Research Article

Efficacy of Essential Oils and Formic Acid in the Management of *Tropilaelaps clareae* in *Apis mellifera* Linnaeus Colonies in Relation to Honey Production

Noor Islam[1], Muhammad Amjad[2], Ehsan ul Haq[3], Elizabeth Stephen[1] and Falak Naz[4]

[1]Honeybee Research Institute, National Agriculture Research Centre (NARC), Islamabad; [2]Plant Sciences Division, Pakistan Agriculture Research Council, Islamabad; [3]Insect Pest Management Programme, NARC, Islamabad; [4]Coordination, Pakistan Agriculture Research Council, Islamabad, Pakistan.

Abstract | The brood ectoparasitic mites, *Tropilaelaps clareae* is causing greater damage to *Apis mellifera* colonies and major economic losses (a serious threat) to beekeeping industry in Pakistan. Seven treatments including five essential oils of basil (*Ocimum basilicum*), lemongrass (*Cymbopogon citratus*), oregano (*Origanium vulgare*), lemon (*Citrus lemon*) and thyme (*Thymus linearus*), formic acid at three different concentrations of 25%, 50% and 100% and control were used against *T. clareae* in naturally infested honey bee, *A. mellifera* colonies in Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan. The percentage of infestation by *T. clareae* on worker brood, number of dead/fallen mites per hive/week on white formica sheet, percent mite mortality and honey yield per colony was determined before and after the treatments in the experimental colonies. The results revealed that formic acid and the highest concentrations (100%) of tested essential oils caused effective control of *Tropilaelaps* mites, whereas the infestation reduction percentage with formic acid, lemongrass, thyme, lemon, basil and oregano oils was recorded more than 96% after the end of treatments on worker brood. The highest total number of dead *T. clareae* mites (761) fallen on the sheet was recorded by formic acid followed by lemongrass (445), thyme (349), lemon (298), basil (283) and oregano (250) respectively. The hives treated with essential oils and formic acid also showed good persistence with over all mean percent mite mortality ranging from 64.37 to 84.33% up to fourth application of treatment. High mean value for honey yield (9.3 kg) was recorded by formic acid, followed by lemongrass (8.8 kg), thyme (8.5 kg), lemon (8.5 kg), basil (7.7 kg) and oregano (7.6 kg) per colony as compared with control colonies (3.8 kg) per colony.

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***Correspondence** | Noor Islam, Honeybee Research Institute, National Agricultural Research Centre, Islamabad, Pakistan; **Email:** khattakni@gmail.com
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Introduction

Different pests and predators attack the honey bee colonies as they live together in a tightly knit social group. Among pests, honey bee mites *Varroa destructor* and *Tropilaelaps clareae* are considered as the major limiting factor in world apiculture (Hosamani et al., 2006; Zhou et al., 2007). These mites can be categorized as parasitic, predatory and phoretic. Amongst these, parasitic mites are quite harmful to honey bees causing brood mortality; reduced colony strength and increase in the number of deformed bees (Kapil et al., 1985). *Tropilaelaps* can cause 50-100% loss of bee colonies (Hosamani et al., 2006). Kiprasert

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(1984) reported that *T. clareae* infests as much as 90% of the brood of *A. mellifera* colonies but smaller brood infestation levels of 3-6% have been consistently reported from *A. dorsata* colonies (Underwood, 1986).

T. clareae was observed in 1981 for the first time in Pakistan and spread to European honey bee, *A. mellifera* in 1991 throughout the country by commercial migratory beekeepers as they shift their bee colonies in different areas, where good honey flora is available for the production of different types of honey. At present *T. clareae* is causing major economic losses and damage to beekeeping industry in Pakistan with *A. mellifera.* The highest infestation of *T. clareae* occurred in February, March and April with a decline from May to August (Camphor et al., 2005). Burgett and Akratanakul (1985) predicted that in the near future *T. clareae* would play a major role in the destruction of commercial honey bee colonies far greater than that caused by *Acarapis woodi* and *Varroa* mites. Poor management of bee colonies, hive microclimate and weak colony has increased the incidence of *T. clareae* in bee colonies (Mahavir and Gupta, 1999). *T. clareae* caused 30-70% colony loss of *A. mellifera* with reduced honey production (Woo and Lee, 1997).

