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Effectiveness of the essential oils of local plant materials for controlling *Varroa destructor* in honeybee colonies under field conditions

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The ectoparasitic mites, *Varroa destructor* is the single factor responsible for the declining of honeybee, *Apis mellifera* health worldwide and causing greater economic losses to apiculture industry in Pakistan. For preventing the population of this mite a better understanding of the association of this parasite and its host is needed for the development of sustainable management practices. The efficiency of five essential oils of Thymol, Eucalyptus, Cinnamon, Garlic and Olive oil at 30%, plant material of Garlic powder at 10 gm per colony and formic acid 65% at 10 ml per colony easily available in the local market was tested against *Varroa destructor* from March 2022 to June 2023 under field conditions. Results indicated that essential oils, plant material and formic acid showed fumigant acaricidal activity against varroa mite in varying degree. The highest (90.75 and 94.0%) mean percent reduction in infestation of *V. destructor* per colony was recorded with formic acid and (85.50% and 88.20%) with thymol, followed by cinnamon (80.70 and 85.27%) after three treatments in worker brood and adult bees, respectively. The highest total number of fallen dead varroa mites after three treatments application was recorded by formic acid (498) followed by thymol (293) and cinnamon (187) mites per colony. Highest (91.54%) mortality of varroa was recorded with formic acid and thymol (86.43%) followed by cinnamon (80.26%). Maximum area of sealed worker brood (1590.4 cm^2 per colony) was observed with formic acid (65%) followed by thymol oil (1362.0 cm^2 per colony) in selected experimental bee colonies. Highest Acacia and Wild Karanda honey yield (9.0 kg) was observed with formic acid followed by (7.8 kg) with thymol and cinnamon (6.7 kg) per colony as compared to (1.5 kg) in untreated colonies.

Keywords: *Apismellifera*, *Varroa destructor*, essential oils, plant material, formic acid

INTRODUCTION

Honeybees including *Apis mellifera* are beneficial insects both for hive products production like pollen, bees wax, honey, royal jelly, bee venom, pollen, and propolis (Tiwari et al. 2014; Qadir et al. 2021; Rehman et al. 2021) and biodiversity conservation (Sajid, et al. 2020). In addition, they also contribute to the pollination of field crops, vegetables, fruits, and wild plants throughout the world (Hung et al. 2018; Stojanov et al.2021; Khan and Ghramh, 2021). Due to many threats like honeybee diseases, mites, *Varroa destructor,* and *Tropilaelapsclareae*, bad weather (Becsi et al. 2021), pesticide exposure (Zhu et al. 2014), and a combination of nutritional stress (Branchiccela et al. 2019), honeybee health is at risk worldwide since the last few decades (Goulson et al. 2015; Steinhauer et al. 2018; Topal et al. 2019; Al Naggar et al. 2020; Hristov et al. 2020) as well as in Pakistan (Aziz et al. 2015; Bakar et al. 2019; Khan and Ghramh, 2021; Qadir et al. 2021; Rehman et al. 2021; Islam et al. 2020). Among them for declining honeybees globally the damage of *V. destructor* is the single factor that results in substantial colony losses (Alattal et al. 2017; Traynor et al. 2020). Now the pest can be found everywhere honey bee *Apismellifera* colonies are rearing for hive production and pollination and have a global dispersal (Boncristiani et al. 2021). So, the services they offer to humanity can lead to severe economic losses (Genersch, 2010; Gray et al. 2020).

It was reported previously that ectoparasitic mites feed on larvae, pupae, and hemolymph of adult worker bees inside the cells of capped brood and weaken its host (Rosenkranz et al. 2010). But (Ramsey et al. 2019) reported that varroa mainly feeds on the honeybee's fat

body not hemolymph affecting the properties of the cuticle and the immature system of response is blocked (Yang and Cox-Foster, 2005). The feeding of mites also facilitates viruses and bacteria transmission in bee hives (Ramsey et al.2019) which leads to the parasitic mite syndrome development (Tantillo et al. 2015). Varroa transmits a large number of virus diseases in *A. mellifera* colonies (Heber et al. 2019) such as deformed wing virus (Roberts et al. 2020), Israeli acute paralysis virus and Kashmir bee virus (Genersch and Aubert, 2010; Di Prisco et al. 2011; Martin et al. 2012; Traynor et al. 2016) and severely reducing the population of bee colonies (Budge et al. 2015). If *A. mellifera*colonies were not treated at the economic threshold level for the control of *V. destructor*, both the chances of viral infection and colony mortality increased (Kulhanek et al. 2021). These viruses are transmitted indirectly in large doses in the brood and adult bee's hemolymph (Yang and Cox-Foster, 2005). As a result, the individual infected bee weakens, the damaged bodies and wings become visible and they have a shorter life span (Genersch, 2010; Meixner, 2010). Therefore, in temperate areas periodic treatment of honeybee colonies several times a year for *V. destructor* is required in order to keep the population of mites below the economic threshold level (Rosenkranz et al.2010).

