



Efficacy of essential oils against *Varroa destructor* infesting *Apis mellifera* Linn. colonies and their impact on brood development

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Abstract: *Varroa destructor* is a dangerous pest directly for beekeeping and indirectly for crops that require insect pollination. The present investigation has been carried out to study the efficacy and persistence of some essential oils and formic acid against *Varroa* mite in colonies of *Apis mellifera* Linn. at Pantnagar, Uttarakhand. The results revealed that highest mite mortality (77.54 %) with highest brood development (21.74 % increase) recorded in garlic oil followed by turmeric oil (75.84 %) with 15.39 per cent increases in brood development. The hives treated with T₁ (tulsi oil), T₃ (turmeric oil), T₄ (ajwin oil), T₅ (cinnamon oil), T₅ (clove oil) and T₇ (formic acid) also showed good persistence with mite mortality ranging from 66.54 to 77.54 % and brood development -3.12 to 21.74 per cent increase after 3 weeks exposure of the treatments.

Keywords: *Apis mellifera*, *Varroa* mite, Essential oils, Formic acid

INTRODUCTION

The honey bee, *Apis mellifera* L., is critical for crop pollination and honey production. The mites (Acari) that parasitize honey bees have become a global problem. They are threatening the survival of managed and feral honey bees, the beekeeping industry and, due to the role of bees in pollination, the future of many agricultural crops. *Varroa destructor* (Anderson and Trueman, 2000) formerly named *V. jacobsoni* Oudemans is potentially the main parasite of *Apis mellifera* L. and it can cause the collapse of untreated colonies in a few years. This mite which feeds on haemolymph of brood and adult bees causes colony disorder, weakness, decrease in brood and deformation of bees. It also reduces colony ability to pollinate plants (De Jong *et al.*, 1984). The parasite destroys the mechanical protective barriers of the integument and impairs the immune system of the bees (Glinski, 1991). The varroa mite has been a threat to world beekeeping industry and now a potential threat to Indian apiculture (Gatoria *et al.*, 2004).

Several chemical substances were used successfully to control mites, and a wide array of chemicals were highly effective, killing more than 99% of the mites present in infested colonies (Ferrer-Dufol *et al.*, 1991). In recent years, resistance to acaricides has become a major problem in the control of *Varroa*. Increased tolerance to the most widely used synthetic active ingredients has been observed. *V. destructor* strains have been reported to be resistant to fluvalinate and flumethrin (Baxter *et al.*, 1998), coumaphos (Spreato

et al., 2001), and to amitraz (Elzen *et al.*, 2000). Also, the use of acaricides should be minimized in beekeeping because of the residues and their breakdown products in honey and wax (Wallner, 1999). There is current concern about contamination of bee products with synthetic substances against the varroa (Howis and Nowakowski, 2009). The problems associated with the use of acaricides proved considerable incentive to develop new treatment strategies and screening for potential acaricides that minimize these problems. Natural products having components with various modes of action might provide effective solution to the problem of Varroaosis (Imdorf *et al.*, 1999). These natural products such as essential oils and their components or organic acids, especially formic acid, oxalic acid and citric acid were used for controlling varroa mites (Mutinelli *et al.*, 1997).

Keeping in view of the serious threat of *Varroa* mite in the beekeeping and several constraints for its control the present investigation was carried out to develop the safe and effective management option for the mite population. The present investigation is aimed to determine the effective control of varroa mite by using some essential oils and compared with widely used acaricide, formic acid in the hive of *A. mellifera* colonies.

MATERIALS AND METHODS

The field experiments were conducted during 2012 at Apiary, Department of Entomology College of

Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand).

For conducting this experiment, in total six oils (Tulsi oil, Garlic oil, Turmeric oil, Ajwain oil, Cinnamon oil and Clove oil) along with formic acid were used for testing their efficacy against varroa mite. The experiment was conducted with 8 treatments (including formic acid and untreated control) replicated three times. Before treatment all the cracks and crevices in the hive were plugged with mud. A thick white paper sheet was placed on the bottom board beneath the frames. In each treatment, two strips of absorbent cotton pad (3x2 inches each) were soaked in equal quantity of 5 ml for 24 hrs at placed at bottom

of hive near to brood @ 2 strips per colony. Each treatment was repeated after seven days of interval and observations were taken daily from one day after treatment to twenty one day by counting the fallen dead mite on white sheet and sheet was changed daily. Data gathered during the experiment, such as the number of dead mites that had mites on the white paper sheet at the bottom of the hives was used to determine mite mortality. The data collected from experiments were statistically analyzed. The randomized block design was used to find out the efficacy of different essential oils against the mite, *V. destructor*.

