

Integrated management of *Varroa* mite, *Apocephalus* fly, and *Aethina tumida* using essential oils in *Apis mellifera* colony

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Abstract

Honey bees play a crucial for sustaining ecosystem stability and biodiversity. Honey bees play a vital role in agriculture field by pollinating about 75% of flowering crops, which provide about one third of the food consumed by the world's human population. Honey bees are also a bioindicator of environmental pollution. The product of *Apis mellifera* provides us with incredible health benefits. Honey has antimicrobial, antiallergic, anti-inflammatory and immune-boosting properties. Bee pollen is a very high-protein-rich diet that is easily digestible. About 300 compounds present in raw propolis have anticancer and antiviral properties. Royal jelly has anti-ageing properties. Beeswax is used in various commercial industries for making paints, cosmetics, and candles, etc. So, this beneficial insect provides us with many benefits, but itself endures the stress of different biotic and abiotic factors throughout the year, causing colony collapse disorder. The main abiotic factors are temperature, humidity, rain, sunlight, nutrition, and pesticides. The biotic factors that affect the *A. mellifera* colony are pests and predators that are present in the colony. The most dangerous one is the *Varroa* mite, which causes the spread of viral illness, including deformed wing virus and it reduces the strength of the colony and leads to colony collapse. The beetle *Aethina tumida* is an ectoparasite mite that damages honeycombs by feeding on honey, pollen, and bee brood. The beetle causes the foul smell in the hive due to its activity. *Apocephalus borealis* is a parasitic phorid fly of honey bees that causes abnormal behaviour, weakening of colonies, and mortality of infected bees. To minimise the damage caused by these pests, three different types of essential oils are used (Eucalyptus oil, neem oil, and mint oil) against the *Varroa* mite, *Apocephalus borealis* and *Aethina tumida*. The sticky board method was used to capture the pest. Results show that neem oil and eucalyptus oil is very effective against *Varroa* mite, while mint showed the highest effectiveness against *Aethina tumida*. Treatments give significant effects against *Varroa* and *A. tumida*, ($p < 0.05$) but with *Apocephalus borealis*, all treatments with essential oil showed non-significant effects ($p > 0.05$).

Keywords: *A. mellifera*, sticky board method, essential oil, pest, *Varroa*, *Aethina*, *Apocephalus*

Introduction

The honey bees play a key role in an ecosystem. Its products offer many health advantages. Honey, venom, propolis, beeswax, bee bread, and royal jelly are among the products made by honey bees that offer defence against pests and diseases. (Easton-Calabria., *et al.*, 2019) [6]. It is known quantitatively about its function as a pollinator outside of controlled ecosystems. The significance of *A. mellifera* in natural environments using a global dataset comprising 80 reported plant pollinator interaction networks and pollinator effectiveness metrics from 34 plant species (Hung, K. L., *et al.*, 2018) [11].

The complexities of the honey bee's social structure in a lively and captivating manner. the complex division of labour and the significance of a temporal caste structure to the colony's efficient functioning. He also describes the highly specialised behaviour of workers, queens, and drones (Winston, M. L., 1991) [24]. Crop quality and quantity are greatly enhanced by bee pollination, which also improves global economic and dietary results. (S. A. Khalifa *et al.*, 2021) [13]. Each beehive contains thousands of foraging bees, which are periodically renewed and can be thought of as thousands of detectors. Since a forager bee's average flight radius is roughly 1.5 km, a colony's foraging area is roughly 7 km² (Crane, 1984). In this context, the honey bee is highly sensitive to the pesticides commonly and heavily

used in both urban and agricultural areas (Porrini *et al.*, 2014) [20].

Natural enemies can infest or harm any living thing, and honey bees in the genus *Apis* are no exception. They have developed a high degree of sociality as a result of their lengthy history of evolution and natural selection, and thousands of individual bees coexist in a close-knit social organisation (Muhammad Sarwar, 2016) [22]. In the last several years, population of honey bees have been threatened by a various environmental factor, and pesticides. (Fouad, M.R. 2026) [7].