The management of varroa depends on the regular application of synthetic miticide treatments in commercial beekeeping like pyrethroids, organophosphates, and formamidine for a long time (Meikle et al. 2012). However, these acaricides are economically convenient, easy to apply for beekeepers, and effective for controlling mites (Gracia et al. 2017) but currently, except for their reducing effectiveness, the varroa mites population developed resistance globally to synthetic acaricides (Price and Lunnis, 2014; Tihelka, 2018; Rinkevich, 2020). Besides, these hard chemicals are slow to break down and remain in the environment for a long time (Rosenkraz et al. 2010). Long-term and overuse of synthetic acaricides has led to residues accumulation which contaminate honey and bees wax with the possibility of risk for the end consumer (Rinkevich, 2020; Agrebi et al. 2020). Until now, it is the only means to control efficiently ectoparasitic mites, *V. destructor* and keep mite infestation below the economic threshold level. Therefore, safe and cheap materials both for humans and honeybees to control *V.destructor* are urgently needed. Several research works have been carried out to test and focus on natural substances like plant extracts and essential oils which offer a very desirable substitute of synthetic acaricides for controlling *V. destructor* in the colonies of honeybees worldwide to minimize the environmental and health problems related with the use of hard miticides in bee hives (Ariana et al. 2002; Meikle et al. 2012).

In reaction, great interest was shown by beekeepers to use soft chemicals against mites in their bee hives instead of hard chemicals to delay resistance problem. Organic acids and essential oils as natural chemicals and their derivatives are therefore increasingly used currently in beekeeping industry (Bahreini, 2003; Emsen et al. 2010). However, several research experimental findings stated that using organic acids to control *V. destructor* is harmful to bees (Hatjina and Haristos, 2005; Gregorc and Škerl, 2007). Besides, regular loss to excretory, digestive and bees glands (Silva-Zacarin et al. 2006; Hernández et al. 2007) queen damage or frequently premature deaths of queens (Underwood and Currie, 2003) or decrease in pH of the honey in the coming honey flow season are some of the detrimental effects of using organic acid against mites in bee hives (Borsuk et al. 2012).

An additional potential method with beekeepers is to decrease *V. destructor* population in honeybee colonies by using essential oils (Conti et al. 2020). Essential oils are lipophilic and may include phenylpropanoids and terpenoids, sesquiterpenes, monoterpenes, alcohols, and aldehydes, (Carson and Hammer, 2011). Essential oils have acaricidal activity and used as an alternatives to control varroa population in *A. mellifera* colonies instead of synthetic acaricides with no toxic effects both to bees and honey quality. Essential oils efficacy for controlling varroa is similar comparing to organic acids, but use of organic acids caused more stress in honeybees as compared to essential oils (Isman, 2000).

The use of essential oils in bee hives also frequently causes antimicrobial effects besides the acaricidal effects which help overall in the improvement of honeybee health (Eguaras et al. 2005). Most research studies proposed that the use of essential oils could be a practical alternate for maintaining low population of mites in bee colonies (Conti et al. 2020; Gashout and Guzmán-Novoa, 2009; Ramzi et al.2017). In addition, essential oils residues in honey do not pose a hygiene risk to human health (Adamczyk et al. 2005).

In spite of a variety of essential oils tested in vitro for the management of *V. destructor* having the promising acaricidal effects (Lindberg et al. 2000; Gashout and Guzmán-Novoa, 2009), but few of them has been tried for their efficacy in field conditions (Gashout and Guzmán-Novoa, 2009; Ramzi et al.2017). For this reason, in many commercial formulations with the exception of some cases, the essential oils have not yet been included (Eguaras et al. 2005).

Different types of essential oils were used to manage *V. destructor* in bee colonies showing different efficiency but under field conditions very few of them were tried for their efficacy. For this purpose thyme, eucalyptus, cinnamon, garlic and olive essential oils at 30% concentration and garlic powder compared to formic acid were tested for their effectiveness against *V. destructor* in honeybees under field conditions.

MATERIALS AND METHODS

Location of the study:

This research work was conducted on the experimental apiary of Honeybee Research Institute, National Agricultural Research Centre, Islamabad from March 2022 to June 2023.

