



## REPELLENT ACTIVITY OF CITRONELLA AND CLOVE OILS AGAINST WORKERS OF THE GIANT HONEYBEE, *Apis dorsata* (HYMENOPTERA: APIDAE)

S.Q. Shoaib<sup>1\*</sup> and N. Nagaraja<sup>2</sup>

<sup>1</sup>Department of Zoology, <sup>2</sup>Malaviya Mission Teacher Training Centre, Bangalore University, Jnana Bharathi, Bengaluru – 560 056, Karnataka (India)  
, \*e-mail: syedshoaibqasim@bub.ernet.in

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### ABSTRACT

Essential oils are most frequently used as natural repellents against a variety of insect pests in contrast to the synthetic repellents. Honeybees are susceptible to most of the toxic insecticides in the environment. In the present study, the repellent activity of citronella (*Cymbopogon nardus*) and clove bud (*Syzygium aromaticum*) bud oils was assessed against worker bees of *Apis dorsata* through electroantennogram (EAG), olfactometer by exposing bees in laboratory conditions. EAG studies showed that, the bees responded to both the essential oils and the mean antennal responses of worker bees towards citronella and clove bud oils were  $0.74 \pm 0.23$  and  $0.67 \pm 0.21$  mV, respectively. Comparatively, citronella showed better antennal responses than clove bud oil. The dual choice behavioural responses of bees showed 100% repellency against citronella in contrast to 80% repellency and only 20% attraction towards the clove bud oil. The field studies showed that application of citronella on the surface of the combs gradually repelled the bees. The present study concludes that citronella is a potential essential oil in repelling the *A. dorsata* colonies.

**Keywords:** *Apis dorsata*, essential oils, citronella, clove, electroantennogram

### INTRODUCTION

The giant honeybee, *Apis dorsata* (Fabricius) is widely distributed across southern Asia from Pakistan to the Philippines, and from southern China to Indonesia (Hepburn and Radloff, 2011). These bee species most commonly build their nests on tall buildings, huge trees, rock cliffs, water tanks, metro pillars etc. *A. dorsata* is a major pollinator of a wide range of flora comprising both crop and wild plants (Nagaraja and Rajagopal, 2009, Raffiudin *et al.*, 2024). It produces a huge amount of honey and beeswax in the regions of the Asian continent including India (Woyke *et al.*, 2008, Nagaraja, 2023). Recently, the population of *A. dorsata* was gradually decreasing primarily due to traditional methods of honey harvesting (Oldroyd and Wongsiri, 2006, Nagaraja, 2022), destruction of forest habitat (Laurance, 2007) and insecticide poisoning (Oldroyd and Nanork. 2009) across the south Asia.

The toxicity of organosynthetic insecticides released into the environment severely affect the survival of non-target organisms such as honeybees (Biondi *et al.*, 2012, Decourtye *et al.*, 2013). The irreparable damage caused by these insecticides demands the use of alternative ecofriendly natural plant products in insect pest management practices (Copping and Menn, 2000, Duke *et al.*, 2010). Obviously, these natural products have a lower persistence in the environment and are considered environmentally safer than organo-synthetic insecticides (Copping and Menn, 2000, Duke *et al.*,

2010). Furthermore, natural products, such as botanical insecticides, inhibit oviposition, and enhance the feeding and hormonal systems of several arthropod pests (Copping and Menn, 2000, Isman, 2006).

Plant based repellents have no or least side effects on bees and animals (Nerio *et al.*, 2010). The repellent activity of citronella (*Cymbopogon nardus*) has been reported against mosquitoes (Ogoma *et al.*, 2014). Citronella has been conventionally used as an insect repellent (El-Helaly *et al.*, 2020) for its unique citrus scent. Its major ingredients such as geraniol, citronellal, and citronellol, are known to repel a wide range of insects including fleas, flies and mosquitoes (Da Costa *et al.*, 2020, Wu *et al.*, 2020). Furthermore, the essential oils from clove buds (*Syzygium aromaticum*) have also been found to be repellent against many insects. It contains eugenols, a substance with strong insect-repelling properties (Salsabila *et al.*, 2023). Nevertheless, considerable efforts have been made to find out suitable honey bee repellents for protection of bees and to ensure the public safety (Abramson *et al.*, 1997) by reducing the effects of harmful agrochemicals (Mayer, 1997). There is a need for development of a natural and eco-friendly products to repel *A. dorsata* to reduce their mortality due to insecticide use (Khurshed *et al.*, 2022). Obviously, natural repellents are most effective in repelling insects in contrast to synthetic chemicals (Ankit *et al.*, 2020). The present study was aimed to assess the repellent activity of citronella and clove bud oils against the workers of *A. dorsata* under both the laboratory and field conditions.