Beekeepers are using low costs/quality synthetic acaricides such as sulphur, flumethrin, fluvalinate strips, coumaphos, amitraz, tobacco, fluvalinate injections and formic acid in order to control *T. clareae* and *Varroa destructor* mites in Pakistan. Camphor et al. (2005) observed that *T. clareae* mite has developed resistance to sulphur, tobacco and naphthalene because in spite of regularly treating *A. mellifera* colonies with these chemicals many colonies were still found infested. Honey bee mites are highly resistant to chemical treatments, therefore, even if necessary, the repeated applications of the same chemical may be avoided. Elzen et al. (2000) indicated that these mites are not only resistant to fluvalinate, but also to the commonly used amitraz. Moreover, the wide spread use of synthetic acaricides has lead to the accumulation of residues in bees wax, propolis and honey (Martel and Zeggane, 2002; Mullin et al., 2010). These concerns have provided considerable incentive to develop new strategies that minimize the potential for acaricides resistance and the accumulation of such residues. The use of pyrethroid and fluvalinate has also made the honey bees more susceptible to pesticides sprayed on crops and block the detoxification mechanisms for some fungicides in bees (Pilling et al., 1995). Another study demonstrated that the combination of some pesticides can increase toxicity in honey bees 1,000 folds (Iwasa et al., 2004). Due to the severity of the residue problems national and international food regulations were established for the consumption and trade of honey bee products. These concerns have led to beekeepers looking for alternative acaricides, such as essential oils, to control *T. clareae* in light of the large colony decline. Essential oils are natural, safer alternatives to chemical control measures. Essential oils are non toxic to non target species and have less resistance problems as to other pesticides (Imdorf et al., 1999).

It is well known that many essential oils and their compounds exhibit acaricidal activity (Lee et al., 1997). Different components of essential oils were tested for their activity against *A. woodi*. Recently, research has shown that several essential oils and individual compounds of essential oils have high acaricidal activity against *V. jacobsoni*. Calderone et al. (1997) suggested that they may be useful in maintaining mite infestation rates below economic injury level. More than 150 essential oils have been used for the control of insect pests and mite; few remain viable at controlling mites without affecting honey bees (Imdorf et al., 1999). Essential oils kill the mites when they come in contact within a few minutes and they also impair their reproduction. If the oil is strong enough, the females are unable to lay eggs. If the oils are used at lower concentration, eggs are laid but development of immature mites is delayed. Young mites do not reach maturity before the bees emerge from the cell. Consequently, the immature mites die (Allam et al., 2003).

Very little work has been carried out by using essential oils to control *T. clareae* in Pakistan. Therefore, the aim of the present study is to investigate the efficacy of essential oils of thyme, lemon grass, lemon, oregano and basil against *T. clareae* in honey bee colonies and their affect on honey production.

Material and Method

The experiment was carried out at the apiary of Honeybee Research Institute, National Agricultural Research Centre, Islamabad during June 2014 to October 2014.

Experimental procedure

Selection of honey bee colonies: Before starting the experiment, 300 honey bee colonies in the apiary were monitored to estimate the consistency of the bee population and mite infestation levels in order to obtain homogenous experimental groups (six for treatment and one for control). Sixty three honey bee *Apis mellifera* colonies in Langstroth standard bee hives naturally infested with *T. clareae*, with a normal brood pattern (eggs, larvae and pupae) and removable bottom inserts were used in this experiment. Each bee colony had 10-11 full depth combs of worker bees and 3-5 brood frames. Honey bee colonies were randomly allocated to seven treatment groups. Three colonies were used for each concentration of essential oils and formic acid and three untreated colonies served as control (Ali et al., 2002; Satta et al., 2005).

