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Research Article

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The efficacy of clove oil to manage *Varroa destructor* and *Apocephalus borealis*, in *Apis mellifera* L. colony

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ABSTRACT
Clove oil is a natural, eco-friendly, and highly effective odorant for triggering hygienic behavior in the colonies of *Apis mellifera*. Bees with highly hygienic behavior can quickly remove *Varroa destructors* and other enemies of bee hives. The estimation of hygienic behavior is estimated by a pin-killed method. 24 hours of experiments were conducted for observation at different months. Observed significant difference at 3 hours ($P < 0.001$) and a non-significant difference at 6,9 and 24 hours ($P > 0.09$), ($P > 0.014$,) and ($P > 0.5$) for the opened cells. For the cleared cells there has been observed significant difference at 3 and 6 hours ($P < 0.03$) and ($P < 0.04$), non- significant difference at 9 and 24 hours ($P > 0.25$) and ($P > 0.012$). clove oil also has acaricidal properties which is very effective in controlling Varroa mites and other parasites of honey bees. For the trapping of *Varroa destructor* and *Apocephalus borealis*, the sticky board method was used, in which a petroleum jelly-coated sheet was inserted at the bottom board for the trapping of mites and flies can be controlled within a week, highly significant differences observed in the first 3 days, P-values are respectively ($P < 0.00013$), ($P < 0.0011$), and ($P < 0.01$), on the fifth, sixth and seventh day no significant difference discern. Mites could control within 40 days, there has been no significant difference between the first and last week, and notice a significant difference between the second and third week ($P < 0.0025$).
Keywords: Essential oil, acaricidal, hygienic bhaviour and sticky board method.

INTRODUCTION

Honeybees remove dead and diseased broods from the hive, this behavior is called hygienic behavior. Hygienic behavior is the crucial mechanism of protection against parasites and pathogens. It is a genetic tendency performed by honey bees, by which bees detect first then uncap and remove diseased, paralyzed broods and also remove their parasitic mite *Varroa destructor*. Goncalves et al., 1970).

Nowadays estimation of hygienic behavior is done by the mechanical killing method which is also known as the "pin-killed" method. The pin test is a low-cost method which can be easily used because of its simplicity. At Present the use of essential oils is being done in the treatment of bee mites (Turek and Stintzing, 2013). They have a repulsive and toxic effect on arthropods through fumigation, topical use or ingestion (Umpiérrez et al., 2011). Clove oil has significant acaricidal properties against Varroa mites (Mahmood et al., 2014; Li et al., 2017; Kloucek et al., 2012). Essential oils are the concentration of a hydrophobic liquid containing multiple volatile aromatic components present in the glands located in different parts of plants: seeds, bark, flowers, fruits, leaves and roots (Bayala et al., 2014).

Essential oils have antiparasitic, antiviral, antimicrobial, and antifungal properties (Hyldgaard et al., 2012; Turek and Stintzing, 2013). Syzygium aromaticum L. (clove) is a dried flower bud member of the Myrtaceae family (Bhuiyan et al., 2012; Cortés-Rojas et al., 2014). It contains various bioactive combinations acting as phenolic propanoids (60-90%) and volatile oil (15-20%) (Chaieb et al., 2007). All known oils worldwide have conveyed variable antimicrobial activity. Clove, besides its use as a perfume and food flavour, has culinary and medical properties through its antiseptic, anti-inflammatory, antioxidant, antifungal, antiviral and antiparasitic actions (Alitonou et al., 2012; Mohamed and Badri, 2017). The antimicrobial activity of clove is because of eugenol, oleic acids and lipids found in its essential oils (Hammer et al., 1999). There are so many synthetic drugs used to control varroosis, specially pyrethroid and organophosphate, but the use of these drugs is limited for reasons such as the development of resistance and contamination of bee products, especially honey and beeswax, this contamination could be endangered to the health of bees and humans. To avoid this contamination, we have discovered new ideas and

safer ways to control these mites (Damiani et al., 2009), by the use of natural essential clove oil in the treatment of mite and fly control. The use of antibiotics has been banned in some countries (Genersch, 2010). So natural essential oils are very much better alternative in comparison to synthetic products because they are generally lowest in cost and have lesser health risks for humans and bees.

The present study aimed to investigate the clove oil effect on the hygienic behavior of honey bees and use the oil against parasite control.

