



## Research Article



# An eco-friendly mixture of thymol, camphor and menthol is used to enhance hygienic behavior in the colonies of *Apis mellifera* L.

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### ABSTRACT

Large-scale pesticide usage is contributing to a decrease in *A. mellifera* colonies due to colony collapse disorder. The accumulation of pesticides in bees has led to an absence of “hygienic behavior,” which is a defensive mechanism whereby honey bees uncap and clean cells. This hygienic behavior encompasses the activities of removing infected larvae, pupae, and adult bees from the hive, as well as external parasites such as mites or flies. This marks my inaugural utilization of a combination of thymol, camphor, and menthol crystals to augment hygienic behavior in *A. mellifera* colonies via the pin-killed (needle-killed) technique. In this procedure, I have amalgamated equal quantities of thymol, camphor, and mint crystals, which, after 24 hours, transform into a liquid solution. Five milliliters of this solution are administered by submerging cotton and situating it in the corner of the hives. The number of open and cleared brood cells is tallied after 3, 6, 9 and 24 hours. The dynamics of observation varied between open and cleared cells a significant increase in the cell cleaning rate was observed at 5% level of significance ( $p < 0.05$ ). It is noteworthy that no dead bees were reported around the cotton ball, indicating that the mixture is not harmful to bee health, unlike other synthetic medicines. Furthermore, this eco-friendly and highly effective method could prove to be a convenient option for beekeepers.

**Keywords:** *Apis mellifera*, thymol mixture, hygienic behavior, pin-killed, Uttar Pradesh

### INTRODUCTION

One of the primary means of controlling *V. destructor* at this time is thymol, an acaricide that has gained popularity quickly (Mondet et al. 2011). Thymol has also been proposed to help prevent the spread of other parasites, such as chalk brood (Grobov et al. 1981; Colin et al. 1989; Costa et al. 2010). Although the effects of thymol on mites and honey bees are still mainly unknown, Price and Lummis (2014) state that it is thought to influence these organisms by increasing GABA responses and interfering with GABA receptors. According to Montag et al. (2011), thymol can influence honey bees and interact with olfactory receptors, which induces fanning behavior in European honey bees. This interaction may affect chemical signals within the colony, triggering hygienic behavior, including removal of sick or dead brood and adults (Mondet et al. 2015). Several scents are signals that indicate larval health and/or decay related to the removal of dead adults and larvae (Wilson et al. 1958, Gordon 1983; Choe et al. 2009; McAfee et al. (2018). Hygienic behaviour is an important behavioral mechanism of resistance to *V.*

*destructor* and other routes genes. Goodwin and Van Eaton 2001; Evans et Spivak (2010).

Only one substance, thymol, is utilized in beekeeping and has an efficiency of 70% to 95% against *V. destructor* (Calderone, 1999; Eguaras et al., 2004; Ruffinengo et al., 2014). Eighty per cent of the essential oils of plants like thyme and *Origanum vulgare* (Linnaeus) contain carvacrol. In earlier investigations, our research group's thyme oil demonstrated good acaricidal action (Eguaras & Ruffinengo, 2006). Additionally, experiments on a variety of bacterial species, including *Bacillus cereus* and *Escherichia coli* (von Escherich), have demonstrated its strong bactericidal activity (Garcia-Garcia & Palau Garcia, 2008). In addition, it is used as an additive by the food industry to stop bacterial infection.

Menthol has gained significant recognition due to its ability to effectively control *A. woodi* (Herbert et al, 1988; Cox et al, 1989; Wilson et al, 1990) when the temperature within a colony reaches levels that allow for evaporation. However, studies have shown that menthol may not be as effective in cooler conditions or during fall

treatments (Moffett et al, 1989). To improve evaporation and enhance its effectiveness, menthol placement on the top bars can be helpful. Although the placement of menthol crystals between frames can increase evaporation rate, it may also lead to elevated temperatures that can melt the crystals and cause them to run. Cox et al (1989) conducted research on methods to enhance the surface area of menthol for faster evaporation at cooler temperatures. In a separate study, Wilson et al (1990) designed and tested a menthol-grease board as a potential solution for increasing evaporation rate.