Percent mite mortality in honey bee colonies was calculated by using following formula

Table 1. Effect of different essential oils and formic acid against *V. destructor* mortality applied on absorbent cotton pad in *A. mellifera* colonies during 2012.

| Treatment | Compounds | Average number of dead/fallen mite/ hive | | | |
|----------------|-----------------|--|--------------------------|--------------------------|----------------------------|
| | | Pre treatment count | After one week treatment | After two week treatment | After three week treatment |
| T ₁ | Tulsi oil | 12.32 (2.12)* | 19.56 (2.53) | 16.32(2.43) | 11.65(2.01) |
| T ₂ | Garlic oil | 14.00 (2.27) | 25.00 (2.94) | 22.67 (2.75) | 18.66(2.57) |
| T ₃ | Turmeric oil | 13.41 (2.22) | 22.67(2.75) | 21.00 (2.56) | 16.20(2.42) |
| T ₄ | Ajwain oil | 15.12 (2.35) | 16.89(2.46) | 15.43 (2.37) | 8.99(1.83) |
| T ₅ | Cinnamon oil | 13.22 (2.20) | 19.44 (2.51) | 17.22 (2.48) | 15.10(2.35) |
| T ₆ | Clove oil | 11.00 (1.97) | 20.43(2.57) | 17.76 (2.52) | 15.65(2.39) |
| T ₇ | Formic acid | 11.11 (1.98) | 25.33(2.96) | 22.34 (2.72) | 16.00(2.41) |
| T ₈ | Control | 9.00 (1.85) | 6.44 (1.62) | 6.30 (1.61) | 6.33(1.61) |
| | SEM± | 0.75 (0.19) | 2.02(0.42) | 1.63(0.34) | 0.66(0.16) |
| | CD at 5% | 2.28 (0.60) | 6.14 (1.27) | 4.95(1.03) | 2.02(0.49) |

*Data given in parentheses are square root transformed values

Table 2. Efficacy and persistence of essential oils and formic acid on the per cent mortality of *Varroa* mite, *V. destructor* in *A. mellifera* colonies during 2012.

| Treatment | Compounds | % mite mortality | | | |
|----------------|-----------------|--------------------------|--------------------------|----------------------------|--------------|
| | | After one week treatment | After two week treatment | After three week treatment | Mean |
| T ₁ | Tulsi oil | 75.23 (27.47)* | 72.14 (25.87) | 69.96(27.27) | 72.44 |
| T ₂ | Garlic oil | 79.76 (29.12) | 78.90 (28.61) | 74.66(27.12) | 77.54 |
| T ₃ | Turmeric oil | 78.71(28.78) | 76.93(28.15) | 71.90(25.70) | 75.84 |
| T ₄ | Ajwain oil | 70.11 (27.32) | 70.84 (27.55) | 58.64(24.92) | 66.54 |
| T ₅ | Cinnamon oil | 75.11 (27.75) | 73.21(26.50) | 70.46(25.05) | 72.92 |
| T ₆ | Clove oil | 76.00 (27.79) | 73.88(26.85) | 71.20(25.95) | 73.69 |
| T ₇ | Formic acid | 79.79 (29.12) | 79.72(28.81) | 71.65(25.52) | 76.72 |
| T ₈ | Control | 0.00 (0.00) | 0.00(0.00) | 0.00(0.00) | 0.00 |
| | SEM± | 3.34 (1.27) | 2.44(1.18) | 1.94 (0.94) | 1.64 |
| | CD at 5% | 8.02(7.32) | 7.52 (6.92) | 15.35(13.17) | 10.40 |

*Data given in parentheses are square root transformed values

Table 3. Impact of essential oils and formic acid used against varroa mite infestation on brood development in *A. mellifera* colonies applied as fumigant during 2012.

| Treatment | Compounds | Average brood development (area in cm ²) | | | | | | | |
|----------------|--------------|--|-----------------------------|--|--|------------------|-----------------------------|--|----------------------|
| | | I st application (July-Aug) | | | II nd application (Oct-Dec) | | | | |
| | | Before treatment | Three weeks after treatment | Per cent increase in brood development | Honey bees mortality | Before treatment | Three weeks after treatment | Per cent increase in brood development | Honey bees mortality |
| T ₁ | Tulsi oil | 987.21 | 1121.30 | 13.58 | Nil | 1032.87 | 1186.72 | 14.89 | Nil |
| T ₂ | Garlic oil | 971.91 | 1174.71 | 20.86 | Nil | 1071.91 | 1304.83 | 21.74 | Nil |
| T ₃ | Turmeric oil | 1042.44 | 1205.13 | 15.60 | Nil | 1117.62 | 1289.71 | 15.39 | Nil |
| T ₄ | Ajwain oil | 933.84 | 984.62 | 5.43 | Nil | 1059.60 | 1180.32 | 11.97 | Nil |
| T ₅ | Cinnamon oil | 1025.62 | 989.34 | -3.53 | Nil* | 1132.43 | 1098.27 | -3.12 | Nil |
| T ₆ | Clove oil | 1132.86 | 1219.70 | 7.66 | Nil | 1089.32 | 1175.30 | 12.74 | Nil |
| T ₇ | Formic acid | 945.27 | 994.13 | 5.16 | Nil | 1106.00 | 1157.66 | 4.67 | Nil |
| T ₈ | Control | 916.18 | 957.72 | 4.53 | Nil | 915.16 | 954.83 | 4.33 | Nil |
| CD at 5% = | | 124.42 | 240.40 | 4.43 | - | 213.43 | 284.0 | 3.25 | -- |