MATERIALS AND METHODS

Estimation of hygienic behavior by pin-killed method

To enhance the hygienic behavior by the clove oil, I have conducted an experiment at the Royal Honey and bee forming society, Chinhat, Lucknow Uttar Pradesh and a local apiary at the Kargaina-kargaini, Pilibhit Uttar Pradesh, in the year 2020 and 2021. The experiment was conducted in the 5-7 framed, Longstroth-size beehives of *A. mellifera* colonies. The pin-killed method is used to estimate hygienic behaviour by the formula

$$HYGIENIC\ BEHAVIOUR = \frac{No.\ of\ open\ and\ cleared\ cells.}{Total\ no.\ of\ pinned\ cells} \times 100$$

In the pin-killed method. We made a rectangle column on the hard cardboard sheet each side of the rectangle was 12 cm, in which 100 brood cells could be fit. The experiment can be conducted on the 17 to 18-day-old brood when pupa converted, red colour eyes. The percentage of 10 % clove oil with 90% coconut or 90% olive oil was placed in the corner of the hive, in the cotton ball. The estimation of open cells and cleared cellshave been recorded separately after 3, 6, 9 and 24 hours.The essential clove oil was purchased from the Unani medicine doctor of Aminabad market of Lucknow Uttar Pradesh, India. The average rate of cleared cells was calculated and given as a percentage. The one-way ANOVA test of SPSS version 25., was used for statistical calculation.

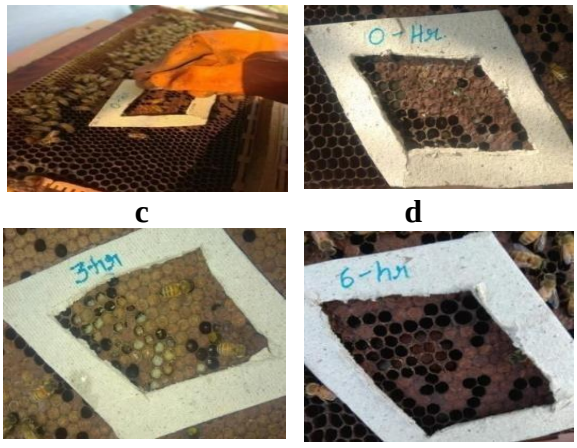

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Fig 1. (a). Pin-kill method (b). Start time of expt. (c). Effect after 3 hours (d). Effect after 6 hours (e). The effect after 24 hours

Counting of *Varroa destructor* and *Apocephalus borealis* by sticky board method.

Sticky board counting technique followed by (Nancy O, Diana S, 2000). For the sticky board, we used a chart sheet coated with petroleum jelly to trap the mite (*Varroa destrector*) and flies (*Apocephalus borealis*). The dimensions of the sticky board are 30 cm by 40 cm. A grid containing 54 cells (6 cells × 9 cells) is drawn with the help of cm scale and graphite pencil, the area of the grid is equal according to the frame width where the mite falls. One extra coating was applied on the sticky sheet for the death of both parasites in which we used a mixture of 5 essential oils mixed with coconut oil. the mixture of oils consists of 90% coconut oil with 3% clove oil, 3% eucalyptus oil, 2% turmeric oil, 2% mint oil and 1% neem oil. The same technique was used in the different hives which were infected by *Varroa destrector* or *Apocephalus borealis*.

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Fig. 2. Petroleum jelly coated sheet at the bottom board

Fig. 3. Varroa infected worker female.

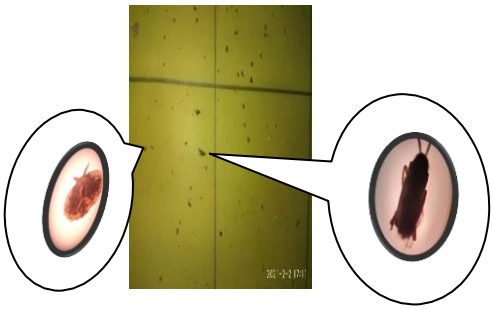

Fig. 4. Fallen *Varroa desructor* and *Apocephalus borealis* on petroleum- coated sheet.