## MATERIALS AND METHODS

### *Experimental design*

The experiments on the response of *Apis dorsata* worker bees to the two selected two essential oils (citronella and clove bud) was conducted both in laboratory and field conditions at Bengaluru (India) during June-August 2023. About 15-20 days old workers of *A. dorsata* (50 bees) were collected from the comb surface of bee nests using a sweep net, kept in 30 × 30 × 30 cm ventilated cages and were fed with honey (40%) under laboratory conditions for 24 h. Citronella and clove bud oils (Grade A) were obtained from Southern Spice Products (India) Ltd., Madurai, Tamil Nadu (India). The electroantennogram (EAG) studies were conducted with an electroantennogram system (Syntech, Hilversum, The Netherlands) to assess the antennal responses of bees to these oils. The EAG system consisted of a dual electrode probe for antenna fixation, CS-05 stimulus controller, and an IDAC-232 for data acquisition. The worker bees were also subjected to olfactory behavioural responses in Y tubes to confirm the selected essential oil either as attractants or repellents under laboratory conditions.

### *Electroantennogram (EAG) studies*

The commercially available steam distilled essential oils of citronella (citronellol and geraniol-70%) and clove bud (eugenol-55%) were prepared by diluting in Dichloromethane (DCM) (Merck, India Ltd) with 1 µg µL<sup>-1</sup> (1% w/v). The solvent dichloromethane without any essential oil served as control. The acclimatized actively flying worker bees of *A. dorsata* were caught safely in a glass test tube and their antennae immobilized by the passage of CO<sub>2</sub> (1 min). Later, bee antennae were dissected below the scape with micro-scissors under a dissection microscope and mounted on the ground electrode and pedicel to the recording electrode using electrogel (Spectra 360, Parker Laboratory, New Jersey, USA) (Reinecke *et al.*, 2005). Bees were continuously flushed with a stream of activated charcoal filtered air. This setup was connected to the stimulus controller [CS 05 Syntech (mv)] by Tygon silicone tube.

A 10 µL aliquot of each essential oil was applied on a strip of Whatman No. 1 filter paper (2-4 mm diameter) and allowed to dry in a fume hood. The bee antenna was exposed to each essential oil separately by inserting a Pasteur pipette containing an oil treated filter paper. A puff of air was blown (30 sec) after loading the filter paper. After 60 sec the antennae were exposed to vapour phase of the stimulus through a pipette placed 15 mm upstream from the antennae that had a continuous air stream

(pulse time 0.5 sec, continuous flow 25 mL sec<sup>-1</sup>, pulse flow 21 mL sec<sup>-1</sup>) (Venugopal and Subaharan, 2019). Between the stimulus puffs, a time gap of 20 sec was maintained. The antennal responses towards respective essential oils were recorded through a high-impedance probe connected to an amplifier (IDAC-4, Syntech), and the signals were analysed using EAG software (standard version). The control stimulus was at the beginning, middle and end of each session. The essential oils were tested on antennae in five replications per stimulus per antennae.

#### ***Dual-choice behavioural studies***

Dual-choice studies were performed in a glass Y-tube olfactometer to determine the behavioural responses of worker bees of *A. dorsata* to essential oils as attractants or repellents. The olfactometer composed of 25 mm-diameter glass tubing with 200 mm-long central tube and two 210 mm-long lateral arms. The angle between the two lateral arms was 60°. The experiment was conducted at room temperature at 25 ± 2°C under red light as per the modified methods of Ravindran *et al.* (2019). A vacuum pressure pump (Tarson Rockyvac 300) was used to force room air through activated charcoal. Airflow through each of the olfactometer arms was kept at 500 mL min<sup>-1</sup> and air was flown into the apparatus through Teflon tubing.