Honeybee *Apis mellifera* colonies:

Forty eight honeybee (*Apismellifera*) colonies infested with *V. destructor* kept in single story with a movable bottom board Langstroth hives were used in this research study. Before carrying out this experimental field trial, these honeybee colonies were not treated for *V.destructor* control for the last six months. In addition, capped worker brood frames infested with varroa mites were also provided in these experimental bee colonies for mite's multiplication during January-February 2022-2023.Two colonies were used for each treatment and two for control (untreated).Treatments were replicated three times with a Randomized Complete Design. All selected *A. mellifera* colonies of each group were equalized two weeks after initiating this study each colony had 10 frames worker bees, 4-5 capped and uncapped worker brood frames, half to one frame pollen and honey per colony and egg laying fertile queen. All experimental honeybee colonies were provided sugar as supplemental feeding at 1:1 (w/v) and pollen as substitute diets at 1:1 (1 part bee collected natural pollen:sugar solution (50%) at weekly interval when natural nectar and pollen was not available to the honeybees during dearth period for honeybee survival and brood rearing and to supplement the nutrition of the selected experimental bee colonies. When honeybee population increased in the colony, then honey chambers were provided to that colony. Honeybee colonies were managed according to the standard beekeeping techniques such as the management of robbing, adding and removing of extra bee boxes, and protection of honeybee colonies from the attack of hornets. Besides honeybee colonies were regularly monitored for the toxic effect of treatment, the performance of the queen, and food availability during the offseason. Honeybee *A. mellifera* colonies were assigned randomly to eight treatment groups.

Essential oils, plant material, and formic acid application method:

Five essential oils of Thyme, Eucalyptus, Cinnamon, Garlic, and Olive were prepared with 30% concentrations by mixing with olive oil. To each mixture, talcum powder was added @1: 2 (Oil: Powders) for making a solid mixture of (3 x 3 x 1 cm) 30 gm weighted. On the top of worker brood frames each mixture was placed. Ten ml of each essential oil was used on two strips of cotton (1 × 2 cm) and placed in two sites on brood frames in each experimental bee hive. Ten-gram garlic powder was dusted on brood frames in each tested bee hive. Formic acid (65%) was applied @ 10 ml per colony on a card board (7.5" × 5.5") kept in a screened bottom board under a brood frame in each colony. Each essential oil, plant material and formic acid was treated three times at 12 days interval.

Assessment of mite's infestation in colonies of honeybees

Determination of varroa infestation on bees

Infestation of ectoparasitic mites on worker bees was recorded after and before treatment in each tested colony using a 70% alcohol wash method. Randomly 100 living worker bees from sealed worker brood frames of each bee colony were collected using a bee brush in each jar containing 70% alcohol and kept overnight for 24 hours. The bees were then shaken with hand for 1 minute and washed with tape water and then bees and alcohol were passed through a 3 mm sieve. The alcohol wash and powdered sugar shake methods are currently extremely recommended to beekeepers because of their high precision ranks (Honeybee Health Coalition, 2018).

Determination of *Varroa destructor* infestation on worker brood

Varroa destructor infestation rate in *A. mellifera* colonies was determined by selecting and removing randomly 50 sealed worker brood cells from brood frames in each colony before and after treatment application. They were inspected individually for the prevalence of *V. destructor* adult female mites on each larva or pupa and were recorded (Branco et al. 2006; Lee et al. 2010).

The percentage infestation in adult bees and in worker brood was determined two times at 3 day intervals before treatment applications and 24 hours, 48 hours, and 72 hours after treatment application. The percent reduction of *V. destructor* infestation after treatment applications on adult worker bees and brood was recorded according to the below equation used by (Floris et al. 2001; Satta et al. 2005):

Percent Reduction in infestation of Mites = 100 (1 ¬ (Bc × At)/ (Bt × Ac)

Where Bt and At are the respective infestation percentages mites before in the treated colonies (Bt) and after treatment (At) and (Bc) and (Ac) the respective parameters measured in the control colonies.

Estimation of fallen mites on screened bottom board

Fallen *V. destructor* mites were collected and counted each three days after three treatments application in each treated and untreated bee hive before and after treatments during the field trial. Each colony was equipped with a screened bottom board having a Formica sheet (Kretzschmar et al. 2015). Fallen naturally varroa mites data was recorded before 24 hours treatment whereas fallen dead mites were

observed after the 1st, 2nd and 3rd day of treatment.

Essential oils efficacy

The percentage effectiveness of each essential oil, plant material and formic acid were calculated with the following formula (Goswami et al.2014; Islam et al. 2020):

$$\text{Efficacy (\%)} = \frac{\text{Dead mites in treatment}}{\text{Dead mites in treatment + control}} \times 100$$

Brood measurement and honey production

The worker brood area was measured after three treatments with a plastic sheet which divided into square inches. Then the data from square inches was converted into cm^2 multiplied with 6.45 according to Ismail et al. (2006).

Honey Production

During spring season multi-flora (Acacia and Wild Karanda) honey was harvested from treated and untreated colonies against varroa. The honey yield data in kilogram per colony was recorded in order to compare treated and untreated colonies yield of honey for determining the treatment's impact.

Statistical analysis of data

All treatments and control data were analyzed statistically by analysis of variance (ANOVA) with Statistix 8.1 computer program by Randomized Complete Design (Analytical Software Statistix 8.1, 2003). For the significance of treatments Analysis of Variance (ANOVA) was used. Through the Least Significance Difference Test (LSD), the means were compared at $P \leq 0.05$.

