, collected data and wrote the manuscript. SA and ZA compiled and analyzed data; WA and GS collected data and conducted the research and MKR and NI were co-researcher and help in all activities. ZAQ provided guideline in collecting data.

Key words
Formic acid, Oxalic acid, Thymol, Screen bottom board tray, Varroa destructor, Apis mellifera

ABSTRACT

The present study was carried out to determine the effects of screen bottom board trays on reducing ectoparasitic mite *Varroa destructor* Anderson and Trueman (Acari:Varroidae) populations in honeybee *Apis mellifera* linguistica (Hymenoptera: Apidae) colonies in the winter, spring and summer season (2013-14). Four honeybee colonies were exposed to oxalic acid (3.2%), thymol (0.5 g) and formic acid (65%) on screen bottom boards in winter, spring and summer seasons. The fourth group had screen bottom board trays but without any chemical. There was a trend of fallen mean number of mites 157±6.28, 97±3.81, 132.75±9.76 and 26±1.68 in winter, 160±20, 149±30, 161.75±18.5 and 27.5±1.04 in spring and 188.25±5.34, 196.25±5.48, 144.25±7.74 and 34.5±4.94 (Mean±SE) in summer, respectively. The average efficacy (%) of the four groups were 86±.58, 66.5±3.93, 75±7.04, 18.25± 10.25 in winter, 77±3.52, 76.75±30, 79±2, 18.25±0.75 in spring and 74.75±1.44, 65.75±1.11, 63±2.04, 18.25±1.11 in summer season, 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 screen bottom board trays alone and with soft chemicals in all three seasons effectively control the *Varroa* mite and can be used without any side effect during all the three seasons.

INTRODUCTION

An Italian strain of honeybee *Apis mellifera* Lngustica 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 more than 4,000 beekeepers are now rearing *Apis mellifera*. There are about 400,000 colonies of *A. mellifera* producing 10,000 metric tons honey annually and 27,000 families are benefiting from beekeeping (PARC, 2010-11).

Each year many honeybee colonies are damaged or destroyed by mites. This causes the economic loss of bees and honey production. Moreover, the infested colony may die or migrate (Needham, 1988). The 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 *A. mellifera* in Hong Kong and the Philippines (Delfinado, 1963) and spread rapidly from there. 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 and damage a large number of bee colonies (Ahmad, 1988).

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Anderson and Trueman (2000) reported that *V. destructor* is one of the most serious pests to hives, causing great economic loss to the bee keeping industry. In *A. mellifera* L. colonies, mortality from *V. destructor* infestation can reach 100% in two years if left untreated (De Jong, 1990).

Non-chemical control measures such as modified bottom boards with screens that can catch mites when they drop from bees have been reported to reduce *Varroa* populations (Pettis and Shimanuki, 1999; Ellis *et al*., 2001; Sammataro *et al*., 2004) others have tested the effects of screened trays on overwintering (Horn, 1990) and brood production (Skubida and Skwronek, 1995; Pettis and Shimanuki, 1999; Ellis *et al*., 2003). The anti-varroa bottom board resulted in on the average 15-30% reduction in the population of *Varroa* mites (Pettis and Shimanuki, 1999; Ellis, 2001; Rice *et al*., 2004; Harbo and Harris, 2004). It is therefore, essential to find other nontoxic and effective methods to suppress mite populations (Pichai *et al*., 2008).

Beekeepers have started to include screen floors with other control measures to create an integrated procedure for controlling *Varroa* mites (Ellis *et al*., 2001; Ostiguy *et al*., 2000; Sammataro *et al*., 2004) and some beekeepers throughout the world have been using screen floors on their hives for decades (Spear, 2002). A screen bottom board has been used in conjunction with paper collecting sheets as a method for monitoring *Varroa* levels (Szabo, 1998, 1999). But the chief merit of the device is its presumed ability to hinder or prevent mites from re-mounting their hosts once the mites fall off the bees and through the screen. Even without miticides, it is not uncommon for mites to drop off their hosts and it is possible for up to 51% of these individuals to be alive (Webster *et al*., 2000).

Keeping in mind the importance of safe and non-contaminated methods to suppress mite populations in beehives the present study was aimed to determine the efficacy of screen bottom board tray when used alone or in conjunction with three soft chemicals *i.e.* oxalic acid, formic acid and thymol against *V. destructor* mite.

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 *Varroa destructor* mites. Treatments were given randomly to all experimental colonies which were requeened with hygienic queens (Spivak and Reuter, 1998; Rashid *et al*., 2012a, b, c) prior to the start of the experiment. 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 by using Randomized Complete Block Design (RCBD) under two factor factorial. 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; Rashid *et al*., 2012a, b, c). 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 were placed at the bottom of the bee colony. The trays were left for 24h period and mites which fell on the trays were counted and used as measure for mite population (Devlin, 2001). Finally, sixteen 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. Each honeybee colony was equipped with a modified bottom board and a mite collection tray (mite excluder) designed by Honeybee Research Institute woodwork shop team (Fig.1) 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 4 colonies each.

Screen bottom board trays were placed in the beehives throughout the treatment period alone and with 3.2 % oxalic acid, 0.5 g thymol, 65% formic acid in winter (Nov., 2013 - Jan., 2014), spring (Feb., 2014 - April, 2014) and summer (May, 2014- July, 2014) seasons. Mite data was collected on fortnightly basis.

Thymol (0.5 gm) crystals finely grinded were placed in Petri dishes (80mm) on top of the brood frame under the top cover of hives. The 65% formic acid was applied by pouring on a cotton cloth placed in the deep bottom board of honeybee colonies (Rashid *et al*., 2011, 2013).