Apis mellifera populations are decreasing due to a number of biotic and abiotic reasons, including habitat loss, the use of pesticides, the emergence of pests and climatic change, this phenomenon is known as colony collapse disorder. It causes in destroying of honey bee populations. The *Varroa* mite, which is already widely dispersed is considered the primary threat to agriculture (Sotelo-Leyva., *et al.*, 2023). The variables linked to honey bee colony collapse disorder examined how many stress factors, including parasites, viruses, pesticides, and environmental changes, lead to colony losses (Van Engelsdorp and Meixner, 2010). The abnormal behaviour of parasitoid's causes the of Colony Collapse Disorder (CCD). The disease, deformed wing virus and *Nosema ceranae* in infected bees, suggesting that the fly may play a role in the spread of pathogens. (Casuso *et al.*, 2015) [3].

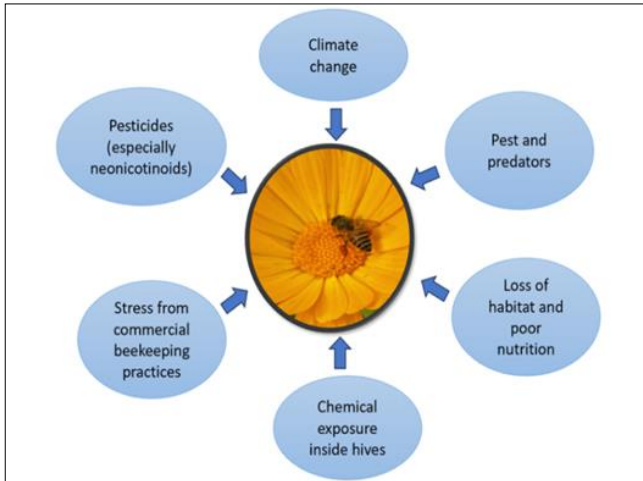


Fig 1: Factors causing Colony Collapse Disorder in *Apis mellifera* colony

Varroa destructor is considered as the most dangerous pest o, also known as *Varroa* mite, which has led to honey bee colony losses worldwide. *Varroa* is an ectoparasitic mite of honeybees of a significant size, it feeds on the hemolymph of bees in the development stages, larvae until hatching to adults (Martin 2001) [14]. The female is a dark reddish mite with a crab-like form that is 1.1 mm in length and 1.6 mm in width. The male is only home to honey brood chambers which is slightly smaller than the female (length: 0.8 mm, width: 0.7 mm) (ZhiQiang Zhang, 2000) [25].

The Varro mite infestation causes the weakening of bees, diminishes longevity, and affects colony productivity. It was emphasised that the significance of *Varroa* act as a vector of viral diseases, deformed wing virus (DWV). Various control methods, including chemical acaricides, essential oils, acaricides and integrated pest management (IPM) (Rosenkranz *et al.*, 2010) [21].

The small hive beetle (*Aethina tumida*), becomes an emerging pest of honey bees. According to the author, the beetle first originated in sub-Saharan Africa. Beetle's life cycle includes, pupation outside the hive in hive, larval feeds on honey and brood, and egg laying occurs in hive cracks (Hood, 2004) [10].

The beekeeping business was becomes seriously threatened by the parasite mite *Varroa destructor*, which causes significant losses in honey bee colonies. When it comes to

controlling *Varroa*, essential oils practice becomes benefit for industrial herbicides. (Mondet, F., *et al.*, 2011) [16].

The biology and effects of "zombie flies" i.e *Apocephalous borealis*, in honey bees are disoriented movement, attraction to light, difficulty flying, and eventual mortality outside the colony, were among the signs of infestation. It seems that when worker bees are foraging, adult female flies inject eggs into them (Casuso *et al.*, 2015) [3].