RESULTS

The efficacy of different essential oils (Eucalyptus, cinnamon, thymol, garlic and olive) plant material (Garlic powder) and formic acid (65%) was evaluated for the mean percent reduction in infestation of *V. destructor* on worker bees and brood in *A. mellifera* is illustrated in Figure 1.Data recorded showed that before treatment per cent infestation on bees and brood ranged from 18.5 to 23.2% and 20.0 to 28.2%, respectively in experimental honeybee colonies. The infestation of *V. destructor* reduced slowly to the lower infestation percentage recorded after 3rd treatment application of essential oils, plant material and formic acid. Statistical analysis showed that the infestation percentage of *V. destructor* was significantly reduced after second and third treatment application on worker bees and brood between colonies treated with essential oils, plant material and control ones. Result showed that formic acid 65% and 30% essential oils caused a highly efficiency against *V. destructor* population per colony after three treatments. The highest (90.75 and 94.0%) mean percent reduction in infestation of *V. destructor* per colony was recorded in worker brood and adult bees with formic acid and(85.50% and 88.20%) with thymol after three treatments, respectively.

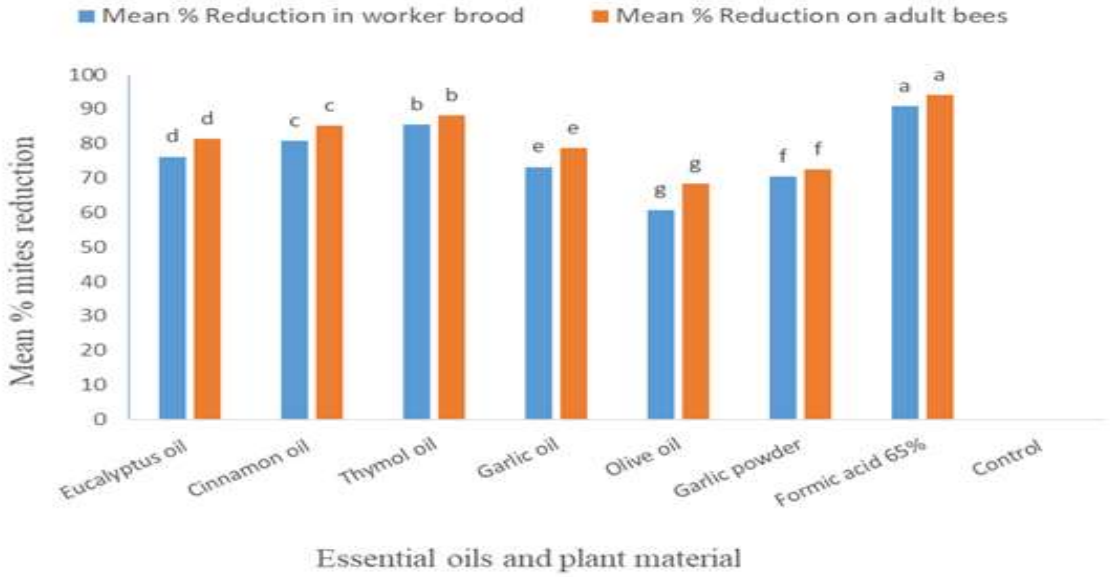

Figure1: Mean reduction in infestation percentage of *Varroa destructor* in worker brood and on adult bees per colony after three treatments with essential oils and plant material
(One way ANOVA using LSD test at p value ≤ 0.05)

Second highest (80.70%) mean percent reduction in infestation of mites in brood of worker and (85.27%) on bees was observed with cinnamon oil followed by eucalyptus oil (76.19%) and (81.35%) and garlic oil (73.25%) and (78.64%) in brood of worker and bees, respectively. The lower (60.80%) mean percent reduction in infestation of varroain brood and (68.45%) on bees was found with olive oil, respectively. Whereas, the infestation percentage of mites on bees and brood in the untreated (control) colonies was increased from 20.5 to 23.9% and 25.65 to 28.70% after first to third treatment applications, respectively. Results showed during three application of treatment, essential oils, plant material and formic acid were more efficient significantly for reducing varroa population as compared to the control (untreated) honey bee colonies.

Mites fallen

The efficacy and perseverance of experimental oils, plant material and formic acid (65%) against *V. destructor* were assessed against *V. destructor* and number of total fallen mites per hive and per cent mite mortality is shown in figure 2. Data revealed that before treatment, the natural fallen mites ranged from 7 to 13 mites per colony. Results revealed that the maximum number of total dead varroa fallen on the Formica sheet was observed after 1st treatment which distinctly reduced during 2nd and 3rd treatments. Among the essential oils, plant material and formic acid tested, the most promising essential oil was thymol and formic acid (65%). The mites during first three days were different statistically in each colony at $p \leq 0.01$ throughout the three treatments. Total highest number of dead fallen varroa after three treatments application was recorded by thymol (293) and cinnamon (187) followed by eucalyptus (148) garlic (112), garlic powder (97) and olive (73) which showed the lowest toxicity against varroa and the control (46). The highest mean total dead fallen mites (498 mites/per hive) was recorded with formic acid after three treatments compared to the treated experimental plant material and essential oils (Figure 2).

