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Effect of sub lethal dose of insecticides on electrophysiological and behavioral response of *Bracon brevicornis* Wesmael (Braconidae : Hymenoptera) a parasitoid of coconut black headed caterpillar, *Opisina arenosella* Walker (Oecophoridae : Lepidoptera)

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ABSTRACT

Bracon brevicornis Wesmael (Braconidae : Hymenoptera) is an effective parasitoid against coconut black headed caterpillar, *Opisina arenosella* Walker (Oecophoridae : Lepidoptera) a major pest on coconut. During outbreaks, chemical insecticides and bioagents are used in tandem to manage *O. arenosella*. Traditionally the impact of pesticides on beneficial arthropods was assessed by determining acute toxicity. In addition to direct mortality caused by insecticides, the sublethal effect on sensory physiology and behavior of parasitoids must be considered for complete analysis of their impact. The present study aims to investigate the impact of sub lethal dose of insecticides on *B. brevicornis*. In olfactory assay, the starved parasitoids oriented to food source, whilst the fed parasitoids responded more to host volatiles. In electrophysiological assay, the antennal responses of *B. brevicornis* to host volatiles were higher in female. Among the volatiles tested, the host frass and host haemolymph was most preferred by both sexes. Exposure of *B. brevicornis* to sublethal dose of imidacloprid, malathion and carbaryl (LD_{20}) altered the orientation of *B. brevicornis* to host volatiles.

Key words: Behavior, *Bracon brevicornis*, EAG, insecticide, *Opisina arenosella*.

INTRODUCTION

Coconut (*Cocos nucifera* Linn.), a perennial crop is attacked by an array of pests round the year. Among the key pests, coconut black headed caterpillar (*Opisina arenosella* Walker) (Oecophoridae: Lepidoptera) is a serious one. The larval stage of the *O. arenosella* feeds on the parenchymatous tissues on the undersurface of the leaflets and constructs galleries of silk and frass. Under favorable conditions the pest multiplies rapidly and devastates the leaf lamina (Venkatesan *et al.*, 2003). In the year following severe outbreak, there is a crop loss of 45.4% in terms of nut yield. It takes around four years for the infested palms to regain their normal yield (Chandrika Mohan *et al.*, 2010).

O. arenosella is managed by spraying dichlorvos @ 0.02% (Nair *et al.*, 1998). In addition to spraying chemical pesticides, the ecto parasitoids are released to target *O. arenosella*. The success of inundative release of *Bracon brevicornis* Wesmael (Braconidae: Hymenoptera) to manage *O. arenosella* was reported by Venkatesan *et al.*, (2009). The parasitoids released in the field are exposed to insecticides through droplets or residues on crop foliage or when feeding on contaminated water droplets and nectar. There are no studies on the behavioral effects of insecticide on *B. brevicornis*. The detection of chemical stimuli and its association to adapted behavior is entirely dependent on nervous transmission (Komez *et al.*, 2001). Considering the above, the present study was taken up to study the response of *B. brevicornis* to host volatiles when exposed to sub lethal dose of insecticides. Considering the above, the present study was conducted to study the response of *B. brevicornis* to host volatiles when exposed to sub lethal dose of insecticides.

MATERIALS AND METHODS

The study was conducted at Central Plantation Crops Research Institute, Kasaragod, India (Latitude – 12^{0}3 0'N; Longitude 75^0 00'E). *Bracon brevicornis* was mass multiplied on alternate host rice moth, *Corcyra cephalonica* larvae. A pair of freshly eclosed parasitoid (male and female) was introduced in a tube (7.5 × 2.5cm) for mating. After a pre-oviposition period of 6 days, a last instar larva of *C. cephalonica* was provided in the vial. The paralyzed larva was removed and a new larva was introduced into the vial. The life cycle of the parasitoid was completed in 10-14 days. The cultures were maintained at 25 °C and 65 – 70 % RH with a constant dim light illumination.