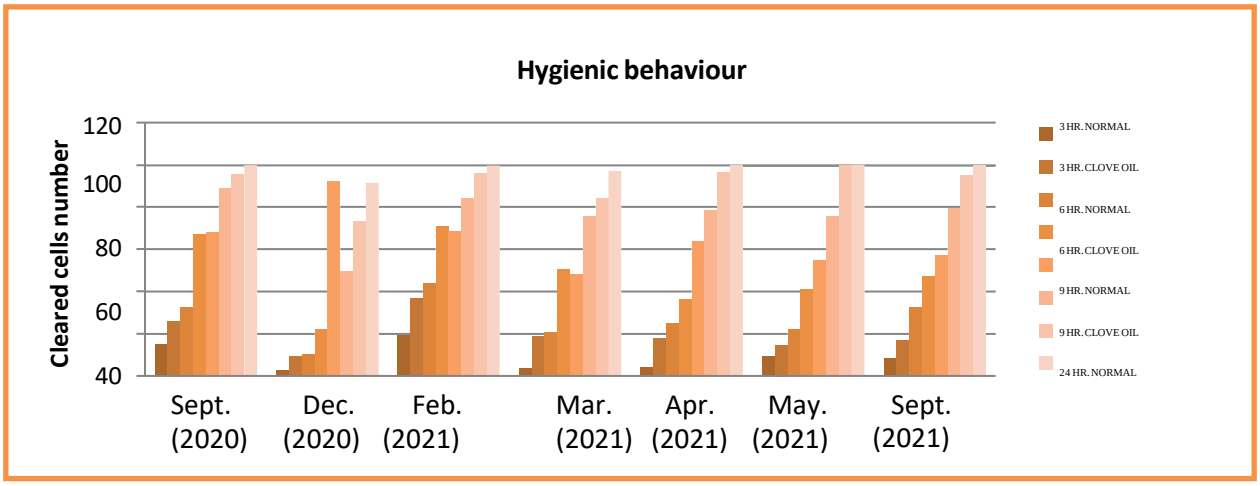

Fig. 5. Dynamic of cells cleaning, with pin-killed brood in different hours (3 hr, 6 hr, 9 hr and 24 hr)

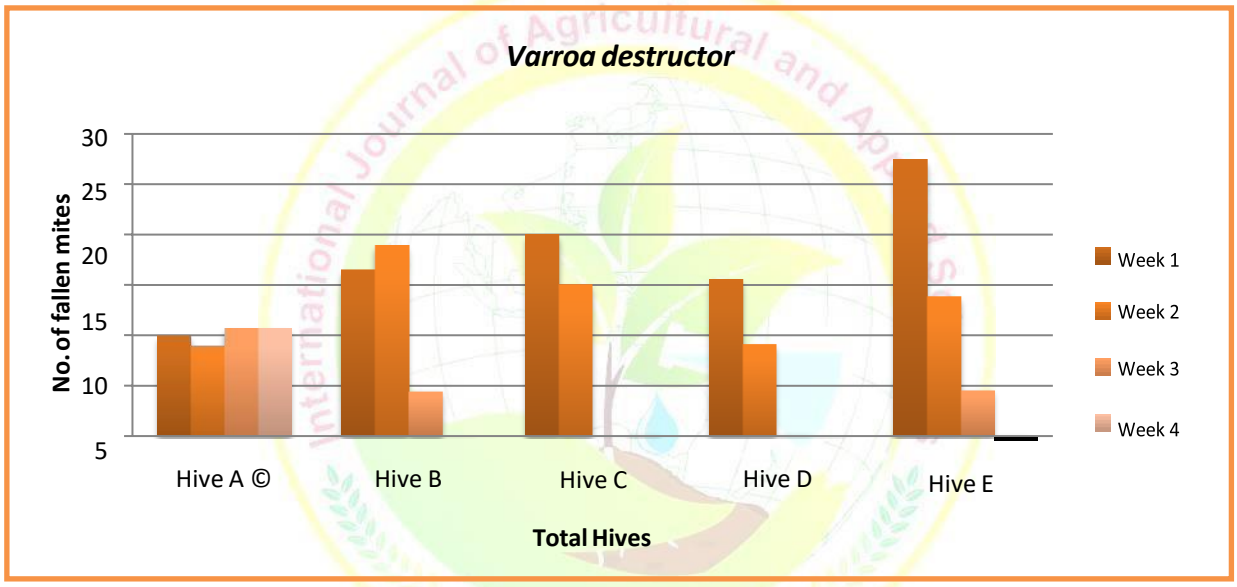

Fig. 6. Dynamics of fallen Varroa mites in different weeks.