The 10 µL of each essential oil was applied on a 20 × 5 mm filter paper strip and immediately placed into an arm of Y-tube. In these assays, each bee was offered a choice between 10 µL of test essential oil and 10 µL of dichloromethane (control). The bees were individually released at the base of the central arm of the Y-tube and observed for 5 min. If a bee did not make a choice after this period, it was removed and recorded as non-responding. The bees that moved into one of the terminal arms and stayed there for at least 5 sec or reached two-third the length of a lateral arm were recorded as having chosen the essential oil offered through the arm. Arms containing the treatment and control were reversed to avoid positional bias for each test. A total of 10 bees were tested for each essential oil.

#### ***Field studies***

Repellent activity of citronella was evaluated on workers of *A. dorsata* under field conditions. Five moderately populated nests of *A. dorsata* found on window ceilings were selected for the field studies. Citronella oil (100 mL) was sprayed uniformly on the surface of the comb (2500 cm<sup>2</sup> area) using a 2 L hand pump sprayer. The response of worker bees towards citronella oil were observed and recorded. Dichloromethane (DCM) was used as the control.

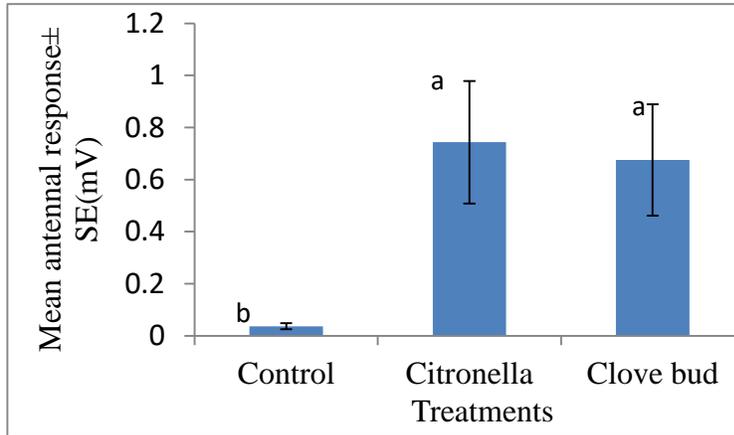
#### ***Statistical analysis***

The data obtained on responses of *A. dorsata* workers to citronella and clove bud oils were analysed by one-way analysis of variance followed by Tukey's HSD test (IBM SPSS statistical version 22), and  $p < 0.05$  was considered significant. Further, t-test was performed to analyse the bee choices towards essential oils as repellents and or attractants.

## **RESULTS AND DISCUSSION**

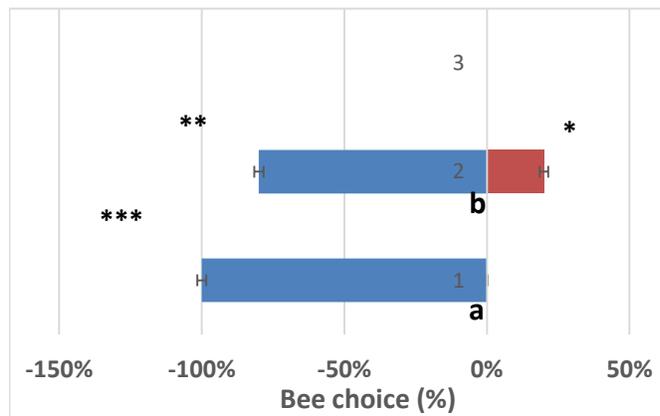
The honeybee antennae are crucial, sensory structures which play a pivotal role in their sensory ecology and behaviour. The electroantennogram technique was used to measure the average output of an insect antenna towards the brain for a known odour. The electroantennogram studies were conducted to determine the responses of *A. dorsata* worker bees towards essential oils of citronella and clove buds by stimulating their antennae. The change in baseline voltage for the bee's antenna exposed to the two different essential oils was recorded. The results showed that *A. dorsata* workers responded to both essential oils with variable rates of antennal responses. The mean responses of worker antennae to citronella and clove bud oils were  $0.74 \pm 0.23$  and  $0.67 \pm 0.21$  mV, respectively (Fig. 1), and were significantly ( $p < 0.05$ ) higher than the control.