Olfactory assay

'Y' tube made of clear glass having two arms of 10 cm length and stem of 20 cm length with an internal diameter of 3 cm was used in the assay. The odor source was placed in one arm and control in other arm. The adult parasitoids were released in the stem of the 'Y' tube and secured with muslin cloth. The airflow was passed into the arms through activated charcoal cartridge @ 30 ml / min. Constant airflow was maintained in both arms. A fluorescent bulb was placed in front of the Y arms. The parasitoids (0 -12 hrs old) fed / starved for 24 hrs were used for the assay. The choice of the parasitoids was assessed from the tracks of the images captured. The parasitoid movements and their choice of the arm was recorded by the JVC TK C 1380 CCD camera, which in turn was connected to Pinnacle video capturing card in the CPU. The data was captured at 640 × 480 resolution at the rate of 30 frames per second and stored in MPEG format. The MPEG video clips were converted into AVI format in 'true color' image type and 'indeo5' video compression. The program was implemented using the functions of Image Processing Toolbox in MATLAB® Release14. The MATLAB script was run with a graphical user interface on a Dell Optiplex GX270, 2.99 GHz, Pentium IV Hyper Threading (HT) processor, with WINDOWS XP Professional (Service Pack 2) and 512 megabyte of memory. All video footages were converted into AVI format using Adobe Premier Pro 2.0 version. Backlight was used to create maximum contrast between the insect and the substrate. Parasitoids that walked 7 cm up into one of the arms were considered to have made choice. Parasitoids not making a choice within five minutes were considered not responding. Ten adults were assessed per treatment with four replications per treatment. After testing ten individuals the tubes were cleaned and treatments were reversed to avoid position effects. The data was subjected to G test.

Electrophysiology

Electroantennogram (EAG) responses of *B. brevicornis* adults were made using a commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe for antenna fixation, a CS-55 stimulus controller and an IDAC 232 box for data acquisition. The antenna was excised at scape level and then fixed with the tip to one of the electrodes and terminal portion of the club of the antennae was fixed to the other electrode as suggested by Reinecke *et al.* (2005). The antenna was fixed between the two electrodes using Spectra 360 conductive gel (Parker, Orange, New Jersey). The antenna was flushed continuously with stream of activated charcoal filtered air.

Olfactory stimulation system

The stimulus to be tested was placed into the microtip pipettes (Tarsons 100–1000 µl). This was connected to stimulus controller by silicone rubber tube. The stimulus was puffed on to the antenna by injecting the vapor phase of the micro tip pipette 15 mm upstream from the antennae in the continuous air stream (pulse time 0.5 s, continuous flow 25 ml/s, pulse flow 21 ml/s). The minimum delay between the stimulus puff was 120 s. Antennal response to host products was recorded from ten adults with three replications per antenna.

Pesticide exposure

The adult females were exposed to dry residue of insecticides. The insecticides *viz.* malathion, imidacloprid, carbaryl, that are commonly used in coconut pest management (Rajan *et al.*, 2009) were obtained from Accustandards, USA. It was dissolved in acetone (Merck HPLC grade) and applied on the inner surface of the glass tubes. To obtain a homogenous residue layer 200 µl of the analyte was applied per tube. This volume aids to achieve full coverage of the internal surface of the glass tube. The tubes after filling with the insecticides were uniformly rotated over a flat surface so as to achieve a full coverage of the internal surface of the glass tube. They were left for 30 min for complete evaporation of acetone before introducing the parasitoid. Ten adults (3- 4 day old) per tube were exposed to insecticides. Three replicates were maintained per treatment. Two to three drops of honey were placed in each tube over a strip of wax coated paper. The neck of the tubes was secured by a muslin cloth so as to enable air circulation. The entire setup was maintained at room temperature 25^{0}C and 12: 12 h light and dark period. After 12 hrs the number of dead parasitoids was counted. For determining the regression line of mortality, four increasing doses of insecticides were used and 30 adults were exposed to each dose. The LD_{20} was estimated from the regression line and this dose was used to assess the sub lethal effect of insecticides on adult *B. brevicornis.*

Residence time on kairomone patch

The sub lethal effect of insecticides on adult female parasitoids towards host frass was determined. For this, the females exposed to pesticides at LD_{20} level were used. Those that were not exposed to pesticides served as control. Two pieces of 2 cm dia filter paper discs were placed in Petri dish. Ten mg frass (equivalent to the amount produced by one larva per day) was placed in one disc and the other was maintained as control. Individual adults were released into the Petri dish and their orientation towards the frass and the time spent on the patch was recorded using JVC

TK C 1380 CCD camera, which in turn was connected to Pinnacle video capturing card in the CPU. The data was captured in 640×480 resolution, 30 frames per second and stored in MPEG format. The time spent on the patches was calculated.

