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Studies on Antennal Response of Giant Honeybees Towards Selected Plant Essential Oils

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Abstract

Essential oils of aromatic plants possess repellent properties against various insect species. Experiments were conducted to evaluate the response of honeybee, Apis dorsata towards 13 different plant essential oils through electroantennogram studies. Surprisingly, honeybees showed three (low, moderate, and high) levels of responses to essential oils based on the change in baseline voltage. The worker honeybees exhibit low antennal response towards patchouli (0.08±0.03mV) and eucalyptus (0.20±0.04mV) oils, moderate response to basil (0.39±0.08mV), Japanese mint (0.36±0.08 mV), ajwain (0.29±0.05 mV), star anise (0.26±0.05 mV) and sweet flag (0.23±0.05 mV) and high antennal response towards palmarosa (0.58±0.13mV), cinnamon (0.49±0.06 mV), peppermint (0.48±0.06mV), geranium (0.44±0.10mV), betal (0.44±0.08mV) and ginger oils (0.41±0.06mV). The findings of the study conclude that, these essential oils may be used to repel A. dorsata colonies from urban areas to safer forest patches to overcome the problem of human-wildlife conflict.

Keywords: *Apis dorsata*; electroantennogram; essential oils; repellents

1 Introduction

Honeybees are eusocial and economically productive insects that live in well organized societies. They play a crucial role in the ecosystem by serving as major pollinators of both wild and crop plants[1]. Among nine honeybee species distributed worldwide, the giant honeybee, *Apis dorsata,* is widely distributed across the Asian continent by building large-sized, vertical nests preferably on tall trees, multi-storied buildings, rock cliffs, water tanks etc[2,3]. These bees successfully establish their colonies by gathering floral resources from both wild and crop plants in the plains of Karnataka, India. *A. dorsata* is a major honey producer and contributes about 75% of total honey production in the Indian subcontinent[4].

The presence of abundant suitable nesting structures coupled with a variety of bee flora attracts a huge number of *A. dorsata* colonies in urban regions seasonally[5]. Distressingly, these colonies are brutally killed by the application of toxic insecticides to overcome their mass attack on human beings especially in urban regions.

Furthermore, the burning of its colonies with fire in the act of traditional honey harvesting during honey flow season in the dark light was responsible for the mortality of thousands of giant honeybee colonies annually in its distribution regions[6,7]. Nonetheless, there is a need to safeguard these bees species by evaluation of eco-friendly natural chemicals to repel these bee colonies from human-habited areas.

The essential oils derived from aromatic plants have long been accepted for their repellent properties against many insect species[8] Honeybees successfully learn and discriminate hundreds of odorants through their sensory system. These volatile compounds contain bioactive molecules that repel insects through olfactory cues or direct contact[9]. The present study investigated the antennal response of *A.dorsata* worker bees toward selected plant essential oils through electroantennogram (EAG). Nevertheless, these studies may be useful in identifying suitable eco-friendly essential oils for repelling *A. dorsata* colonies to safer zones.

2 Materials and Methods

The experiment on the response of giant honeybees colonies (Figure 1) towards selected 13 commercially available plant oil essential oils from different aromatic plants was conducted in laboratory conditions. The worker bees of *A. dorsata* were collected from the comb surface of their nests using a sweep net, kept in ventilated cages (30×30×30 cm), and fed with honey (40%) for acclimatization. The essential oils (Grade A) used in the studies were obtained from Southern Spice Products (India) Ltd., Madurai, Tamil Nadu, India.

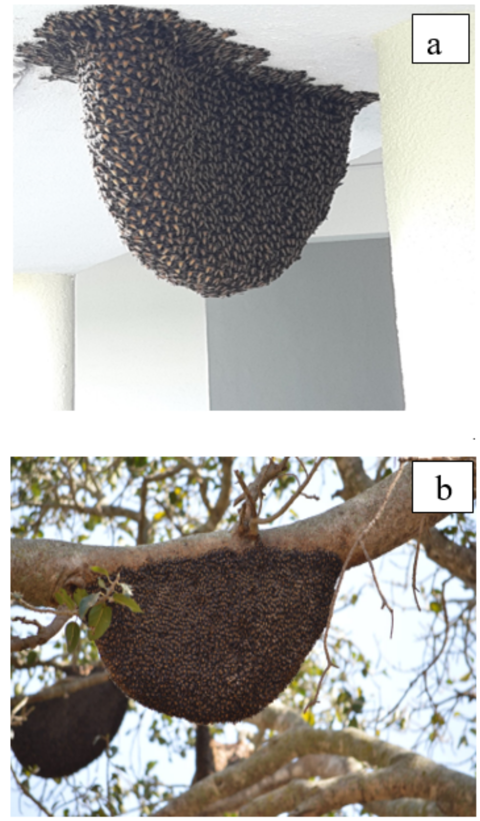

Fig. 1. Gaint honeybee *Apis dorsata* colonies on (a) building and (b) tree branch.