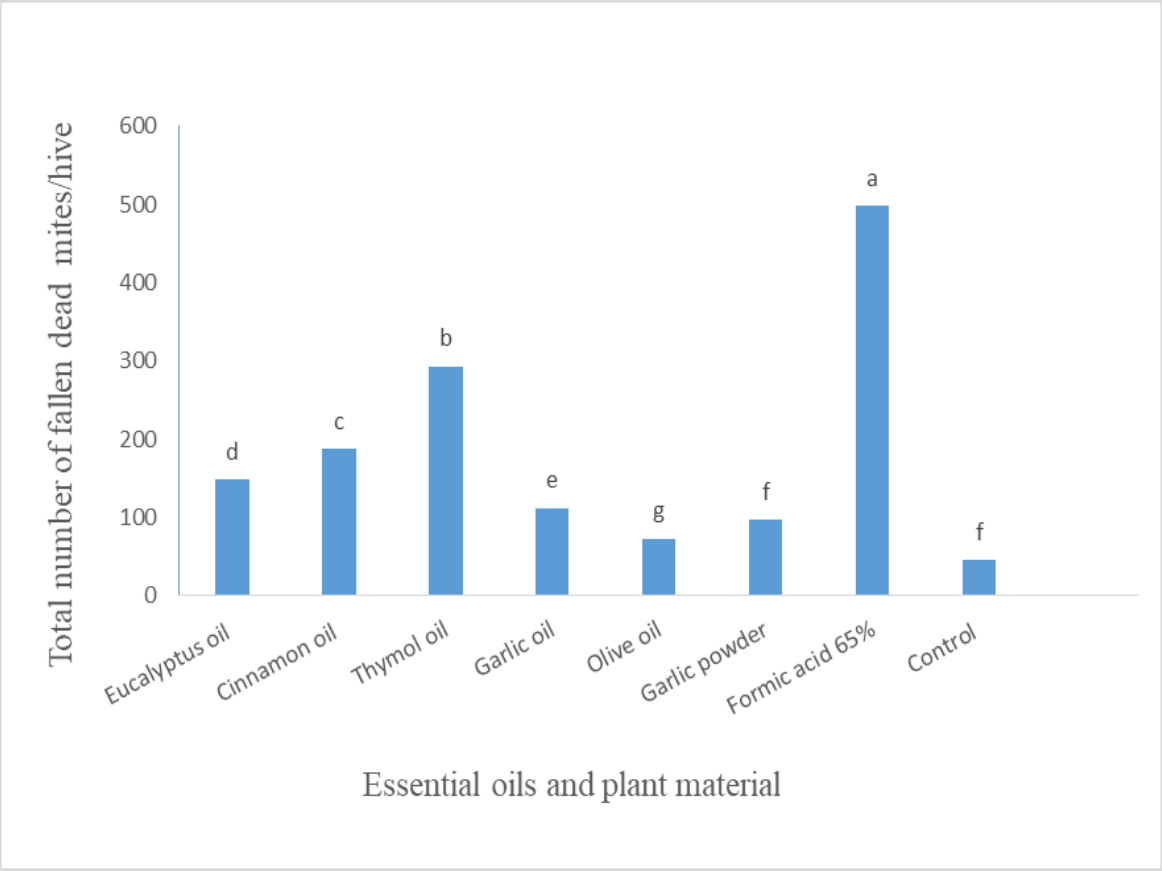

Figure 2: Total number of fallen dead *Varroa destructor* mites/hive after three treatments with essential oils and plant material

(One way ANOVA using LSD test at p value p value ≤ 0.01**).**

Percent mortality

Data analysis regarding the percent mortality of *V. destructor* with essential oil, plant material and formic acid revealed that thymol and formic acid were better significantly in controlling mite population in honeybee colonies. Different essential oils and plant material effectiveness against varroa mites recorded were in the range of 61.34 to 86.43%. Thymol oil performed best (86.43%) which was followed by cinnamon oil (80.26%), eucalyptus oil (76.29%), garlic oil (70.89%), garlic powder (67.83%) and olive oil (61.34%). Data analysis indicated that formic acid was very efficient as compared to the oils and plant material showing 91.54% mortality of *V. destructor* mites in honeybee colonies (Figure 3).

Worker brood area (cm^2) per colony

Results showed that significant increase in worker brood area (cm^2) in honeybee colonies treated with different essentials oils and plant material was recorded predominantly after third treatment application. Maximum area of sealed worker brood (1590.4 cm^2 per colony) was recorded in those colonies which were treated with formic acid (65%) which was followed by thymol oil (1362.0 cm^2 per colony), cinnamon oil (1257.6 cm^2 per colony), eucalyptus oil (1132.2 cm^2 per colony), garlic oil (1022.5 cm^2 per colony), garlic powder (904.5 cm^2 per colony) and olive oil (794.8 cm^2 per colony) as compared to untreated colonies (624.7 cm^2 per colony). An increase in the area of worker brood was very clear in those colonies which were treated with essential oils and plant material for the management of *V. destructor* (Figure 4).