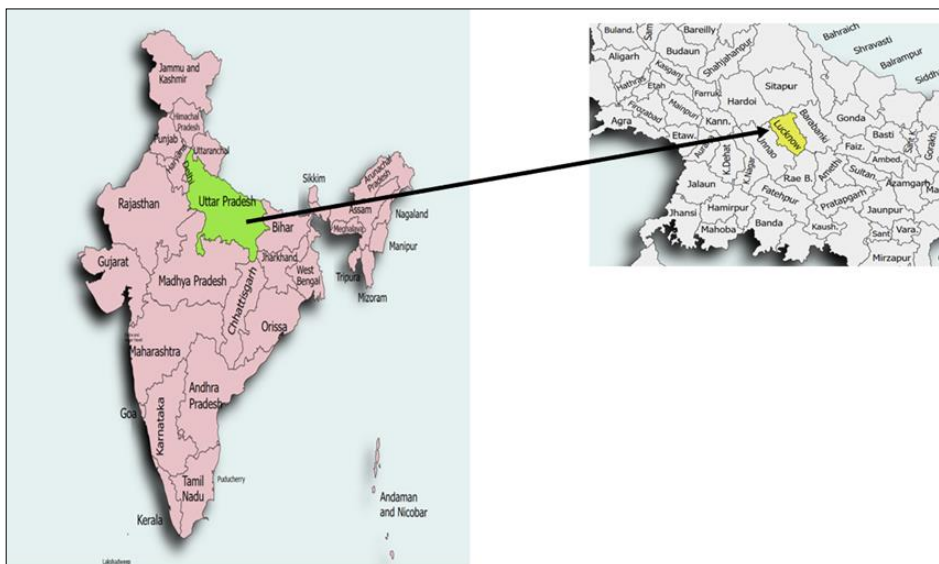
In order to lessen stress on bee colonies, their findings demonstrated the significance of integrated pest management tactics that include colony monitoring, sanitation, and environmentally friendly control techniques (Core *et al.*, 2012) [5].

To assess the efficacy of various miticides and essential oil compounds against *Varroa jacobsoni* (now known as *Varroa destructor*). The study showed, clove oil, thymol, carvacrol, methyl salicylate, and other botanical substances caused relatively moderate mortality in honey bees, they demonstrated considerable mite mortality. Thymol and clove oil, two of the substances that were examined, showed a high degree of mite expiration (Lindberg *et al.*, 2000) [4]. The usage of synthetic acaricides leads to the development of mite resistance and also results in increase toxicity in hive products, endangering both bees and consumers. These drawbacks have drawn interest in plant-based essential oils as environmentally beneficial mite control options. Numerous laboratory and field studies demonstrate the potent acaricidal, fumigant, and repellent properties of essential oils rich in terpenoid components, such as thymol, menthol, eucalyptol, and linalool, against *Varroa destructor* (Bava *et al.*, 2023).

The significance of using plant extracts, and essential oils in integrated pest management (IPM) strategies leads to inhibit the chemical use. Botanical chemicals such as thymol, clove oil, eucalyptus oil, and menthol may have potential for safely treating developing bee pests, despite the lack of specific studies on essential oils against *Apocephalus borealis* (Abou-Shaara and Staron, 2019) [1].

Material and method

The present investigation on the integrated management of honeybee pests, namely *Varroa destructor*, *Apocephalus borealis* and *Aethina tumida* in colonies of *Apis mellifera*, was carried out in selected apiaries located in Isabella Thoburn College, Lucknow, Uttar Pradesh, India.



The Healthy and naturally infested honeybee colonies maintained under similar environmental conditions were selected for the study. The colonies were regularly inspected throughout the experimental period for the occurrence of mites, flies and hive beetles. Standard beekeeping practices were followed during the study to maintain colony strength and hygiene.