The antennae of honeybees respond in various behavioural contexts towards many stimuli, including odorants. Nonetheless, young bees express limited antennal responses as compared to the old bees suggesting that the responses are acquired during bee's behavioural development (Chole *et al.* 2022). In present study, the worker bees showed antennal response towards both citronella and clove bud. However, greater antennal responses were recorded towards citronella than clove bud oil.



**Fig. 1: Antennal response of *Apis dorsata* workers towards essential oil. Data is mean of five replications. Different lower case letters having same letter do not differ significantly at  $p < 0.05$  according to ANOVA followed by Tukey's test**

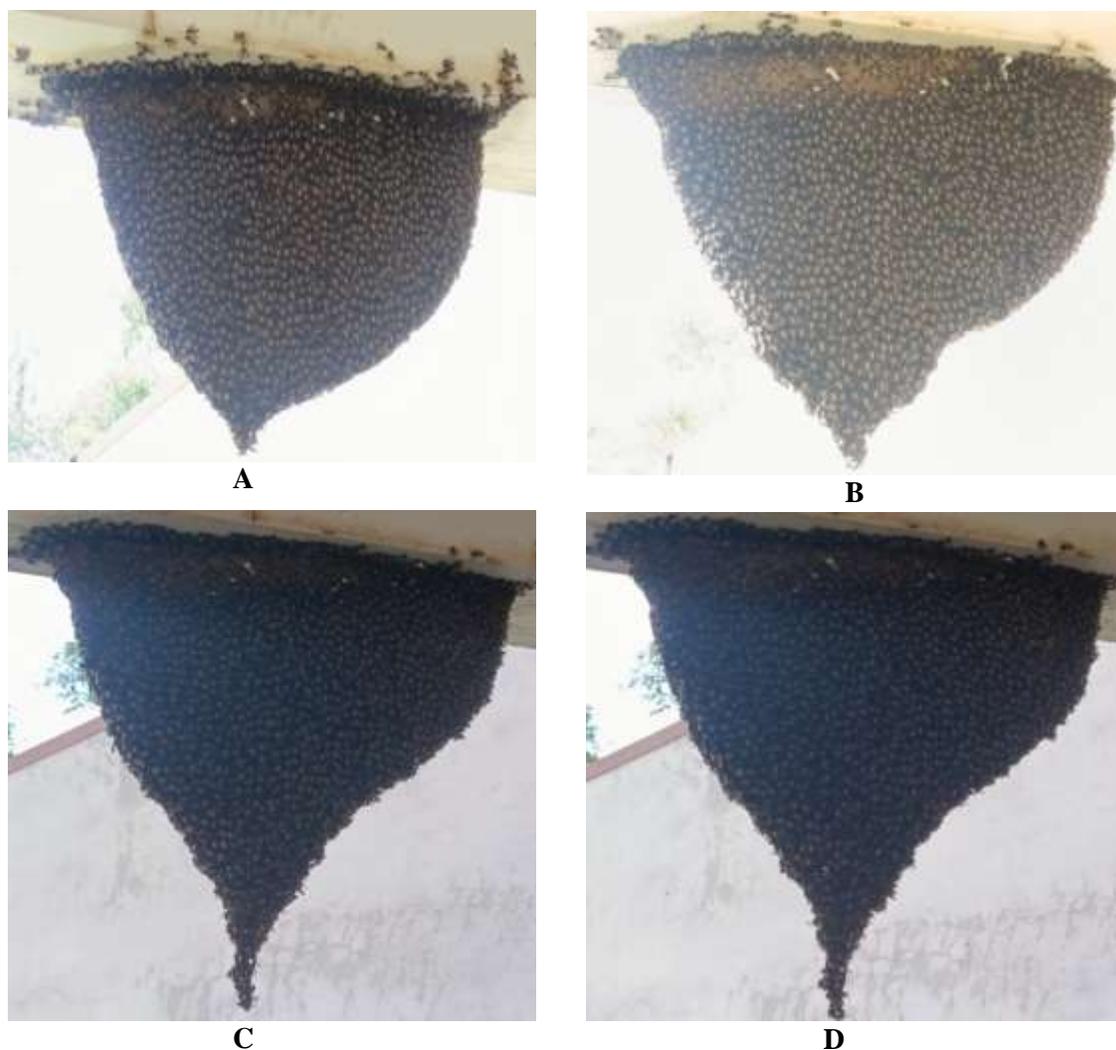
Experiments were conducted to assess the behavioural response of *A. dorsata* workers towards citronella and clove bud oils to evaluate as an attractant or repellent and or both using Y-tube olfactometer. The observation revealed that the bees showed 100% repellency towards citronella, but only 80% towards clove bud oil. Citronella showed significantly better response ( $p < 0.01$ ) than clove bud ( $p < 0.02$ ) [Fig. 2]. Studies have confirmed that, eugenol, a component of clove bud, affect insect's nervous system and behaviour by acting on its octopamine receptors (Enan, 2005).