## MATERIALS AND METHODS

In order to improve hygienic behavior through the use of thymol mixture, I conducted an experiment in Uttar Pradesh, India at the Royal Honey and Bee Farming Society in Chinhat, Lucknow. The experiment was carried out in 2020 and 2021 using 5-7 framed, Longstroth-size beehives containing *A. mellifera* colonies. A total of ten healthy and uniform colonies of *A. mellifera* were chosen for the experiment. Each colony included a laying queen, approximately equal adult bee strength, brood area, and food reserves.

To ensure uniformity, 100 sealed worker brood cells were selected from each colony to record hygienic behavior. The same experiment was repeated six times, with an average temperature range of 28°C and an average relative humidity was 65%.

The pin-killing method was utilized to accurately measure hygienic behavior using a specific formula. In the pin-killed method, we have made a rectangular column on a hard cardboard sheet, each side of the rectangle is 12 cm, and 100 brood cells can fit in the column. The experiment can be done on 14 to 18-day-old brood, when the pupa develops red colored eyes.

Mixed the same amount of crystals of thymol, camphor and menthol, after 24 hours, it converts into a liquid solution. Five ml. of this solution is used by the cotton ball and put it, in the corner of the hives. Count the open and cleared brood cells after 3-24 hours. Dr Ruchira Tiwari's Method followed for counting cells (2012). The average rate of open cell and cleared cell is calculated separately and reported as a percentage. Univariate analysis (GLM) is used for statistical calculation. Sidak multiple analysis (Post Hoc) test is used to check significance at different hours. SPSS v29 software is used for data analysis.

$$\text{Hygienic behavior} = \frac{\text{No of cleared cells count at differen hour}}{\text{Total no of pinned cells}} \times 100$$

The proportionate share of the components of the volatile mixture was analyzed using equipment software from the total peak area (TIC). The components in the volatile oil were identified by comparing their mass spectra and retention times to those of the real compounds, matching the mass

spectral data on a computer with the NIST and WILEY libraries, and comparing the fragmentation pattern with those published in the literature.



Fig 1. Parallelogram block for 100 brood cells.

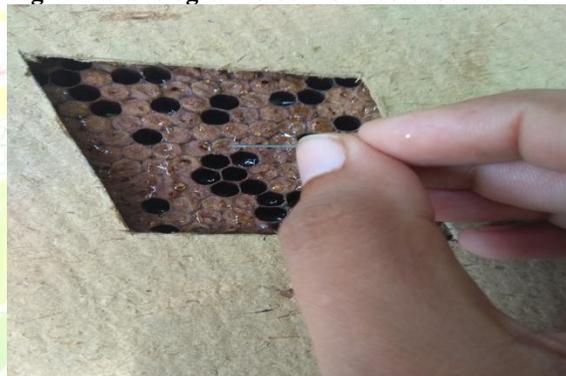


Fig 2. Brood killing by a sterile pin



Fig 3. Thymol crystals (a), Menthol crystals (b), Camphor crystals (c), whole mixture(d).

### GC-MS analysis of compound mixture

The proportionate share of the components of the volatile mixture was analyzed using equipment software from the total peak area (TIC). The components in the volatile oil were identified by comparing their mass spectra and retention times to those of the real compounds, matching the mass spectral data on a computer with the NIST and WILEY libraries, and comparing the fragmentation pattern with those published in the literature.

### RESULTS AND DISCUSSION

Composition of Thymol (*Thymus vulgaris*), Menthol (*Mentha piperita*) and mixture (*Cinnamomum Camphora*) obtained by GC/MS analysis resulted under our test conditions in the identification of 4 different components: Butyl isocyanatoacetate, Isoborneol, 2-Isopropyl-5methylcyclohexylethylphosphonofluoridate and Thymol were present. (Table 1) representing compound name with chemical formula, library score and retention time.

