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Evaluation of Few Essential Oils for the Management of Parasitic Bee Mites, *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* L. Colonies

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Authors' Contribution
MAB designed and conducted study. MAA helped in review and proof reading; ABMR helped in data collection and analysisand MIU helped in review and data interpretation. MA helped in statistical analysis and preparation of manuscript; MS helped in literature and experimentation and JMO critically reviewed and interpreted results

Key words
Essential oils, Percent mortality, Varroa mites.

ABSTRACT

Varroa destructor (Anderson and Trueman), is a greatest rigorous and economical pest of *Apis mellifera* L. worldwide. Many control measures including application of chemicals are adapted to resistor the influx of mites in hives of honey bee. The indiscriminate use of pesticides involve many problems; cause resistance in mites, residual in honey, wax and potentially hazardous to man and environment. Plant-derived mixtures are normally more easily degradable and could show a minor undesirable impact on environment with respect to synthetic compounds. This study was planned to check the efficacy of four different essential oils (neem oil, lemon oil, eucalyptus oil and orange oil) at different doses against percent mortality of *Varroa* mites. The essential oils were applied at two different doses (2.5ml and 5ml) and percent mortality data was recorded after 12, 24, 48 and 72 h of the application. As seen from results, all essential oils gave satisfactory control of *Varroa* mites except orange oil. The percent mortality was observed higher in eucalyptus oil (76.13%) at 2.5ml dose. Similarly, at 5ml dose, eucalyptus oil (90.27%), lemon oil (85.54%) and neem oil (82.69%) gave significant control of *Varroa* mites. With the passage of time, mite mortality percentage increases. Overall, eucalyptus oil at 5ml dose rate gave best result and can be recommended to manage the varroa mite in apiculture.

INTRODUCTION

The haemolymph serving mite, *Varroa destructor* (Anderson and Trueman), is a supreme rigorous pest menacing honeybee worldwide (Lodesani *et al*., 1992). The mite is a parasite on brood of bees causing brood abnormality, loss of the bees and consequent colony weakening or escaping (Hosamani *et al*., 2006). *Varroa* mites are external parasites of honey bee that violence both brood, honeybee's adult and with a divergent preference for drone brood (Peng *et al*., 1987). They slurp the body fluid from both the adults and developing larva, waning them and curbing life period of the bees which they nourish on. Developing brood might stay instinctive with deformed wings. Unprocessed in fluxes of *V. destuctor* can reason of colonies of honeybee to downfall (Martin, 1994). Mites instigated 30-70% colony loss of *A. mellifera* and also condensed the honey production and (Woo and Lee, 1997). Unfortunately, ectoparasitic mites attack on honeybees, resulting low yield of honey and also cause absconding and swarming (Mahmood *et al*., 2011).

Altered approaches have been functional to accomplish *Varroa* mite in modern hives including chemical, mechanical and ordinary measures. While organic techniques condensed mite influx and flourished to a countless level, resistance of mite to most acaricides used for control was a major problem. Similarly, misuse of organic compound clues to inappropriate excesses in hive yields *e.g.,* beeswax and honey (Wallner, 1999).

Liable on unconventional practices in monitoring

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Varroa mites, natural constituents *e.g.,* botanical extracts and vital oils were employed and flexible efficacies were succeeded as well as integrated controlling of the mite (Balhareth *et al*., 2012).

A variety of vital oils have been originated to revelation acaricides action opposing to *V. destructor*. These are distilled from fragrant plants, have passionate smell, exhibit little injuriousness to animals and bees and have less detrimental influence over surroundings and aeclectic public approval (Isman, 2000). A significant quantity of necessary oils and their constituents have been proven to control the mite, with diverse consequences. Their miticides or attractant/ repellent properties on insects and their impact on its reproduction have been calculated. Vital oils are being locally ordered, useful in liquidation or in an inert vaporization form (Imdorf *et al*., 1999).

The beekeepers have been mandatory to practice unapproved chemicals such as amitraz, sulphur, phenothiazine, chloro benzilate or diverse pyrethroids to switch the mite influx. Unrestrained use of these compounds ran to the expansion of resistance, revival of the influx and the hazard of residues in the honey which potency pose a hazard for human consumption (Farooqi *et al*., 2016). Keeping in vision the prominence of nontoxic and non-stained control approaches to destroy mite residents in bee colonies as well as to emission from resistance problematic, the present study was planned to define the efficacy of different essential oils as miticides against *V. destructor*.

MATERIALS AND METHODS

The field experiment was conducted at Apiary of University of Sargodha. For conducting this experiment, four essential oils (neem oil, lemon oil, eucalyptus oil and orange oil) at two different doses (2.5ml and 5ml) were used to evaluate their effectiveness against *varroa* mite. The experiment was replicated three times. Before application, entirely the crevices and cracks in the hive were plowed with mud. Mite collection trays (mite excluders) were placed through the back side of the hive covered by a wire screen to prevent the bees from coming into contact with the debris. A white sticky paper piece was hired on the lowest line of each box covered with wire mesh. Two strips of staining paper (5x2 inches each) were drenched for 24 h in each dose of all treatments (Goswami and Khan, 2013). Treated strips were hanged between frames of each hive. Four colonies of equal bee population with ten frames were selected for each treatment and control. Data was recorded once before treatment application to estimate the initial population of *varroa* mites in each colony. Sugar shake method was used to estimate mite infestation by the method used by Ellis and Ellis (2005). From each colony, 250 adult bees were evaluated to check the effects of different essential oils. Acetone was applied on bee hives as control treatment.

