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Effectiveness of Acaricidal Treatments against *Varroa destructor* (Acari: Varroidae) Affecting Honey Bee, *Apis mellifera* L. Colonies

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A B S T R A C T

A study was carried out to determine the effectiveness of thymol (powdered form) with oxalic acid against an ectoparastic mite, *Varroa destructor* populations in honey bee, *Apis mellifera ligustica* (Hymenoptera: Apidae) colonies in the autumn. Thymol 2, 3 and 4gm with 3% oxalic acid was applied to twenty honeybee colonies thrice on different dates. The mean number of *Varroa* mite fallen by treatments T1, T2 and T3 was found 348±2.34, 412±2.87 and 523±3.24respectively. The highest number of mites fell in T3and was found significant (F= 34.40, $P < 0.05$) from all other treatments. The percent efficacy of *Varroa* mite by different treatments T1, T2 and T3 was found 82±1.45, 86±1.46 and 92±1.48 respectively. The results showed a significant difference between efficacies (F = 233.28, P < 0.05). The amount of honey produced from different colonies treated with T1, T2 and T3 was found 12±0.11, 15±0.13 and 24±0.16respectively and was found significant (F= 45.35, P < 0.05).The treatment T3 showed maximum number of fallen mites, highest percent efficacy and amount of honey produced which clearly showed that treatment T3 (Thymol 4gm + 3% OA)to be the most effective treatment against *Varroa* mite.

Introduction

The ectoparastic mite, *Varroa destructor* is considered as one of the most serious pest, causing tremendous damage to honey bee (*Apis mellifera* L) and great economic loss to the beekeeping industry (Anderson and Trueman, 2000;Al-Abbadi and Nazer, 2003). Parasitism can result in a loss of up to 25% of adult weight, severe deformations of the wing and reduced longevity of workers and drones (Kanga and James, 2002). Colonies infested with *Varroa destructor* have significantly reduced worker bee populations and eventually die, if left without controlling (Sammataro, 1997). The *Varroa* mite has a negative effect on honey production as well as other bee products and can facilitate the horizontal transmission of Deformed Wing Virus, Sac Brood Virus and Kashmir Bee Virus from nurse bees to larvae through larval food and via brood to adults (Chen *et al.*, 2004). It can also be transmitted vertically by drones via semen and by queens via virus infected eggs (Yue *et al.*, 2006, 2007). In addition, there is evidence for horizontal mite-to-mite transmission of viruses (Bowen-Walker *et al.*, 1999; Chen *et al.*, 2005). One interesting observation is that at the time of collapse, *Varroa* mite populations were not at

levels known to cause economic injury or population decline (vanEngelsdorp *et al.*, 2009). Efforts to control varroosis have been focused on the use of synthetic acaricides. However, these acaricides have some disadvantages, they may promote the mites to develop resistance against their active ingredient; they are toxic to bees and humans and may leave chemical residues in honey which is a product for human consumption (Miozes- Koch *et al.*, 2000; Ruffinengo *et al.*, 2007; Adjlane *et al.*, 2013). In the short term, mite control may be achieved by using some natural acaricides, which have low toxicity and low environmental impact, because no residues are left in honey and breakdown or volatilized rapidly (Mattila and Otis, 1999). Few natural products have shown effectiveness against varroa i.e. formic acid, oxalic acids, thymol and essential oil are among them (Stanghellini and Raybold, 2004; Espinosa-Montano, and Guzman- Novoa, 2007).The aim of this work was to study the effectiveness of thymol and oxalic acid against *Varroa* mites in honey bee populations.

Materials and Methods

The present study was carried outon *Apis mellifera ligustica* honey bee colonies naturally infested with *V. destructor* mite. Treatments were given randomly to all experimental colonies which were requeened with hygienic queens prior to the startof the experiment. About 200 adult workers and sealed brood populations were assessed for infestation level before selecting the experimental colonies. The samples were collected from *Varroa* infested colonies and the alcohol wash technique was used (De Jong *et al*., 1982). The capped brood infested with mite was evaluated by opening 100 cells of sealed brood before treatment, while for the assessment of mite population in debris; white sticky paper was placed at the bottom board of the bee colony. The sticky paper was left for24h period and mites fell on the paper were counted and used as measure for mite population. Twenty queen right honey bee colonies were used that had been standardized (100 mites) for bee frame+ brood+ debris infestation levels (Rashid *et al*., 2011). The bee hives were placed at a distance of 3 meters. The experiment was started in the month of July which isthe peak time of mite population. Colonies were divided into 4 groups of 5 colonies each. Colonies of the first group were treated with 2gm finely grinded thymol plus 3% OA (T1), the colonies of the second group received 3gm finely grinded thymol plus 3% OA (T2), the third group was treated with4gm finely grinded thymol plus 3% OA (T3) and the fourth group served as control (untreated) group(C).