**Table 1.** GC-MS compound list, score match with online NIST and WILEY libraries

S. n o	Compound Name	Chemical Formula	SCO RE	Retention time
1	Butyl isocyanatoacetate	(C7 H11 N O3)	81.35	3.170
2	Isoborneol	(C10 H18 O)	73.34	11.334
3	2-Isopropyl-5methylcyclohexyl methylphosphonofluoridate	(C11 H22 F O2 P)	82.43	11.563
4	Thymol	(C10 H14 O)	69.58	13.275

Previously, we have found thymol, menthol and camphor separately effective for *Varroa* control and promising materials to induce hygienic behavior. To test the effectiveness of these odorants on the needle-killed assay, we have developed a mixture of the crystals of these three compounds to test hygienic behavior. GLM univariate analysis indicated that time had a highly significant effect on measurement ( $F = 278.26$ ;  $p < 0.01$ ), and the effect of the group was significant ( $F = 30.66$ ;  $p < 0.01$ ). Also, the interaction between time and group was significant ( $F = 3.79$ ;  $p < 0.05$ ). In the multivariate analysis, for uncapped brood, there were significant impact occurring at 3 hours ( $p = 0.01$ ), whereas at 6 hours ( $p = 0.02$ ), 9 hours ( $p = 0.03$ ), and 24 hours, the effect was not significant ( $p = 0.99$ ).

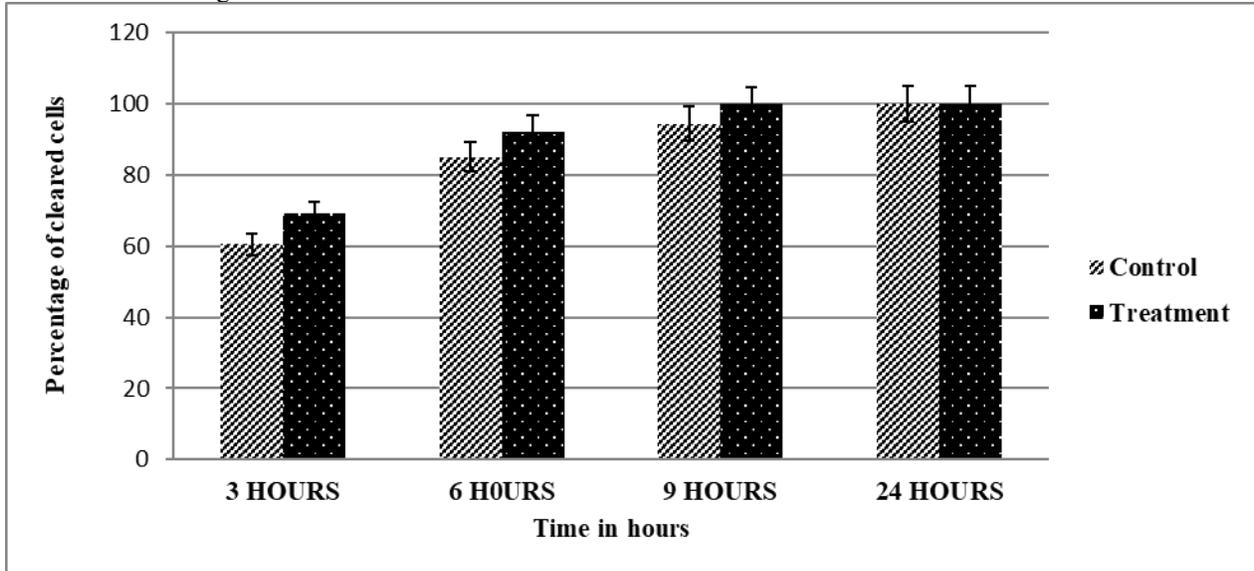
Likewise, for the cleaning rate of brood, the results showed a very significant overall model ( $F(7, 16) = 495.71$ ,  $p < 0.001$ ). It explained 99.5% of the total variation in the data. There were significant effects for both time ( $F(3, 16) = 1123.87$ ,  $p < 0.001$ ) and treatment groups ( $F(1, 16) = 76.94$ ,  $p < 0.001$ ). Additionally, there was a significant interaction between these factors ( $F$