Chemicals and concentrations used: Five essential oils of lemongrass (*Cymbopagon citrate*), lemon (*Citrus lemon*), origano (*Origanium vulgare*), basil (*Ocimum basilicum)*, thyme (*Thymus linearus*) and formic acid (65%) at three different concentrations of 25%, 50% and 100% were used for conducting this experiment.

Plants identification and essential oils extraction

Plants were identified from the National Herbarium Programme, NARC, Islamabad. These plants were dried in the shade at room temperature and chopped into small pieces with electric grinder. The essential oils were extracted by the process of water steam distillation using a clevenger-type apparatus (Richard et al., 1992) within three hours. An average of 100 g plant material was used for each extraction and several distillations were made until obtaining an appropriate volume for all the trials. After oil extraction, anhydrous sodium sulphate was used for the removal of excess water. These oils were transferred to dark brown glass vials with aluminum foil and stored in refrigerator at 4^0C until used in the experiment.

Percent infestation of *Tropilaelaps clareae*

The ectoparasitic bee mite, *T. clareae* infestation in worker brood was determined before and after treatment. The level in worker brood was recorded by randomly choosing fifty sealed worker brood cells from three combs per hive (Satta et al., 2005; Rashid et al., 2011; Islam et al., 2016). The capped brood cells were unsealed and then the pupae were withdrawn from the cells with the help of a forceps and picked up in petri dishes. Samples were transferred to the laboratory and the body surfaces of the brood were inspected for *Tropilaelaps* infestation under stereomicroscope, which were counted and recorded.

Mortality of *Tropilaelaps clareae*

The number of dead/fallen mites per hive from treated and control colonies were counted on a weekly basis using a white formica sheet under mesh at the bottom of each hive (Floris et al., 2001; Gregoric and Planincy, 2005). The metal sheet was covered by a wire screen to prevent access by bees (Calderone and Lin, 2003; Gregoric and Planincy, 2005). On the sampling dates, the number of dead mites was recorded and the sheets were emptied (Gregorc and Smodis Skirl, 2007). *T. clareae* mite mortality was observed at different times a week before the treatment, at one week intervals during the entire period of the experiment and finally after the treatment.

Honey yield

It is known that there is a strong correlation between a colony's strength and food supply and honey yield provides a good indication of this relationship (Jevtić, et al., 2009). By measuring the total honey yield from each colony, we can determine whether the treatments had any long term effects.

Data analysis

The efficacy of the treatments was calculated on the basis of the percentage of *T. clareae* mite mortality (Floris et al., 2001; Satta et al., 2005) using the following equation:

$$\%\text{ age mite Mortality} = 100\left(1 - \frac{Bc \times At}{Bt \times Ac}\right)$$

Where Bt and At are the respective percentages of infestations in the treated colonies before (Bt) and after treatment (At) and Bc and Ac the respective parameters measured in the control colonies. Percent mite mortality in *A. mellifera* colonies was determined by using the formula (Pawar, 2008; Islam et al., 2016):

$$\textbf{Perecent mite mortality} = \frac{\text{Mite mortality in treatment} \times 100}{\text{Mite mortality in trearment} + \text{Mite mortality in control}}$$

Analysis of variance (ANOVA) was carried out by using F- test and least significant difference (LSD) test at 1% probability level was used to compare between the different treatments (Fisher, 1950; Snedecor and Cochran, 1972).

Results and Discussion

Plant essential oils of Basil, Thyme, Lemongrass, Lemon, Oregano and organic acid formic acid were evaluated at three different concentrations to determine their effect on the reduction percentages infestation of *Tropilaelaps clareae*, number of dead/fallen *Tropilaelaps* mites on white formica sheet, percent mite mortality and honey yield (Figure 1 to 10).