Production of Honey

Results indicated that the mean production of Multi-flora (Acacia and Wild Karanda) honey during spring 2022 and 2023 in kg per colony differed significantly after treatment with different essential oils and plant material against varroa mites. The honey in each colony was harvested and weighted. Data indicated that healthy and strong colonies gave more yield of honey than weak colonies. Maximum yield of honey (9.0 kg/colony) was extracted from formic acid (65%) treated colonies followed by thymol oil (7.8 kg/colony), cinnamon oil (6.7 kg/colony as compared with untreated colonies (1.5 kg/colony). Whereas the lowest (2.6 kg/colony) honey yield was extracted from colonies treated with olive oil (Figure 5).

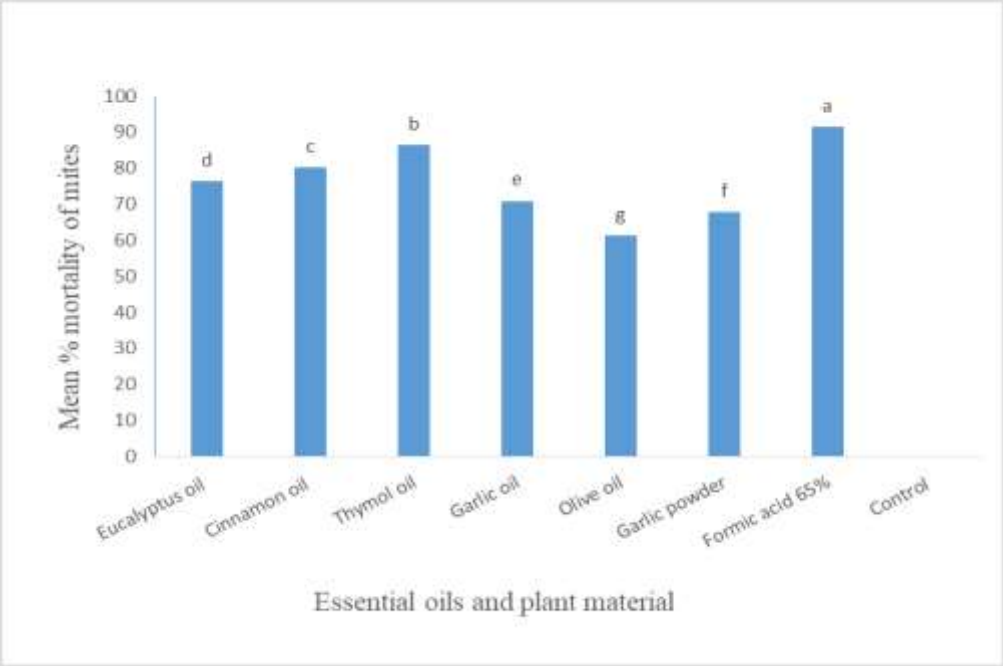

Figure 3: Mean percent mortality of *Varroa destructor* after three treatments with essential oils and plant material

(One way ANOVA using LSD test at p value p value ≤ 0.05).

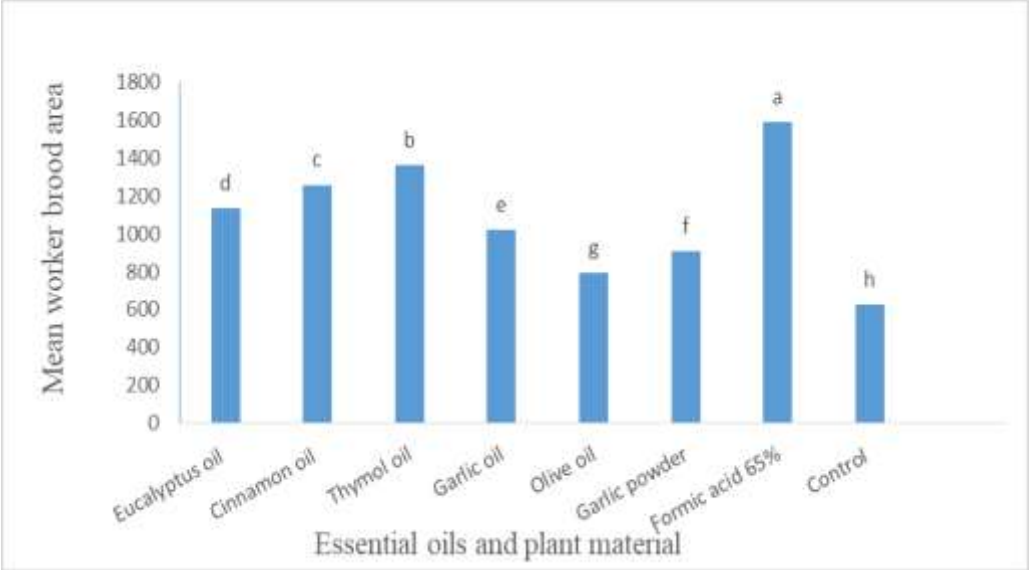

Figure 4: Mean worker brood area cm^2 per colony after treatment with essential oils, plant material and formic acid against *Varroa destructor* in honey bee, *Apismellifera* colonies
(One way ANOVA using LSD test at p value ≤ 0.05)