(3,16) = 7.13,  $p = 0.003$ ). So, the multivariate analysis showed the mixture had a significant effect at 3 hours,  $p$ -value is  $p = 0.03$ . Similarly, the mixture also showed significant effects at 6 hours  $p = 0.03$ - and 9-hours  $p = 0.05$ . However, at 24 hours, the effect of the mixture on brood cell cleaning was not significant  $p = 0.8$ , indicating that the effect of the thymol mixture on brood cell cleaning varied significantly over time, so the increasing rate of cleaning cells was highly significant at the first six hours of start and was neutral in 24 hours. Mixture-enhanced hygienic behavior also helps to maintain colony temperature.

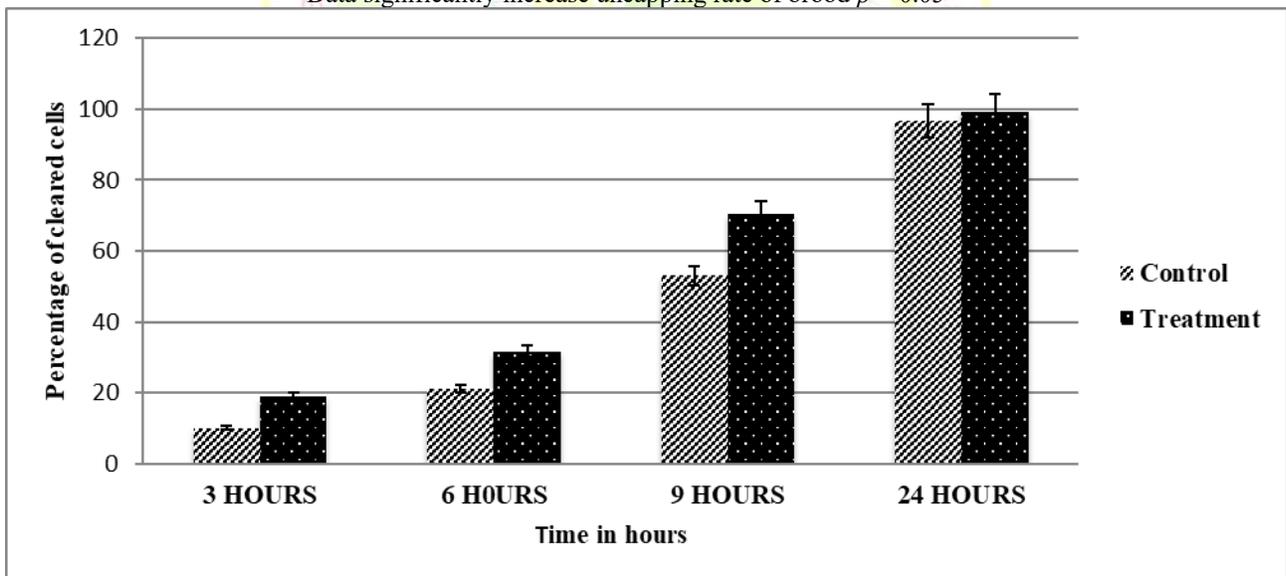
According to Calderone (1990), the use of a thymol mixture in bee colonies led to a higher mortality rate among ticks than in control colonies. The variation in tick mortality between colonies treated with Thymol and Apistan was 67.15, while for those treated with Formic Acid and Apistan it was 117.65. These results suggest that Apistan is a more effective and reliable treatment option compared to other methods tested in this study. Based on the findings of Coli ME (1997), substances such as Thymol, Menthol, and Camphor have been proven effective in controlling mites when applied under specific conditions. However, these conditions may be influenced by regional and climatic factors. Therefore, further research should be conducted in Mediterranean hives to better understand the application of these substances. It is crucial to accurately assess the level of parasitic infestation in colonies after treatment before considering these alternatives to chemical control methods. According to Damiani et al (2018), the thymol-menthol-camphor mixture appears to be a valuable resource for improving hygienic behavior in honey bees, potentially contributing to the health and resilience of the colony. Johnson et al. (2019). Findings suggested, combination of thymol, menthol, and camphor has demonstrated a positive effect on hygienic behavior in honey bees. This formulation might engage the bees' olfactory system, thereby affecting their capacity to identify and react to diseased or deceased brood. Furthermore, it may possess antimicrobial characteristics that aid in diminishing pathogen levels within the hive. Colin T (2019) reports that after 48 hours in both locations, thymol increased dead brood closure and removal by 24–36%, but had no effect on dead adult bee removal. Increased brood clearance could enhance thymol's effect on *V. destructor*, particularly if bees remove mite-infected cells first. In summary, the thymol-menthol-camphor mixture appears to be a valuable resource for improving hygienic behavior in honey bees, potentially contributing to the health and resilience of the colony. Menthol, extracted from peppermint oil, has historically been employed to address certain parasites affecting honey bees. Studies have evaluated its efficacy when combined with thymol to fight *Varroa* mites in real-life conditions, yielding encouraging outcomes; specifically, the infestation rate decreased from 21.86% to 1.32% following treatment. Additionally, utilizing menthol at a concentration of 2.5