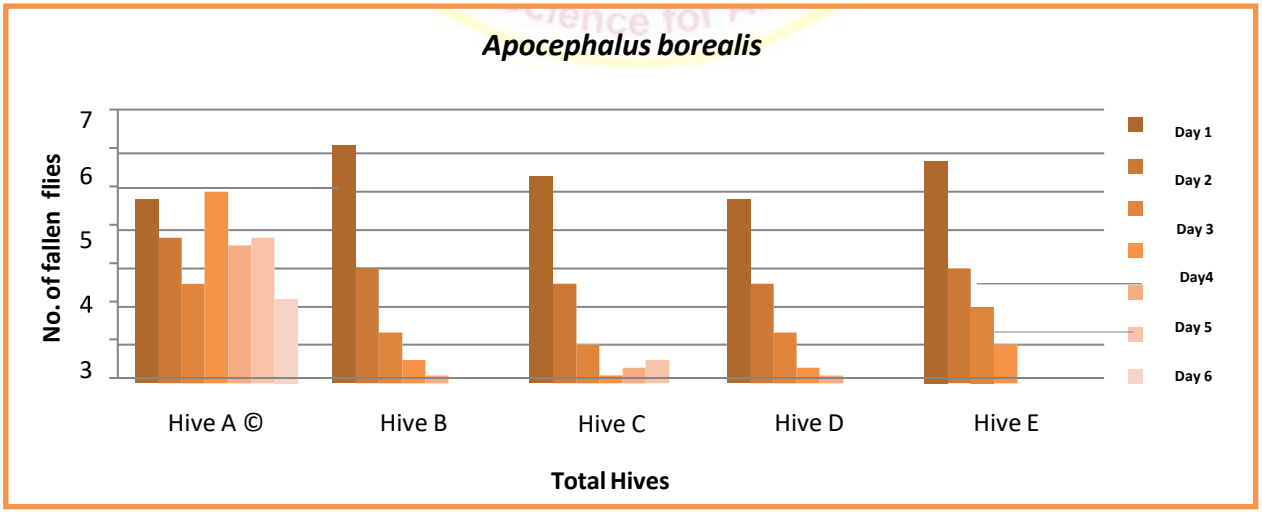

Fig. 7. Dynamics of fallen *Apocephalus* flies on different days of the week

RESULTS AND DISCUSSION

Dynamic of cells opening and cleaning, in different hours (3 hr, 6 hr, 9 hr and 24 hr)

Detailed analysis of the cells opening was done on different months of the year 2020-21 at different hours. During the first 3 hours, after applying clove oil, cell openings are significantly higher than normal openings. The P-value of 3-hour open cells was ($P < 0.001$) which is highly significant than normal cells opening. After 6 hours there is no significant difference were found in the cell's opening rate so the P-value of open cells of 6 hours, 9 hours and 24 hours were ($P > 0.09$), ($P > 0.014$) and ($P > 0.5$) respectively, that was non-significant values.

So, the result analysis of the cell cleaning was done in different months of the year 2020-21 at different hours. During the first 3 hours, after applying clove oil, cells cleaning are significantly higher than normal cleared cells. The P-value of 3-hour open cells was ($P < 0.03$) which shows a significant difference from normal cell cleaning. In the cleaning of the cells, there was also a reported significant difference after 6 hours so t h e P-value of 6-hour cleaning cells was ($P < 0.04$) which showed a significant difference. After 9 hours there is no significant difference were found in the cell cleaning rate so the P-value of cleared cells at 9 hours and 24 hours were ($P > 0.25$) and ($P > 0.12$) respectively, that was also non-significant values. So, clove oil is more affected by cleaning cells than open cells.

In previous studies, we have been concerned with many scientific approaches where different essential oils like clove oil, thymol and organo were used against Varroa control (Marian Hybl et al. 2021). Younger bees and drones have a higher chance of Varroa infestation than other stages of bees (Winsto, 1995). Varroa mites were more fallen when the temperature was below 17˚C at that time bees formed clusters and groomed fast and removed Varroa from other bees (Shalaby et al. 1996). If the temperature is high Varroa does not leave the brood cell to the adult bees (Sazabo and Walker 1996). Clove oil, thymol, organum oil, carvacrol and menthol were the most effective compounds for killing mites in the Laboratory assay (Sammatraro et al. 1998). Clove oil is less toxic for Varroa killing than menthol (Hanan A et al. 2009).

Vimal Goswami and M.S. Khan suggest Other than Clove oil, Garlic oil significantly reduced Varroa population within three weeks, having $P < 0.005$ which was higher than turmeric oil, cinnamon, tulsi and ajwayan and also observed acaricidal activity of clove oil and formic acid against Varroa. Lindberg et al. (2000) concluded that clove oil and thymol were toxic for the Varroa mite, caused high mortality (>70%) for mites, and produced Low mortality (<30%) for bees. Although Ariana et al (2022) performed the same experiment and showed thymol oil caused mite mortality without affecting adult honey bees. Ebert et al. (2007) fed bees with different natural products and concluded that menthol and clove oil were less toxic for bees than oxalic acid.

CONCLUSION

Varroa destructor is a major problem for apiculture, there are various synthetic prescriptions available in the market which contaminate, a product of apiary. But natural essential oils are the best substitute for treating apiary without contamination.

Clove oil is a non-synthetic treatment for apiculture which can enhance hygienic behavior rapidly and control parasites like *V. destructor* and *A. borealis*. It can quickly influence worker bees and enhance hygienic behaviour rapidly. Clove oil is easily available in Unani medical store and it is a cost-effective method for beekeepers.

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CONFLICT OF INTEREST

The author here declares that there is no conflict of interest in the publication of this article.

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