[(-) =decreased]; *Queen died in one replication.

% mite mortality = Mite mortality in treatment / (Mite mortality in treatment + Mite mortality in control) X 100

The effect of essential oils on brood development in *A. mellifera* colonies, brood measurement was done before and after 5 days of each treatment. Measurement of brood was done with the help of scale. Both the side of the frame in hive having brood was measured with the help of scale. Percent change in brood development in honey bee colonies was calculated by, using following formula

% change in brood development = (Final brood – Initial brood / Initial brood) X 100

RESULTS AND DISCUSSION

The efficacy and persistence of essential oils and formic acid were evaluated against varroa mite and number of fallen mites/hive/week, per cent mite mortality and their impact on brood development are summarized in tables 1, 2 and 3.

First week observations revealed that highest no. of dead/fallen mites per colony (25.33: 79.79%) and (25.00; 79.76 %) mortality was recorded in treatment T₇ (Formic acid) and T₂ (garlic oil) followed by treatment T₃ (turmeric oil), with from 22.67 dead / fallen mites giving 78.71% mite mortality. Both these treatments gave significantly higher mite mortality as compared to other treatments. The number of fallen mites recorded after one weeks in the treatment T₆ (clove oil), T₁ (tulsi oil), T₅ (cinnamon oil) and T₄ (ajwain oil) treated colonies were 20.43, 19.56, 19.44 and 16.89 fallen mites/hive/week with and per cent mite mortality of 78.71, 76.00, 75.23, 75.11 and 70.11 respectively.

The observations after second week indicated that number of fallen mites recorded in treatment T₇ (formic acid) and T₂ (garlic oil) 22.34 and 22.67, dead / fallen mites / hive / week with 79.72 and 78.90 % mite mortality which were significantly higher than in T₃ (turmeric oil), T₆ (clove oil), T₅ (cinnamon oil), T₁ (tulsi oil), and T₄ (ajwain oil) treated colonies with 21.00, 17.76, 17.22, 16.32 and 15.43 fallen mites/hive/week giving 76.93, 73.88, 73.21, 72.14 and 70.84 percent mite mortality, respectively. Similar pattern was observed in third week after treatments but with deceased number of fallen mites and percent mortality. Rana *et al.* (2010) also reported that placing formic acid sponge pads during summer months resulted in 83 -90 % mite mortality. These findings are also in line with the earlier reports of Hoppe *et al.* (1989) and Stangellini and Raybold (2004).

The results showed that among seven treatments applied for *Varroa* mite control, Garlic oil (T₂) and formic acid (T₇) treatments were significantly better. garlic oil (T₂) treatment gave highest mite mortality (79.76 %) after one week which decreased to 78.90 per cent after two weeks and then to 74.66 per cent after three weeks giving an overall mortality of 75.54

percent with the highest brood development 20.86 per cent. This per cent increased brood development was significantly higher ($P < 0.005$) than the treatments of clove oil (7.66), ajwin oil (5.43), formic acid (5.16) and untreated (4.53). During the three weeks, no queen and worker bees loss was observed.

However, in treatment T₅ (cinnamon oil) after first application brood development was decreased by -3.53 per cent three weeks after treatment. This decrease in brood development was due to high mite infestation and loss of one queen in the bee colonies. Abd El-Wahab *et al.* (2012) reported that the highest concentration of tested oils resulted in high amount of sealed worker brood particularly in anise and lemon grass oils, while, the cinnamon oil and formic acid recorded the lowest one.

It was concluded that garlic oil is effective in causing varroa mite mortality to a level of 78.90 % upto two weeks and the application need repetition thereafter for achieving effective control. The effectiveness of garlic oil can be an alternative to formic acid, a chemical treatment widely being used in *A. mellifera* colonies against *Varroa* mite. The use of essential oil may fit well into Integrated Pest Management (IPM) programs for alternative use with other control measures for the management of *Varroa* mite and other pests in honeybee colonies although they enhance chances for colony survival and ensure residue-free hive products.

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