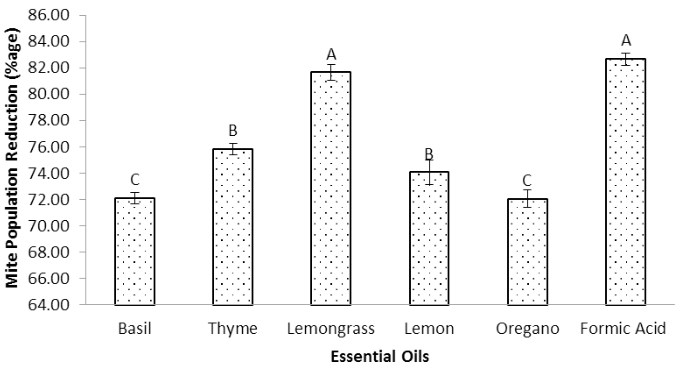

Figure 1: *Mean population reduction percentage of honey bee ectoparasitic mite Tropilaelaps clareae in worker brood by using 25% concentration.*

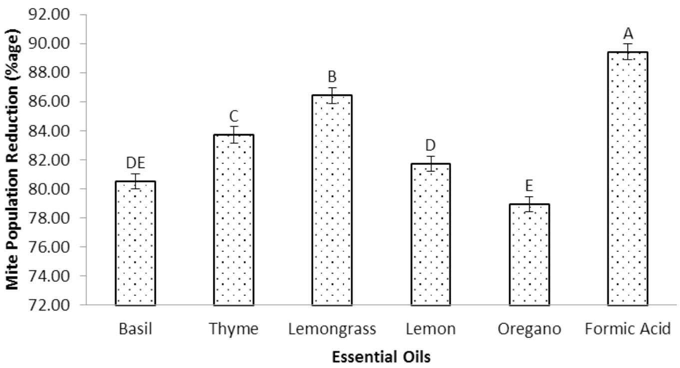

Figure 2: *Mean population reduction percentage of honey bee ectoparasitic mite T. clareae in worker brood by using 50% concentration.*

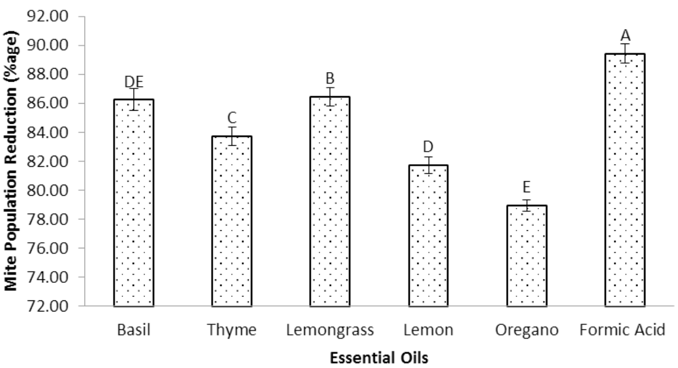

Figure 3: *Mean population reduction percentage of honey bee ectoparasitic mite T. clareae in worker brood by using 100% concentration.*