In this experiment, the sticky board method was used to capture the *Varroa* mite, *Apocephalous borealis* and *Aethina tumida*. In this method, a sheet was used, and a proper grid was drawn to make counting easier. The dimensions of the sticky board sheet are 2.5 cm by 2.5 cm, and it is quoted with petroleum jelly and inserted at the bottom of the hive below the wire mesh frames. A sticky board trapping method was adopted by (Nancy O, Diana S, in 2000) [17]. A cotton ball was used to put the essential oil in the hive to capture the pest, and a layer of essential oils was also applied over the adhesive layer in order to kill the pest and examine the role of essential oil in controlling the pest population. The visual view inspection was used to see the population of beetles in the hive box was proposed by (Neumann and Elzen, 2004) [18].

The essential oils used in this experiment were eucalyptus oil, mint oil, and neem oil. They were purchased from Unani medicine doctor of Aminabad market of Lucknow Uttar Pradesh, India. The essential oil was prepared by the method given by (Girisgin, A. O., 2014) [9], 50 milliliters of 1%

essential oils were produced in distilled water with the use of 50 milliliters of Tween 80 solution and emulsified with the cup method (2 milliliters of essential oil + 44 milliliters of distilled water + divided into two paper cups (2×25 ml) and poured 4 ml tween 80.

The univariate analysis in General Linear Model (GLM) was used in SPSS version 32 for statistical data analysis. In comparison between the essential oils Tukey's HSD post hoc analysis was done.

Results and Discussion

There were differences in average infestation at various times (24, 48, and 72 hours) between treatments. Treatment type and exposure time significantly impacted infesting *Varroa* mites. Neem oil and eucalyptus oil had higher mean infestation values than the control and mint oil at all time levels. Results from a two-way ANOVA showed a main effect of time ($F = 26.657$, $p = 0.001$) and treatment ($F = 19.470$, $p = 0.001$) and a treatment-time interaction ($F = 6.158$, $p = 0.001$). Results show significant treatment-time interactions. Tukey's HSD post-hoc analysis showed that both neem oil and eucalyptus oil were significantly different from the control ($p < 0.05$). There was no significant difference in mint oil and the control ($p > 0.05$). The conclusion is that both neem oil and eucalyptus oil treatments were the most effective.



Fig 2: Petroleum jelly quoted sheet



Fig 3: The petroleum jelly quoted sheet is placed at the bottom of the hive box



Fig 4: Three types of Essential oils used in the experiment- mint oil, neem oil, Eucalyptus oil