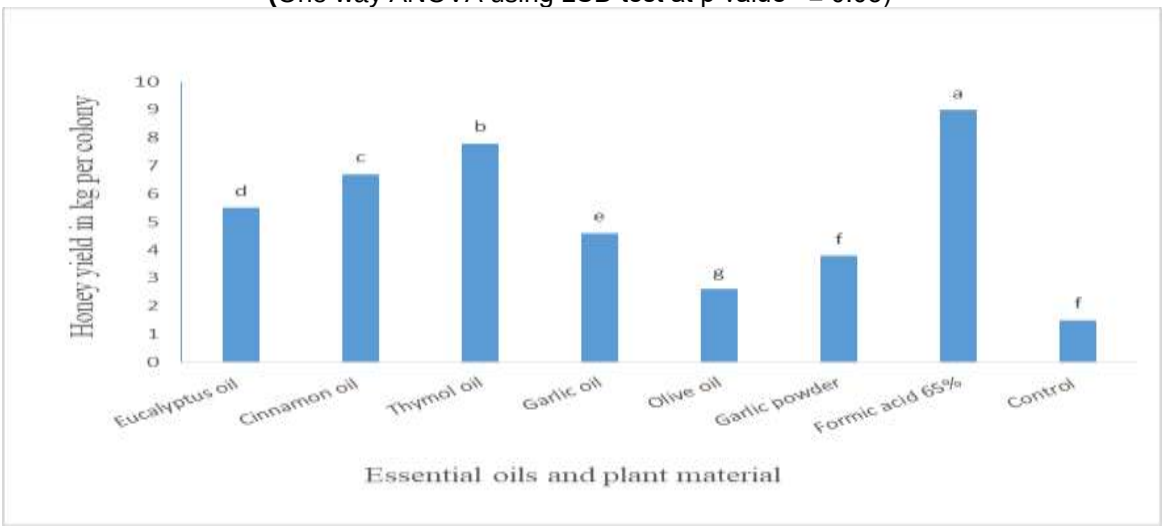

Figure 5: The effect of essential oils, plant material and formic acid on honeyyield in kg/ colony against *Varroa destructor* mites
(One way ANOVA using LSD test at p value ≤ 0.05)

DISCUSSION

Ectoparasitic mite, *Varroa destructor* is an economically significant pest in honeybee *Apismellifera* colonies. Nowadays, different essential oils and plant materials are used as soft acaricides for managing *V. destructor* in *A. mellifera* colonies in many countries of the world. Researchers have conducted several studies on essential oils effectiveness against varroa mites and they reported encouraging positive results both in laboratory and field conditions. When these oils used at proper dosages they do not have any toxic effect on the honeybees neither any adverse effect on the colony development. Moreover, essential oils prevent residue problem in bee hive products such as honey, beeswax and others as well as supports organic hive production in beekeeping. When *A. mellifera* colonies are infested with *V. destructor* become more susceptible to secondary infection like viruses and bacteria. Different acaricides such as coumaphos, flumethrin, strips, fluvalinate strips and injection, sulphur and amitraz, have been used as hard chemicals to reduce the population of varroa mite in *A. mellifera* colonies in the country by beekeepers. Long-term continuous and misuse of these synthetic acaricides has led to resistance development in varroa against these chemicals, residues accumulation in honey and bees wax which increased the chances of