Percent infestation of *Tropilaelaps clareae*

The data indicated that the percent infestation of sealed worker brood before application of tested oils was 34.5 to 37.6% at 25%, 34.7 to 38.5% at 50% and 35.8 to 39.0% at 100% concentrations in the experimental honey bee colonies. After treatment infestation decreased gradually to the lowest infestation %age observed after 4th treatment application. The results showed that at 25% the highest mean percent reduction infestation in worker brood by *T. clareae* was 82.67% (Formic acid), 81.66% (Lemongrass), 75.81% (Thyme), 74.07% (Lemon), 72.10% (Basil) and 72.06% (Oregano), respectively. At 50%, the highest mean percent reduction infestation in worker brood by *T. clareae* was 89.42% (Formic acid), 86.43% (Lemongrass), 83.72% (Thyme), 81.73% (Lemon), 80.51% (Basil) and 78.94% (Oregano), respectively. Whereas at 100% concentration, the highest mean percent reduction infestation in worker brood by *T. clareae* was 93.47% (Formic acid), 91.44% (Lemongrass), 88.70% (Thyme), 86.39% (Lemon), 86.24% (Basil) and 83.94% (Oregano), respectively. While this percent infestation in worker brood was increased from 40.2% to 47.4% in the control colonies. The analysis of variance indicated that throughout the first three applications, all the essential oils and formic acid were significantly more effective against *Tropilaelaps* mites as compared to the untreated colonies. The highest concentration (100%) of all the essential oils and formic acid was highly efficient in reducing the mean infestation percentage of *Tropilaelaps* on worker brood. The results also showed that mean percentage of *Tropilaelaps* infestation on the worker brood reduced to 100% after the fourth application with formic acid, lemon, thyme and basil. These results were in agreement with the findings of Allam et al. (2003) who found that formic acid caused 91.7% mortality of the *Varroa* mites. Abd El-Halim et al. (2006) observed significant differences in mean infestation percentages of *Varroa* in worker brood after 1st, 2nd, 3rd and 4th week of treatments between the sour orange, lemongrass and citronella oils at three different concentrations (25, 50 and 100%) and control. Abd El-Wahab et al. (2012) recorded highest reduction infestation percentage in adult worker bees at the highest concentration (100%) of Formic acid, Thyme, Cinnamon, Lemongrass and Anise whereas more than 96% reduction infestation percentage of worker brood with Formic acid, Lemon grass, Thyme and Anise oils were recorded against *Varroa* mites.

Mortality of *Tropilaelaps clarea*

The results showed that highest number of fallen/

dead *Tropilaelaps* mites was recorded after 1st and 2nd treatments, particularly at 100% concentration of the essential oils and Formic acid in comparison with control colonies. It gradually decreased from 2nd to 4th treatment. These results were in accordance with those of Shoreit and Hussein (1994) who determined that maximum number of dead mites was observed after the first treatment with Coriander extract in both of winter and spring feedings which steadily decreased during 2nd and 3rd week of treatments. The results showed that the total maximum number of dead *T. clareae* mites/hive fallen on formica sheet after treatment of four application was 352, 472, and 761 (formic acid), 220, 316 and 445 (lemongrass), 172, 264 and 349 (thyme) 134, 227 and 298 (lemon) 133, 200 and 283 (basil) and 99, 155 and 250 (oregano) at 25%, 50% and 100% concentrations, respectively as compared to control with 62 dead mites/hive. There were highly significant differences between formic acid, essential oils and control in the total number of dead/fallen *Tropilaelaps* mites between the treatments during the experimental period. These results also indicated that the number of dead/ fallen *Tropilaelaps* mites was higher after 1st application than 2nd and 3rd application whereas the 4th application resulted in the lowest number of mites. These results are in agreement with Islam et al., (2016) who found that the highest total number of dead *Varroa* mites (323) fallen on the sheet was recorded by formic acid followed by lemon grass (306), thyme (263), mint (204) and rosemary (188) respectively. This reduction in the number of fallen mites could be attributed to either the reduced rate of infestation or the reduced efficiency of the control agents (Shalaby et al., 1996).

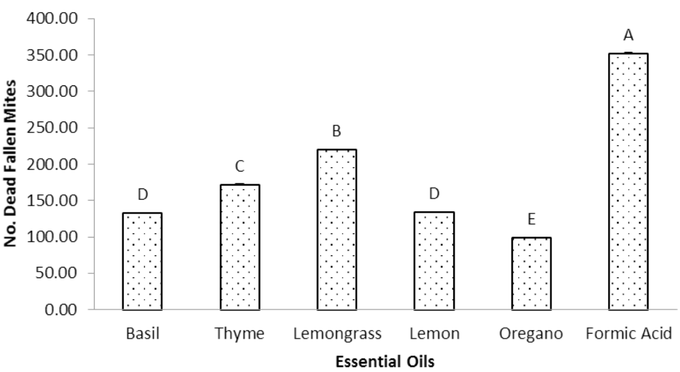

Figure 4: *Mean number of dead/fallen T. clareae mites in honey bee colonies by using 25% concentration.*