mL has proven effective against tracheal mite infestations. Topal E, Cornea-Cipcigan M, Tunca R. I., Kosoglu M, Margaoan R: The use of medicinal aromatic plants against bee diseases and pests. Essential oils and specific compounds extracted from them, including camphor, carvacrol, eucalyptol, menthol, thymol, and various sesquiterpenes, have been utilized in topical treatments to manage *Varroa destructor* mites and as

nutraceutical agents against *Nosema* spp. Spores, Kumar et al. (2022), results suggested the improvement of hygienic practices through the thymol-menthol-camphor blend holds significant relevance for beekeeping and the health of bee colonies. By encouraging these behaviors, beekeepers could potentially decrease disease prevalence and enhance the overall resilience of their colonies.



**Fig 4.** Effect of thymol mixture on uncapping of brood cells in colonies of *Apis mellifera*.  
Data significantly increase uncapping rate of brood  $p < 0.05$



**Fig 5.** Effect of thymol mixture on brood cells cleaning in colonies of *Apis mellifera*.  
Data significantly increase cleaning rate of brood  $p < 0.05$

**CONCLUSION**

The findings of this study indicate that a mixture of thymol, camphor, and menthol is highly effective in promoting hygienic behavior among bees. Additionally, this formulation did not negatively impact the health of the bees. As a natural compound, it is completely eco-friendly and proves to be effective in controlling parasites within the hive.