risk for human health (Rinkevich, 2020; Agrebi et al. 2020). These problems need the implementation of effective, safe and ecofriendly control against varroa mites in honeybees. Tutun et al. (20l8) reported that many essential oils are alternative to synthetic miticides for controlling *V. destructor* in order to avoid resistance in varroa and residue problems in hive products. In addition, many authors in their research studies used different controlling agents in combinations in an integrated pest management program successfully against *V. destructor* mites. By avoiding unnecessary use of synthetic acaricides and suitable treatment against *V. destructor* as a result can reduce economic losses and contamination of residues in bee hive products (Ayan et. al. 2019). This study explored the efficacy of essential oils and plant material against *V. destructor* in field conditions. Our results indicated that effectiveness of essential oils and plant material were varied according to the type of essential oil and plant material. Generally, essential oils are broad spectrum having several active ingredients that may work through different modes of action (Chiasson et al. 2004). Results of the present study revealed that Formic acid at 65% and essential oils at 30 concentrations were highly effective against varroa mites. Among the tested essential oils and plant material thymol was found best for decreasing varroa population both in brood and bees. Our findings are in accordance with (Floris et al. 2004) who found that thymol at 30% concentrations significantly reduced the infestations level of mites in brood and on bees in field conditions, followed by cinnamon and eucalyptus oil. Similar results were reported by (Allam et al. 2003), they reported that 91.7% varroa mortality was observed with formic acid in honeybee colonies. (Hamaad et al. 2008) reported 65.9% mortality of varroa after Thyme oil spray and reported that it has the potential to control mites under Egyptian climatic conditions. Melathopoulos and Gates (2003) found that formic acid and thymol have great efficiency against varroa mites in honeybee colonies in field conditions and have no harmful effect on the honey bees.

The results of the application timings showed that higher dead fallen mites were observed after the 1st application than the 2nd and 3rd applications both in treatment of winter and summer. These results agreed with the findings of (Islam et al. 2022) they reported that higher total fallen mites were recorded after first treatment than second and third treatment. Formic acid was efficient against *V. destructor* as compared to the oils used by showing (91.62%) in January to February and (87.50%) in August to September. These results are also in accordance with (Rashid et al. 2011) reported that formic acid was statistically effective in falling higher number of mites as compared to thymol (Calderon et al. 2014) also reported that formic acid 86% was 96.9% effective in controlling varroa mite's whereas thymol was 94.7% effective against varroa in honeybee colonies.

Our achieved results concerning honey yield in kg per colony are in line with (Rashid et al. 2011), (Abd El-Wahab et al. 2012) and (Rehman et al. 2021) they also reported that higher honey yield was extracted from colonies treated with formic acid in comparison to essential oils and untreated colonies.

Results regarding the essential oils and plant material effect on rearing of brood are not in line with the findings of (El-Hady et al. 2015), they found that brood rearing area ($inch^2$) increased after using Cinnamon and Anise oils treatments than thymol at 30% concentration compared to formic acid and the control colonies while we recorded significantly different maximum worker brood area in cm^2 in colonies treated with formic acid 65% followed by thymol, cinnamon and eucalyptus oils at 30% concentrations after three treatments application, respectively.

The most common essential oils used in beekeeping for the control of varroa and tropilaelaps both in laboratory as well as in field conditions are Rosemary, Cinnamon, Wintergreen, Citronella, Peppermint, Spearmint, Tea tree, Thyme and Lemongrass oil (Tutun et al. 20l8, Hassan et al. 2008, Damiani et al. 2009).The most used components are menthol, eucalyptol, camphor, thymol, citral, and citronellal and (Tutun et al. 20l8, Gashout and Guzmán-Novoa, 2009).

CONCLUSIONS

We tested five essential oils of thymol, cinnamon, eucalyptus, garlic and olive oils and plant material of garlic powder locally available and were found that thymol and Cinnamon more effective for decreasing the infestation rate of *V. destructor* in honeybee colonies after three treatment applications while Olive oil indicated the lowest effectiveness against the mites. This finding encourages the researchers to investigate honestly to find suitable concentrations of the screened essential oils and plant material for the varroa management in the colonies of honey bees. We recommend that the tested essential oils and plant material might have a promising potential to use as an alternative chemicals against *V.destructor* and that may be used as useful tool in sustaining lower mite infestation in bee hives. These oils and plant materials might be used singly like synthetic acaricides as a practical treatment for the control of varroa by beekeepers. These chemicals might be delivered as an important component in integrated pest management program for preventing varroa infestation in honey bee colonies. Moreover, in our study, no negative effect of these essential oils and plant material was observed on adult bees, brood and queen survival and performance during the experimental period. Therefore, to investigate the components and suitable concentration of these essential oils and plant material for better evaluation and confirmation of toxicity to honey bee worker brood

(larvae and pupae) more laboratory and field studies are needed on commercial beekeepers apiaries.

Supplementary materials

The supplementary material / supporting for this article can be found online and downloaded at: https://www.isisn.org/article/10.3390/antiox12081524/s1,

Author contributions

Noor Islam conceived this research study and designed experiment. Rashid Mahmood supervised the overall research work and provided honeybee *Apismelliferacolonies* infested with *V. destructor* mites for conducting the field trial. Noor Islam collected data and performed analysis while GhulamSarwar and Zafar Islam help in this work. Noor Islam wrote the paper. Syed AhqabullahKakakhel comments on earlier drafts and edited the manuscript. Before publication all authors read the final manuscript and approved.

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Institutional Review Board Statement

The study was approved by the Bioethical Committee of the …….

Informed Consent Statement

Not applicable.

Data Availability Statement

All of the data is included in the article/Supplementary Material.

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Conflict of interest

All the authors declared that this research study was accomplished without any conflict of interest.

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