2.1 Electroantennogram studies

The concentrations of each essential oil (10 ml) were prepared by dissolving in dichloromethane (DCM) (Merck, India Ltd) with 1 μg/μL (1% w/v). Dichloromethane without any essential oil was used as a control. The actively flying worker bees of *A.dorsata* that were acclimatized, in the cages were caught safely in a glass test tube and immobilized their antennae by the passage of carbon dioxide (1 min). The electroantennogram (EAG) studies were conducted with an electroantennogram system (Syntech, Hilversum, The Netherlands) consisting of a dual-electrode probe for antenna fixation, a stimulus controller, and an Intelligent Data Acquisition Controller (Figure 2). The antennae of each bee were dissected below the scape with micro scissors under a dissection microscope and were mounted on the ground electrode and pedicel to the recording electrode using electrogel (Spectra 360, Parker Laboratory, NJ, USA)[10]. They were flushed with a stream of activated charcoal-filtered air continuously. This setup was connected to the stimulus controller (CS 05 Syntech (mv)) by Tygon silicone tube.

Fig. 2. Electroantennogram (EAG) system used in the experiment

A strip of Whatman No. 1 filter paper was applied with 10 μL of each essential oil separately, dried in the fume hood, and inserted into a Pasteur pipette. Further, the bee antenna was exposed to a Pasteur pipette containing an essential oil-treated filter paper. A puff of air was blown after 30 s of loading filter paper. After 60 s, the antennae were exposed to the vapor phase of the essential oil through a pipette placed 15 mm upstream from the antennae that has a continuous air stream (pulse time 0.5 seconds, continuous flow 25 ml/s, pulse flow 21 ml/s)[11]. Between the stimulus puffs, a time gap of 20 s was maintained. The antennal responses were recorded through a high-impedance probe connected to an amplifier, and the signals were analyzed using software. The control stimulus was at the beginning, middle, and end of each session. The essential oils were tested on antennae in five replications. The data obtained on responses of *A. dorsata* workers to different essential oils were analyzed by one-way ANOVA followed by Tukey's HSD test. (IBM SPSS statistical version 22) and $p < 0.05$ was considered significant.

3 Results

The electroantennogram studies are effective in measuring the average output of an insect antenna to its brain for a known odor. The electroantennogram (EAG) studies were conducted to determine the responses of *A. dorsata* worker bees towards selected 13 different essential oils by stimulating their antenna. The results showed that responses of worker bees to essential oils varied from 0.08±0.03mV (patchouli) to 0.58±0.13mV (palmarosa). The antennal responses of honeybees towards all tested essential oils were significantly ($p < 0.05$) higher than the control. Based on the change in baseline voltage and also for the interpretation of results, the response of bees exposed to different essential oils were categorized into low antennal response (0.01mV-0.20 mV), moderate antennal response (0.21mV-0.40 mV) and high antennal response (0.41mV-0.60mV).

Honeybees showed low antennal response towards patchouli (Pogostemon cablin) followed by eucalyptus (Eucalyptus teriticornis) oils and were found to be 0.08±0.03mV and (0.20±0.04mV respectively (Figure 3). Furthermore, bees showed moderate antennal response to a few essential oils ranging from 0.21mV to 0.40mV. Amongst these, the minimum response was found towards sweet flag (0.23±0.05mV) and the maximum towards basil (0.39±0.08mV). The increasing order of antennal response of essential oils from the moderate group was basil (Ocimum basilicum) > Japanese mint (Mentha arvensis) > ajwain (Trachyspermum ammi) > star anise (Illicium verum) >and sweet flag (Acorus calamus) with responses of 0.39±0.08mV, 0.36±0.08 mV, 0.29±0.05 mV, 0.26±0.05 mV, and 0.23±0.05 mV respectively (Figure 4).

The high antennal response of worker bees toward a few selected essential oils is presented in Figure 5. The results showed that the high antennal response was varied from 0.41mV to 0.60mV. The observations showed a minimum response towards ginger (0.41±0.06 mV) and a high response towards palmarosa (0.58±0.13mV). The increasing order of antennal response recorded in high response category were palmarosa (Cymbopogon martinii) (0.58±0.13mV) > cinnamon (Cinnamomum zeylanicum) (0.49±0.06 mV) > peppermint (Mentha piperita) (0.48±0.06mV) > geranium (Pelargonium cucullatum) (0.44±0.10mV) > betal (Piper betle) (0.44±0.08mV) >and ginger (Zingiber officinale) (0.41±0.06mV).