Data was recorded after 12, 24, 48, and 72 h of application by counting the fallen/ dead mite on white sheet. Sheet was changed daily after data recording. The number of fallen/dead mites was used to determine mite mortality percentage. Percent mite mortality in bee colonies was calculated by using formula (Abbott, 1925):

$$\text{Percent mortality} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in Co after treatment}}\right) \text{x } 100$$

Where, n is mite population, Co is control and T is treated.

Statistical analysis

The data of percent mortality was statistically analyzed by three factor factorial analysis of variance considering essential oils, time interval and doses as variables. Means of percent mortality were separated using tukey HSD all pair wise comparison test. All the experimental analysis was performed using Minitab 16.1 software.

RESULTS AND DISCUSSION

Analysis of variance for percent mortality of *Varroa* mites for different essential oils at different doses after different time interval showed in Table I. The results showed that treatment (F=146.76, *P<0.005*), dose (F=1350.1, *P<0.005*) and time interval (F=965.8, *P<0.005*) was highly significant. Interaction of doses with treatment and time interval (F=2.95, *P<0.05*, F=3.95, *P<0.05*), respectively, also showed significant variation in percent mortality of mites. Interaction between treatment, dose and time interval was not significant at 5% level of significance.

Table I.- Efficacy of different essential oils against *Varroa* mite at different time interval.

Source	DF	SS	MS	F-value	*P*-value
Dose	1	3700.17	3700.17	1350.63	*P<0.001*
Treatment	3	1206.04	402.01	146.74	*P<0.001*
Time	3	2897.46	965.82	352.54	*P<0.001*
Dose × Treatment	3	24.25	8.08	2.95	*P<0.05*
Dose × Time	3	32.50	10.83	3.95	*P<0.05*
Treatment × Time	9	31.13	3.46	1.26	*P>0.05*
Dose × Treatment × Time	9	31.08	3.45	1.26	*P>0.05*
Error	64	175.33	2.74		
Total	95	8097.96			

P<0.05, Significant; P<0.001, highly significant; P>0.05, non-significant.

Mean percent mortality data showed that eucalyptus oil was best compound against *Varroa* mites as compared to other treatment. At 2.5ml dose, the highest mortality of *Varroa* mite was observed 72.54 % and 76.17% at 48 and 72 h, respectively, which was significantly different from other treatment. Overall percent mortality was observed less in case of orange oil (Fig. 1A).

At 5ml dose rate, percent mortality of *Varroa* mites remains similar. The percent mortality of *Varroa* mites was observed higher (84.18%, 90.27%) after 48 and 72 h, respectively, in case of eucalyptus oil. After 72 h, there was no significant difference among treatments. Percent mortality was also observed significant (85.54%, 82.69%) in lemon oil and neem oil, respectively, at 72 h (Fig. 1B). Maximum effect of orange oil at 5ml dose did not exceed 79.2% after 72 h (Fig. 1B). Percent mortality of mites was increased with the passage of time in all selected essential oils.

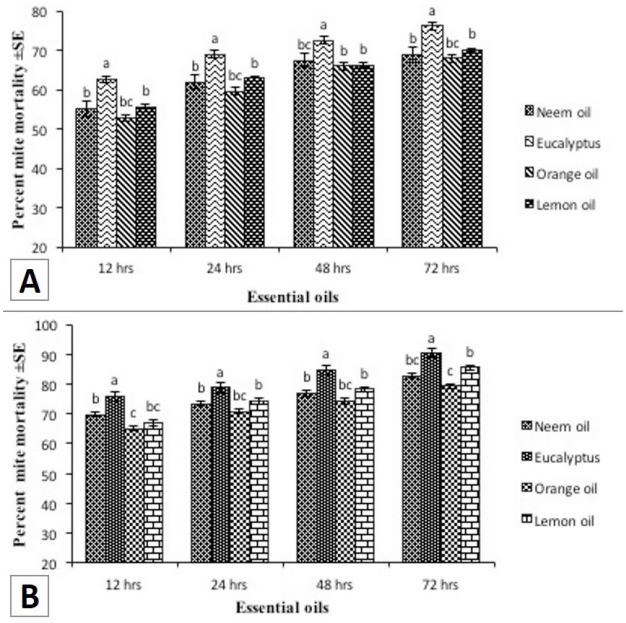

Fig. 1. Mortality (%) of *Varroa* mites at 2.5 ml (A) and 5 ml (B) dose after application of essential oils at different time interval, $P<0.05$. (Similar letters show that means are not significantly different from each other within each h after application).