Percent mite mortality data (Figure 7, 8 and 9) showed that among seven treatments, formic acid and lemongrass were significantly better in reducing the mite's population. Formic acid treatment gave maximum percent mite mortality (93.33%) at 1st application which was reduced to 90.15%, 86.47% 3rd application and 67.37% after 2nd, 3rd and 4th application represented with overall mean mortality of 84.33%. Similarly, lemon grass oil treatment recorded 91.42% mortality after 1st application which was dropped to 83.88% after 2nd application, 76.69% after 3rd application and further to 61.17% after 4th application withan overall mean mortality of 78.29%. On the basis of present study, it was apparent that the equally successful in producing desired and intended control of *Tropilaelaps* mites' infestation especially when applied at 100% concentration (100%).

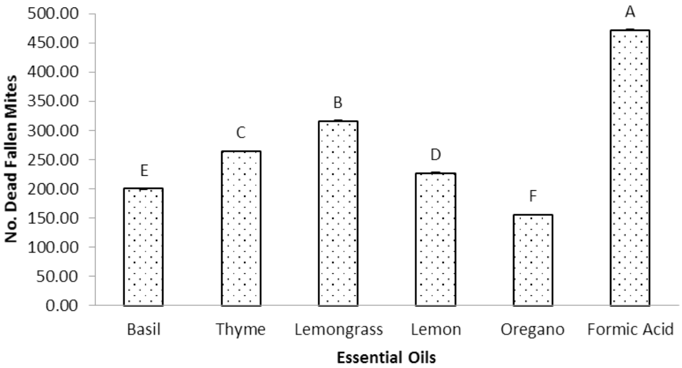

Figure 5: *Mean number of dead/fallen T. clareae mites in honey bee colonies by using 50% concentration.*

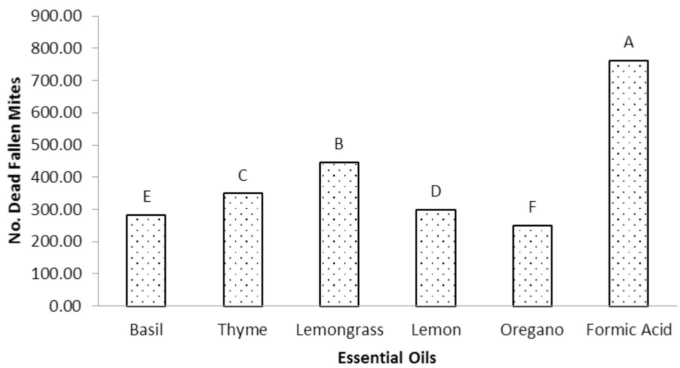

Figure 6: *Mean number of dead/fallen T. clareae mites in honey bee colonies by using 100% concentration.*

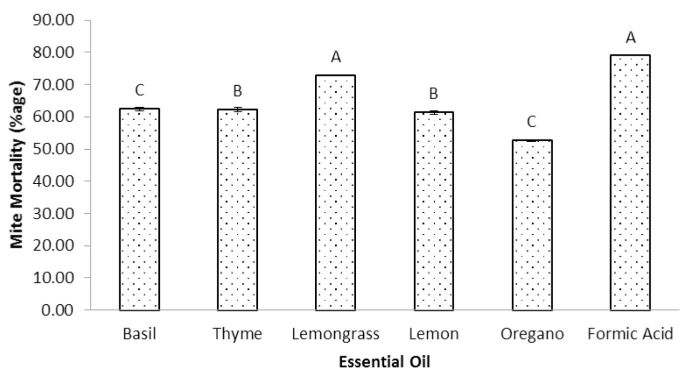

Figure 7: *Mean mite mortality percentage of T. clareae in honey bee colonies by 25% concentration.*