**Fig. 2: Behavioural responses of *Apis dorsata* worker bees to essential oils in Y-tube olfactometer, A) Citronella, and b) Clove bud. The positive scale represents attractants i.e. treatment with either clove bud or citronella and negative scale represents repellents i.e. control (dichloromethane). Asterisks show a preference that is significantly different at  $p < 0.05$ . (binomial test) from a 50:50 distribution; \* =  $p < 0.05$ , \*\* =  $p < 0.02$ , \*\*\* =  $p < 0.01$ .**

The antennal response of bees may be due to their learning and memory recall abilities through sensilla on their antennae. Greater antennal response of bees towards citronella might be due to the detection of volatiles such as citronellol, citronellal and geraniol in citronella by the bees. These findings are in confirmatory with the observations of Aquino *et al.* (2004) who found repellence of bees towards citronella. Helaly *et al.* (2020) have recommended the addition of citronella in nicotinoid pesticides to keep the bees safe.

About 70-90% of clove bud oil is made up of eugenol, which is the major active ingredient. Phenolic compounds like eugenol have a wide range of biological actions, such as insecticidal, antifungal and antibacterial properties (Chaieb *et al.*, 2007). Numerous studies have demonstrated the influence of eugenol and other minor ingredients, like  $\beta$ -caryophyllene and eugenyl acetate, in repelling a variety of insect pests such as houseflies, ticks and mosquitoes (Pavela, 2015). In present study, the worker bees showed 100% repellency against citronella while clove bud oil showed both repellency (80%) and attractant (20%) properties. Dual choices of bees towards citronella and clove bud oils may be governed by their volatile components.



**Fig. 3: Effect of citronella on *A. dorsata* worker bees under field conditions; A) Colony before spraying of citronella oil; B) Colony 30 min after application of citronella oil; C) Formation of tail-like structure by bee at the base of colony after 2 days; D) Elongation of tail-like structure formed by bees after 1 week until absconding**

The effect of citronella oil on repellent activity of *A. dorsata* workers was conducted by direct application on the nest surface. The studies showed that, immediately on the application of the oil, about 25% of the adult population flew away from the nest with bemused flight activity for almost 30 min, later the swarm settled temporarily on the window ceilings more or less 1 m away from the original nesting site. However, after 1 h of application they gradually began to resettle on the original nest and settled fully in 2-3 h. Further, it was observed that the bees formed a tail-like elongated chain-like structure (30-50 cm) at the bottom of the nest (Fig. 3). The queen stopped laying eggs however the foraging bees continued their foraging activity. Finally, the colonies abandoned the nest after 10-12 days from the day of application of citronella oil. During the study period no mortality of bees was observed due to the application of essential oil citronella. The citronella oil would stimulate the queen to stop laying eggs and finally abandoning the nest after rearing the developing larvae and pupae in the colonies.

**Conclusion:** The studies showed that *A. dorsata* workers showed antennal response towards both citronella and clove bud oils. Similarly, citronella acts as repellent and clove bud oil showed dual response. Citronella is a potential repellent which can effectively be used to repel *A. dorsata* workers

in diverting these bees from human populated areas to safer zones. However, further studies are needed to evaluate the optimize the doses of essential oils in repelling these bee species.

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**Author's contribution:** S.Q. Shoaib conceptualized, designed the study and executed the experiments. S.Q. Qasim and N. Narayanappa finalized the manuscript.

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