Overall higher dose (5ml) gave significant mortality of *Varroa* mites at different time interval as compared to low dose (2.5ml). Overall, the essential oils at 5ml dose showed 13.83% greater response compared to 2.5ml dose after 72 h of application. Essential oils have fumigant action (Kim *et al*., 2003) and volatile oil could penetrate organism via the respiratory system resulting in enhanced efficacy (Choi *et al*., 2004). Essential oils can inhibit with basic behavioral functions of certain arthropods (Imdorf *et al*., 1999). Some reveal acute noxiousness while others may action as repulsive substances (Watanabe *et al*., 1993), antifeedant (Hough-Goldstein, 1990), or may stay the development or reproduction or affect with physiological and biological manners.

The unadventurous pesticides show noxiousness to the surroundings and injurious effects on human health. In this situation, there is an emergent attention in botanical pesticides due to their negligible charges and absence of environmental side effects (Khater, 2012), which brands them required replacements to synthetic compound for controlling pests.

As the risk of resistance growth in mites and dangerous residues in bee yields, practice of carbon-based mixtures has develop prevalent all over the world.

Botanic extracts gained from diverse plant species have been given away to have a wide-ranging variety of acaricidal action counter to varroosis and also against other creatures such as bacteria, mites, insects, nematodes and fungi (Damiani *et al*., 2009).

Eucalyptus oil was proved as best to control *Varroa* mites at 5ml dose. Minimum mortality percentage was recorded in orange oil at both dose rate of application. Our findings were similar to Su *et al*. (2006) who also reported the insect killing activity of eucalyptus oils against *Varroa* which has been owed to the constituents such as citronellyl acetate, eucamalol, 1, 8-cineole, p-cymene, citronellal, limonene and citronellol. Gonzalez-Gomez *et al*. (2006) worked with crude extracts of neem seeds and evaluated the effects on mites and bees separately. They also found an important repellency effect against mites. No toxic effect was found for bees. Similarly, Gonzalez-Gomez *et al*., (2012) found more repellency of mite population using neem extract. According to Melathopoulos *et al*., (2000a) neem and canola can be used to suppress the population of parasitic mites on bees. Kraus *et al*. (1994) also described repulsive properties of necessary oils on *Varroa*. Colin (1990) assumed that long-term repellency may diminish *Varroa* fertility. Djenontin *et al*. (2012) reported that Azadirachtin is the supreme vigorous constituent for preventing and killing pests and can be taking out from neem oil. Active ingredients in neem oil are azadirachtin, quercetin nimbin, sodium nimbinate, nimbidin, gedunin, nimbidol, and salannin.

With the passage of time, mite mortality percentage increases using essential oils. Calderone and Spivak (1995) and El-Zemity *et al*. (2006) also found that essential oils showed good result against *Varroa* mite after 48 h of exposure.

Increasing the mean number of *Varroa* mite fallen

on the sheet in tested honey bee colonies treated with essential oils may be due to the activation of the defense behavior mechanisms of honey bee workers by these plant oils against *Varroa* mite. We succeeded to achieve significant *Varroa* mites mortality from eucalyptus and neem oil without toxicity to the bees. Ghasemi *et al*. (2011) reported that essential oil, *Thymus kotschyanus* gave better management of mite population with least adverse effects of insecticidal activity against honeybees. General insect mortality is dose and exposure time dependent. With the passage of time the toxicity of essential oils against *Varroa* mites increase and proved more potent against *Varroa* mites. Salem *et al*. (1998) initiate that honey bee clusters which nourished on neem extract exposed the maximum number of *Varroa* mite dropped on the sheet. Our result is closely related with Mahmood *et al*. (2014) who found that the efficacy (85.3 %) of neem oil against varroa mites. They proposed that these extracts affected deviations in the blood of worker bees and consequently amplified the number of *Varroa* mite fallen on the sheet. Neem oil has the ability to kill phoretic adults of *varroa* and also disrupt the reproduction and growth of mites within the cell (Melathopoulos *et al*., 2000b). However, Abd El-Wahab and Ebada (2006) indicated that specific protection behavior appliances counter to *Varroa* mite were noticed in some hybrids and races of honey bee. These appliances caused in growing the number of fallen *Varroa* mite on the end board of bee box. According to Lee *et al*. (2003), the monoterpenes that may be lipophilic and volatiles can enter over breathing and rapidly interfere in physiological functions of insect. These mixtures can also action openly as neurotoxic compounds, disturbing octopamine receptors or acetylcholine esterase activity (Isman, 2000).

CONCLUSION

Essential oils gave best result to control *Varroa* mites and did not affect the colony strength of honeybees. The finding of present study indicated that all essential oils except orange oil are promising as safe natural product for control of *Varroa* mites. Also these essential oils proved to be harmless to the bees and quite save to the environment. The use of essential oils 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 while they enhance probabilities for colony existence and ensure residue-free hive products.

Statement of conflict of interest

Authors have declared no conflict of interest.

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