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**CONFLICT OF INTEREST**

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

## REFERENCES

- McAfee, A., et al. 2018. A death pheromone, oleic acid, triggers hygienic behavior in honey bees (*Apis mellifera* L.). *Scientific Reports*, **8**, 5719. <https://doi.org/10.1038/s41598-018-24054-2>
- Borges, D., Guzman-Novoa, E., & Goodwin, P. H. 2020. Control of the microsporidian parasite *Nosema ceranae* in honey bees (*Apis mellifera*) using nutraceutical and immunostimulatory compounds. *PLoS ONE*, **15**, e0227484. <https://doi.org/10.1371/journal.pone.0227484>
- Costa, C., Lodesani, M., & Maistrello, L. 2010. Effect of thymol and resveratrol administered with candy or syrup on *Nosema ceranae*. *Apidologie*, **41**(2), 141–150. <https://doi.org/10.1051/apido/2009071>
- Gorden, D. M. 1983. Dependence of necrophoric response to oleic acid on social context in ants. *Journal of Chemical Ecology*, **9**, 105–111.
- Damiani, N., et al. 2018. Antimicrobial activity of essential oils against honey bee pathogens. *Journal of Essential Oil Research*, **30**(3), 257–265. <https://doi.org/10.1080/10412905.2018.1437417>
- Wilson, E. O., Durlach, N. I., & Roth, L. M. 1958. Chemical releasers of necrophoric behavior in ants. *Psyche*, **65**, 108–114.
- Mondet, F. 2011. Behavioural response of honeybees to Apiguard®. *Journal of Comparative Physiology A*, **197**, 1055–1062. <https://doi.org/10.1007/s00359-011-0674-9>
- Mondet, F., & Alaux, C. 2015. Antennae key to hygienic behaviour in honey bees. *Scientific Reports*, **5**, 10454. <https://doi.org/10.1038/srep10454>
- García, G., & García, P. 2008. Antimicrobial mechanisms of thymol and carvacrol. *Temas Selectivos de Ingeniería de Alimentos*, **2**(2), 41–51.
- Herbert, E. W., et al. 1988. Menthol placement in honey bee hives. *American Bee Journal*, **128**, 185–187.
- Evans, J. D., & Spivak, M. 2010. Socialized medicine in honey bees. *Journal of Invertebrate Pathology*, **103**, 62–72. <https://doi.org/10.1016/j.jip.2009.06.019>
- Johnson, R. M., et al. 2019. Olfactory cues and hygienic behaviour. *Insects*, **10**(11), 373. <https://doi.org/10.3390/insects10110373>
- Price, K. L., & Lummis, S. C. R. 2014. Varroa destructor receptor modulation. *Insect Biochemistry and Molecular Biology*, **55**, 19–25. <https://doi.org/10.1016/j.ibmb.2014.09.002>
- Kumar, S., et al. 2022. Thymol-menthol-camphor mixture enhances hygienic behaviour. *Journal of Apicultural Research*, **61**(2), 253–262. <https://doi.org/10.1080/00218839.2021.1954443>
- Eguaras, M., et al. 2004. Effectiveness of thymol against Varroa. *Natura Neotropicalis*, **34**, 27–32.
- Spivak, M., & Downey, D. L. 1998. Hygienic behavior assays. *Journal of Economic Entomology*, **91**, 64–70. <https://doi.org/10.1093/jee/91.1.64>
- Colin, M. E., et al. 1989. Essential oils against *Ascospaera apis*. *Apidologie*, **20**(3), 221–228.
- Eguaras, M., & Ruffinengo, S. 2006. *Strategies for Varroa control*. DOI: Not available
- Palacio, M. A., et al. 2010. Hygienic behaviour in honey bees. *Apidologie*, **41**, 602–612. <https://doi.org/10.1051/apido/2009065>
- Calderone, N. W. 1999. Formic acid and thymol blends. *Journal of Economic Entomology*, **92**(2), 253–260. <https://doi.org/10.1093/jee/92.2.253>
- Grobov, O. F., & Gousseva, V. V. 1981. Thymol broad-spectrum activity. *Apiacta*, **16**, 64–65.
- Cox, R. L., et al. 1989. Menthol treatment effects. *American Bee Journal*, **129**, 547–549.
- Goodwin, R. M., & Eaton, V. C. 2001. *Control of Varroa: Guide for beekeepers*. DOI: Not available
- Ruffinengo, S., et al. 2014. Integrated pest management for Varroa. *Zootecnia Tropical*, **32**(2), 149–168.
- Topal, E. 2020. Medicinal plants against bee diseases. *Bee Studies*, **12**(1), 5–11. <https://doi.org/10.51458/BSTD.2021.2>
- Wilson, W. T., et al. 1990. Menthol treatment and colony survival. *Bee Science*, **1**, 48–54.

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