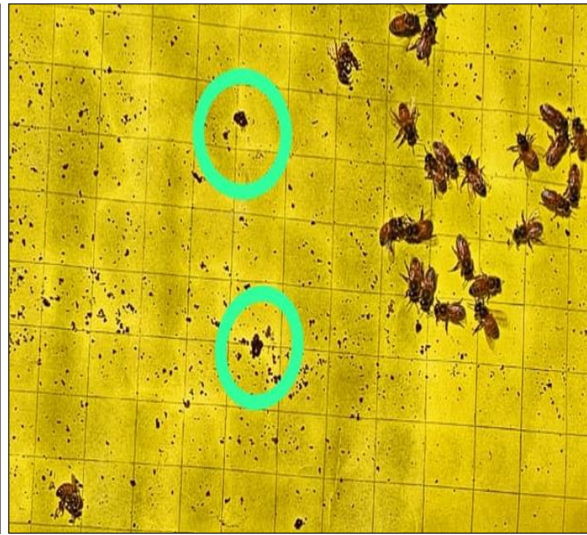


Fig 5: Small hive beetle found at the bottom of the hive box on a sticky sheet

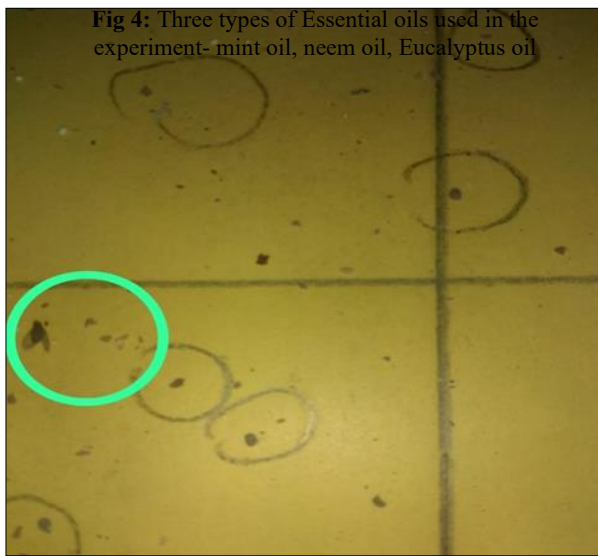


Fig 6: Capturing of dead *Apocephalus* on the sticky sheet

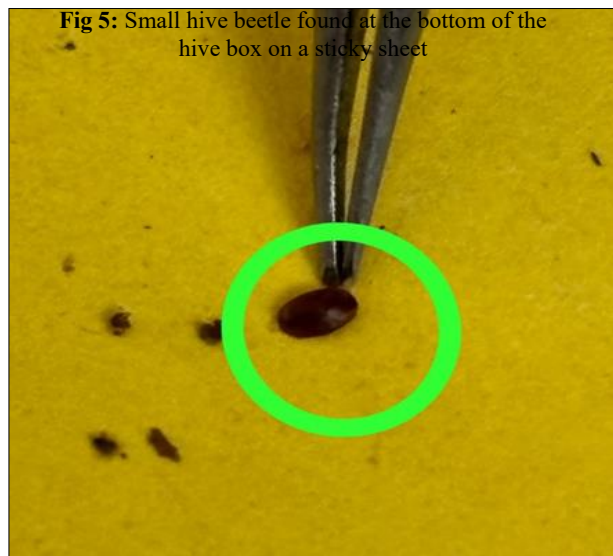


Fig 7: Capturing of dead *Varroa* mite on the sticky sheet

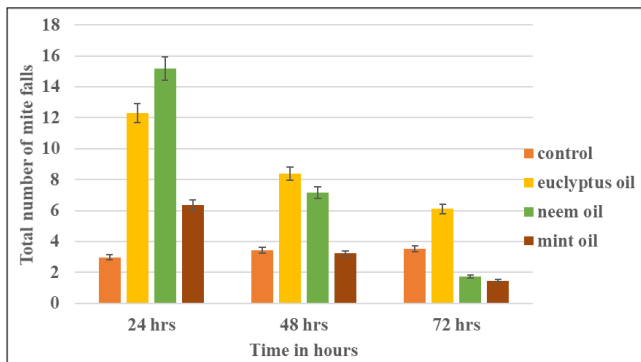


Fig 8: Effects of different essential oils on *Varroa* mite falls
Data are statistically significant (df=6, P< 0.05)

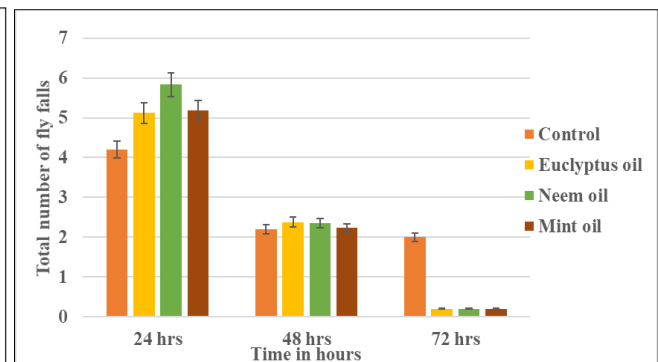


Fig 9: Effects of different essential oils on *Apocephalus borealis* falls
Data are statistically non-significant (df=6, P>

Two-way ANOVA of infestation levels and time against *Apocephalus borealis* showed that while time impacted infestation levels significantly ($p < 0.05$), the treatment ($F = 0.093$, $p = 0.963$) and time-treatment interaction ($F = 1.863$, $p = 0.107$) were both statistically non-significant. Tukey's HSD post-hoc analysis showed that both eucalyptus, neem, and mint oil and control treatments ($p > 0.05$) were statistically similar. Statistically, all treatments were the same; however, they showed that all the volatile compounds used in the treatments had no effect on controlling the infestation in comparison to the control.