Honey Yield

Regarding the results of honey yield, the honey bee colonies treated with essential oils and Formic acid yielded higher honey yield in comparison with control colonies during experimental period. In control (untreated) colonies 3.8 kg honey per hive was extracted due to the increased mite infestation on bees causing low honey yield. Formic acid gave higher honey yield with a mean value of 9.3 kg per hive followed by lemon grass 8.8 kg, thyme 8.5 kg, lemon 8.5 kg, basil 7.7 kg and oregano 7.6 kg honey per hive. Rashid et al. (2011), Abd El-Wahab et al. (2012) and Islam et al. (2016) also recorded that formic acid treated colonies produced more honey as compared to the essential oils treated and control colonies.

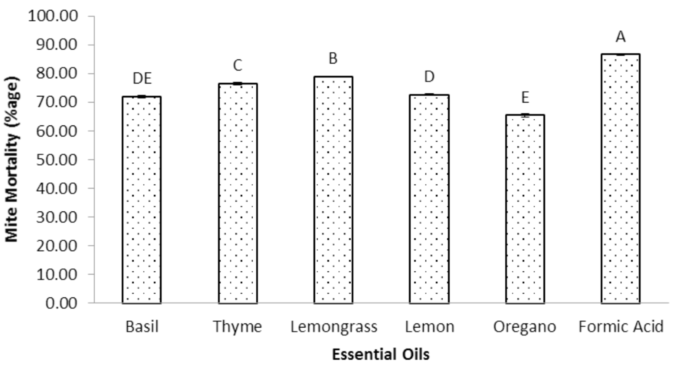

Figure 8: *Mean mite mortality percentage of T. clareae in honey bee colonies by 50% concentration.*

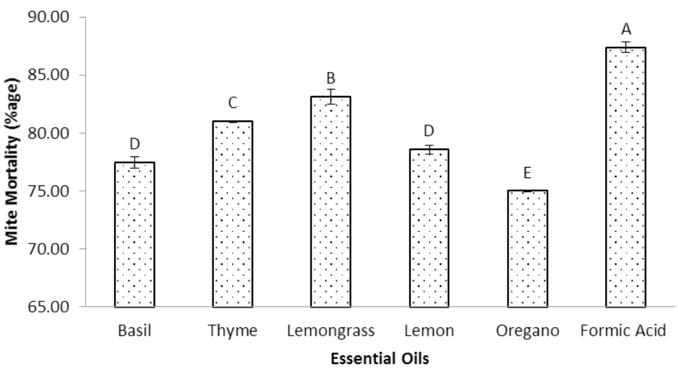

Figure 9: *Mean mite mortality percentage of T. clareae in honey bee colonies by 100% concentration.*

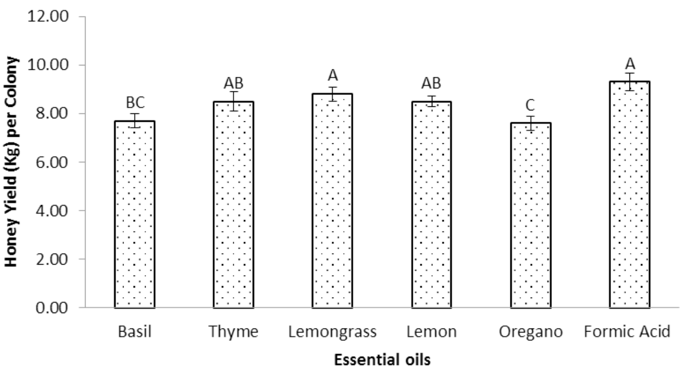

Figure 10: *Comparative mean honey yield (Kg) per colony by using different essential oils.*

Authors Contribution

ES designed the study. NI collected and analysed data while FN helped him. NI conducted reseach and wrote the article. FN contributed in graph prepration. MA supervised the work, evaluated and editted the manuscript. EH did review and critical revision of the article.ES contributed in finalization of "Results and Discussion".

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