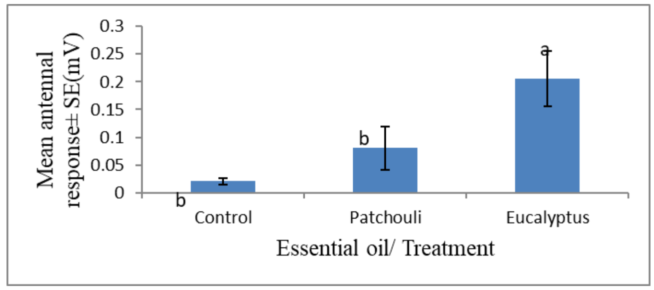

Fig. 3. Antennal response of *Apis dorsata* honey bees towards essential oils of Patchouli and Eucalyptus. Data is mean of five replications. Different lower case letters having same letter do not differ significantly at P<0.05 according to ANOVA followed by Tukey's test.

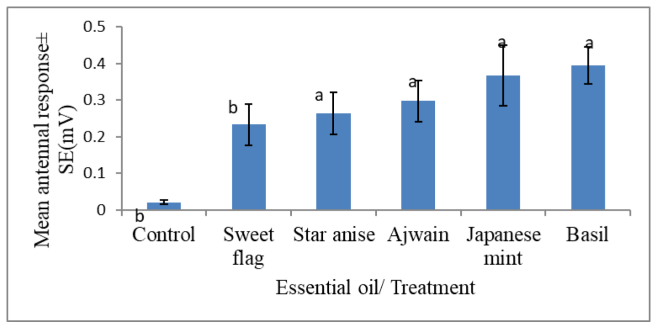

Fig. 4. Atennal response of *Apis dorsata* honeybees towards essential oils of Sweet flag, Star anise, Ajwain, Japanese mint and Basil. Data is mean of five replications. Different lower case letters having same letter do not differ significantly at P<0.05 according to ANOVA followed by Tukey's test.

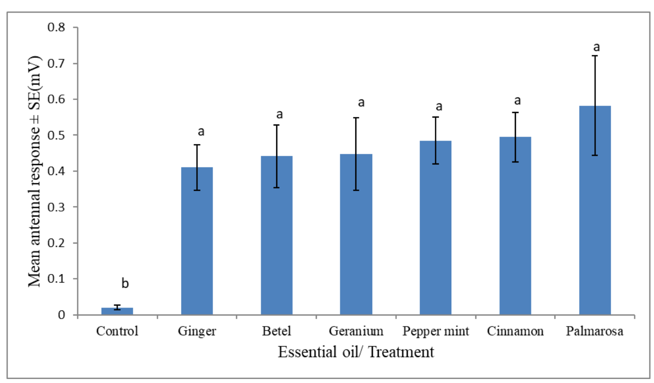

Fig. 5. Antennal response of *Apis dorsata* honeybees towards essential oils of Ginger, Betel, Geranium, Pepper mint, Cinnamon and Palmarosa. Data is mean of five replications. Different lower case letters having same letter do not differ significantly at P<0.05 according to ANOVA followed by Tukey's test.

4 Discussion

The present study investigated the antennal responses of *A.dorsata* worker bees towards 13 essential oils through EAG studies. The results showed that essential oils such as patchouli and eucalyptus elicited low antennal responses to *A. dorsata* worker bees. The low response of bees to these essential oils would be due to low concentration of eliciting bioactive compounds in these essential oils. The minimum efficacy of some essential oils is due to variation in environmental conditions [(12)], material standards [(13)] and characteristics of essential oils [(14)].

Essential oils such as basil, Japanese mint, ajwain, star anis, and sweet flag showed moderate antennal responses in *A.dorsata.* The variability in response amplitudes among these oils may reflect differences in their chemical composition [(15)]. Essential oils such as palmarosa, cinnamon, peppermint, geranium, betel, and ginger elicited high antennal responses in *A.dorsata*. The high antennal response suggests that these oils may play a significant role in repelling the bees. Our results are confirmatory with the findings of Tyagi et al. [(16)] who found 100% repellence for 12 h against Anopheles mosquitoes in a field test [(17)].

The responses of *A. dorsata* provide insights into the olfactory sensitivity of honeybees to different aromatic compounds, which have implications for understanding their behavior for the development of repellents. Such that, the essential oils with high responses may be tested in field conditions for repelling of honeybees to safer zones.

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