The results for *Aethina tumida* show that the application of essential oils achieved considerable success, depending on the time interval over which they were measured. All mean values of the parameters measured in different time intervals decreased, in the order of effectiveness, mint, neem, and eucalyptus oil. Two-way ANOVA showed a significant main effect of time ($p < 0.05$), a main effect of treatment ($F = 9.448$, $p = 0.001$), and an interaction effect of time and treatment ($F = 2.301$, $p = 0.049$). This means that the effectiveness of the treatment changed significantly over time. Tukey's HSD showed that mint oil had a significant

effect when compared to the control ($p < 0.05$), and that neem oil had a significant effect when compared to the control ($p = 0.034$). Eucalyptus oil had no significant effect when compared to the control ($p > 0.05$). Therefore, among all the treatments, mint oil was the most effective, followed by neem, and finally, eucalyptus oil had the least effectiveness.

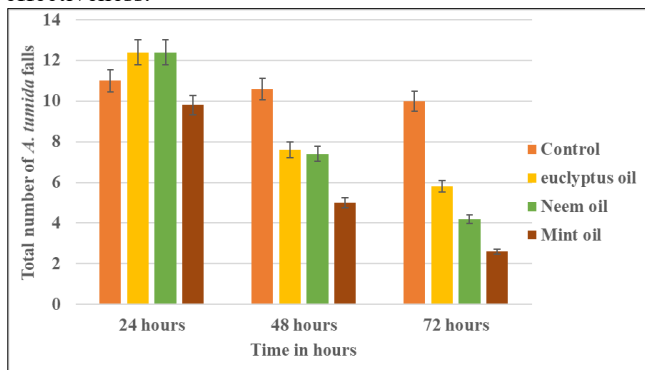


Fig 10: Effects of different essential oils on *Aethina tumida* falls. Data are statistically significant ($df= 6, p < 0.05$)

For *Varroa destructor*, eucalyptus oil and neem oil showed significant effects compared to the control ($p < 0.05$), whereas mint oil showed a non-significant effect ($p > 0.05$). These findings agree with (Ghasemi *et al.*,2011) [8] and (Melathopoulos *et al.*,2016) [15], who also reported higher effectiveness of eucalyptus and neem oils against Varroa mites. However, the present result for mint oil differs from (Castagnino and Orsi 2012) [2], who observed better control with mint oil. Such differences may be due to variations in concentration, environmental conditions, and application methods.

In *A. borealis*, all treatments showed non-significant effects ($p > 0.05$), indicating that eucalyptus, neem, and mint oils performed similarly to the control. This result differs from (Hybl *et al.*,2021), who reported stronger activity of essential oils against bee pests. The variation may be related to differences in pest susceptibility and experimental conditions.

For *Aethina tumida*, the treatments showed significant differences ($p < 0.05$), with mint oil being the most effective, followed by neem oil, while eucalyptus oil showed a non-significant effect. These findings support earlier studies reporting strong insecticidal activity of mint and neem oils (Castagnino and Orsi, 2012 [2], Melathopoulos *et al.*, 2016) [15]. However, the lower effectiveness of eucalyptus oil contrasts with (Ghasemi *et al.*,2011) [8], who found eucalyptus oil to be highly effective against bee-associated pests.

Conclusion

The experiments done in this study show the ability of different oils to combat different pests of honey bees. Out of the oils tested, Eucalyptus and Neem oils were able to combat *Varroa destructor*, while mint oil proved to have the most combat ability against *Aethina tumida*. Every oil tested proved to have no significant impact on *A. borealis*. Though essential oils can be environmentally friendly substitutes to honey bee pest control, more systematic tests should be done to establish the correct dosage and methods of application in fields.

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