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; Rashid *et al*., 2012a). Treatments were only delivered to frame spaces that contained bees; any 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 (Imdorf *et al*., 1997; Brodsgaard *et al*., 1999). The rate of ectoparasitic mites *V. destructor* infestation

Fig. I. Screen bottom board tray insert from the back of bee hive.

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 knock-down. Apistan strips were removed from the colonies after four weeks and dropped dead mites were counted. Treatments efficacy was calculated for each colony as by Marinelli *et al*. (2004), Rashid *et al*. (2012a), (2012b) and (2012c).

$$\text{Efficacy (\%)} = \frac{\text{No of mite during treatment (per treatment)}}{\text{No of mite during treatment(per + pest treatment)}} = \text{X 100}$$

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, thymol and oxalic acid (OA) have shown potential effectiveness against these mites, which have no negative effect on the development of colonies. There was a trend of fallen mean number of mites 157±6.28, 97±3.81, 132.75±9.76 and 26±1.68 in winter, 160±20,149±30, 161.75±18.5 and 27.5±1.04 in spring and 188.25±5.34, 196.25±5.48, 144.25±7.74 and 34.5±4.94 (Mean±SE) in summer respectively (Table I). Our results are in agreement with Rashid *et al*. (2012a) who concluded that 3.2% OA concentration are very effectively control varroa mite and can be used without any side effect during broodless condition. Average efficacy (%) of oxalic acid, thymol, formic acid as against control (screen bottom tray without any chemical) was 86±.58, 66.5±3.93, 75±7.04, 18.25±10.25 in winter, 77±3.52, 76.75±30, 79±2, 18.25±0.75 in spring and 74.75±1.44,65.75±1.11,63±2.04,18.25±1.11 in summer season, respectively (Table II). No queens were lost, and there was no adult honeybee mortality in any of the colonies during the experiment. Rashid *et al*. (2012b) also confirmed 4g thymol + 3.2% OA combination is effective for the control of both types of ectoparasitic mites in broodless condition. Amrine and Noel (2007) concluded that 50% formic acid, seperamint, lemongrass, essential oils with use of screen bottom boards trays collectively effective for the control of *Varroa* mites. Harold *et al*. (1989) reported that 94% mites were killed by application of 4 treatments of formic acid and the most effective treatment (62% of mites killed) was with 40 ml of 65% formic acid (Greatti *et al*., 1993). Thymol was effective against mites but safe to honeybees (Bollhalder, 1998; Calderone, 1999). Chiesa (1991), Gal *et al*. (1992) and Lensky *et al*. (1996) reported that 30% thymol was harmful to bee colonies during summer. OA was found very effective for control of mites is in confirmation with the results showing that OA is very effective against *V. destructor* (Gregorc and Planinc, 2001, 2002; Gregorc and Poklular, 2003; Marinelli *et al*., 2004). Imdorf *et al*. (1997) claimed that OA did not effect brood area and

Table I.- Mortality of mite (*Varroa destructor*) after different treatments during winter, spring and summer seasons.

Treatments	Winter	Spring	Summer	Overall Mean
3.2% Oxalic acid	157.25 ± 6.28 BC	160.00 ± 20.0 ABC	188.25 ± 5.34 AB	168.50 A
65% Formic acid	97.00 ± 3.81 D	149.00 ± 30.0 C	196.25 ± 5.48 A	147.42 B
0.5g Thymol	132.75 ± 9.76 CD	161.75 ± 18.5 ABC	144.25 ± 7.74 C	146.25 B
Screen bottom board tray	26.00 ± 1.68 E	27.50 ± 1.04 E	34.50 ± 4.94 E	29.33 C
Overall Mean	103.25 B	124.56 A	140.81 A	

LSD (α = 0.01, 0.05).
For Treatment = 4.79.
For Season = 4.15.
For Treatment x Season (interaction) = 8.30.
Means that follow similar letters do not differ significantly.

Table II.- Average efficacy (%) of different treatments on *Varroa destructor*, a parasite on *Apis mellifera*, during different seasons.

Treatments	Winter	Spring	Summer	Overall Mean
3.2% Oxalic acid	86.00 ± 0.58 A	77.00 ± 3.52 AB	74.75 ± 1.44 BC	79.42 A
65% Formic acid	66.5 ± 3.93 CD	76.75 ± 30.0 B	65.75 ± 1.11 D	69.67 B
0.5g Thymol	75.00 ± 7.04 B	79.00 ± 2.00 AB	63.00 ± 2.04 D	72.33 B
Screen bottom board tray	18.25 ±0.25 E	18.25 ± 75 E	18.25 ± 1.11 E	18.08 C
Overall Mean	61.44 A	62.75 A	55.44 B	

For statistical details, see Table I.

Wachendorfer *et al*. (1985) observed no bee mortality, no loss of queen and no supersedure. The efficacy of screen bottom board tray was 18.25±10.25, 18.25±0.75 and 18.25±1.11 (Mean±SE) in winter, spring and summer season, respectively. No queens were lost, and there was no adult honeybee mortality in any of the colonies during the experiment. Our result with the anti-varroa bottom board resulted in an average reduction of 15-30% on the population levels of *Varroa* mites (Pettis and Shimanuki, 1999; Ellis, 2001; Rice *et al*., 2004; Harbo and Harris, 2004).

CONCLUSION

It can be concluded that screen bottom board trays alone and with soft chemicals in all three season are very effectively control *Varroa* mite and can be used without any side effect during all three seasonal conditions.

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Conflict of interest statement

We declare that we have no conflict of interest.

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