Thymol crystals finely grinded were placed in Petri plates (80mm) on top of the brood frameunder the inner cover of hives. The5 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 *V. destructor* infestation and treatment efficacy was calculated by counting fallen mites on white sticky paper. At the end, all the experimental colonies were checked for dead worker bees and queens at the end of experiment. The efficacy of all the treatments was calculated by using following formula (Marinelli *et al*., 2004).

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

The results were analyzed using R-Software statistical program. Comparisons between means were made using the least significant difference at $P< 0.05$ probabilities. For

statistical data, standard descriptive statistics were performed for each of the quantitative parameters.

Results and Discussion

The mean number of *Varroa destructor* fallen for each treatment are shown in Table-1. When different treatments were compared for *Varroa*, a highly significant difference was found for the number of fallen mites (F= 24.40, $P < 0.05$). The highest number of mites fell in T3 and was found significantly different from all the other treatments, which clearly showed that T3 to be the most effective treatment against *Varroa* mite. For the *Varroa* mites, the efficacy range for each treatment is shown in Table-2.The results showed a highly significant difference between efficacies (F = 206.28, P < 0.05). The T3 again showed the highest efficacy of 92% against *Varroa* mite. The mean amount of honey produced by different groups is shown in Table-3 where significantly more amount of honey was obtained from the colonies treated with T3 (F= 32.35, P < 0.05).

In the present study combination of 3% OA with different quantities of thymol was tried against *V. 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 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). The usefulness of OA as mite control agent has been known since the end of 20th century (Popov *et al*., 1989). The overall OA was found to be quiet effective against *Varroa* (Brodsgaard *et al*., 1999; Gregorc and Planinc, 2001; Gregorc and Poklular, 2003).

Table.1 The mean number of mites, Varroa destructor fallen by different treatments

Treatments	Number of fallen mites (%)		F ratio
	Range	Mean ±SE	
Thymol 2g + oxalic acid 3% (T1)	130-492	348±2.34	24.40
Thymol 3g + oxalic acid 3% (T2)	174-612	412±2.87	
Thymol 4g + oxalic acid 3% (T3)	235-789	523±3.24	
Control (C)	12-35	16±0.33	

P< 0.05

Table.2 Percent efficacy of Varroa destructor by different treatments

Treatments	Efficacy (%)		F ratio
	Range	Mean ±SE	
Thymol 2g + oxalic acid 3% (T1)	80-86	82±1.45	206.28
Thymol 3g + oxalic acid 3% (T2)	84-89	86±1.46	
Thymol 4g + oxalic acid 3% (T3)	90-94	92±1.48	
Control (C)	18-21	20±0.37	

P< 0.05

Table.3 The mean amount of honey produced from colonies treated with different treatments

Treatments	Honey produced (kg)	F ratio
	Mean ±SE	
Thymol 2g + oxalic acid 3% (T1)	12 ±0.11	32.35
Thymol 3g + oxalic acid 3% (T2)	15 ±0.13	
Thymol 4g + oxalic acid 3% (T3)	24 ±0.16	
Control (C)	7 ±0.06	

P< 0.05

Both thymol and OA could provide beekeepers with an effective tool for controlling *varroa* mite (Gregorc, 2005). Calderone *et al.,* (1997) and Imdorf *et al.*, (1996, 1999) studied thymol as main constituent of several commercially available medicinal products and demonstrated its efficacy at controlling mite infestations in honey bee colonies. Rashid *et al*., (2012) studied that 3.2% OA with 4g thymol was the best treatment for controlling two ectoparasitic mites, *Varroa destructor* (Anderson and Trueman) and *Tropilaelapsclareae* (Delfinado and Baker) populations in honeybee, *Apis mellifera* colonies. 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. These 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. Charriere and Imdorf (2002) evaluated OA and lactic acid as an alternate mite control treatment. It has been observed from our pervious experimental studies that 3.2% OA could be effectively used for controlling the honeybee mites.

In conclusion, the beekeepers of the Kashmir valley have used some unapproved chemicals such as chlorobenzilate or some synthetic pyrethroids for the control of mite. 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 populations in bee colonies and to escape from resistance problem, the present study was aimed. However, it can be concluded that the ectoparastic mite, *V. destructor* cause a severe damage in honey bee colonies either through colony losses or less honey production. The beekeepers of the Valley are in severe trouble to manage this mite. Besides that thymol and oxalic acid are effective against *Varroa* mite and can be safely used together without any side effect in controlling the mite.

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