RESULTS AND DISCUSSION

Olfactory response of *B. brevicornis*

In olfactory assay the starved adults of *B. brevicornis* when provided a choice between food and host volatiles they oriented to food source (honey) (Fig. 1). In case of fed parasitoids the orientation was more to *Opisina* larval haemolymph and frass than towards food source (honey). When choice was offered between larvae and damaged leaf containing larval frass the parasitoid preferred the latter. Feeding of the parasitoids in laboratory prior to field release would aid them to search for host than wasting its energy towards foraging for food (nectar) (Fig.2). Orientation of an animal is affected by its internal condition. Host searching behavior of *Pterostichus* spp. was affected by its hunger (Wallen and Ekbom, 1994). The orientation of fed *B. brevicornis* was more towards the larval frass and haemolymph. Hexane wash of larval body and frass of *O. arenosella* elicited positive response from *G. nephantidis* and *Elasmus nephantidis* in terms of number of parasitoids entering the arm of 'Y'tube containing kairomone (Bakthavatsalam *et al.*, 1999). In case of *O. arenosella* larvae that live near their feeding site, accumulation of frass can be disadvantageous as they expose themselves to natural enemies. The damaged leaf with the larval frass is used by the parasitoids as a cue to locate its host (Reddy *et al*, 2002; Rogers and Potter, 2002). Attraction of parasitoid to host frass has been observed in *Microplitis* and *Trichogramma* (Gross *et al.*, 1975). Ghosh and Abdurahiman (1996) observed that gallery washings of *O. arenosella* served as a vital cue in host searching and oviposition behavior in *Apanteles taragamae.*

Antennal response of *B. brevicornis*

The antennal response of *B. brevicornis* to volatiles was assessed by electroantennography. The antennal response of females was higher than that of males. In case of female the antennal response was more for damaged leaf with frass (0.7 mv) whilst in male it was higher for the larval haemolymph (0.33 mv). Antennae of both sexes caused good responses to *Opisina* larval haemolymph followed by the volatiles form larval frass. Volatiles from undamaged leaf caused minimum antennal response in both male and female parasitoids (Table 1). The investigation on host finding by parasitic Hymenoptera is mostly done at behavioral level. The EAG experiments represent an alternative and convenient method to assess the overall sensitivity of insects to a range of compounds at physiologically relevant concentration under the rationale that the peripheral olfactory system has evolved sensitivity to behaviorally important odors (Their and Marion-Poll, 1998).

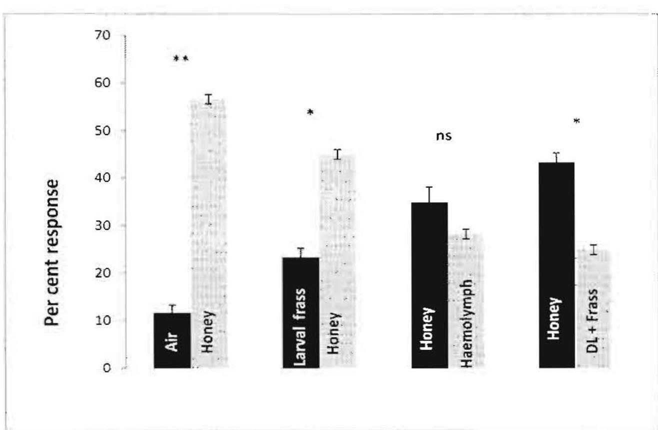

Fig. 1: Response of starved female parasitoid to odor from food source (Honey). Asterisks indicate significant difference (*p <0.05, **p< 0.01) by G test. DL – Damaged leaf Black bar represents arm-1 and grey bar represents arm- 2

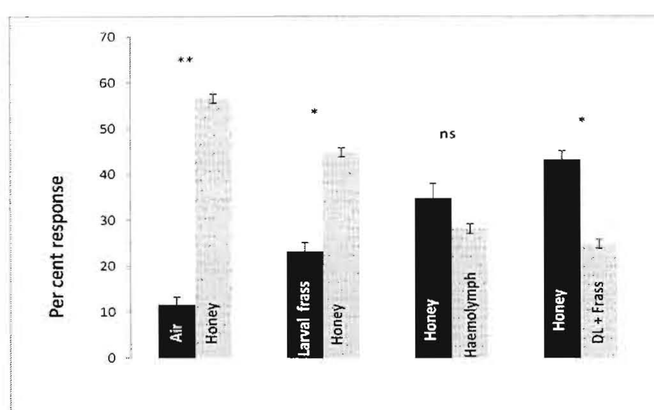

Fig. 2: Response of female parasitoid (fed) to odor from food source and host volatiles. Asterisks indicate significant difference (* p <0.05, ** p< 0.01) by G test. Black bar represents arm-1 and grey bar represents arm- 2

Table 1: EAG response of female *B. brevicornis* antennae to host associated volatiles

Stimulus	*B. brevicornis* antennal response (mV)	
	Female	Male
O.arenosella larvae	0.39 + 0.004 [d]	0.12 + 0.004 [c]
Larval haemolymph	0.67 + 0.007[b]	0.33 + 0.009 [a]
Larval frass	0.53 + 0.008 [c]	0.28 + 0.005 [b]
Damaged coconut leaf let + frass	0.7 + 0.009 [a]	0.35 + 0.006 [a]
Undamaged coconut leaf let	0.15 + 0.008 [f]	0.08 + 0.005 [d]
Mechanically damaged coconut leaf let	0.27 + 0.007 [e]	0.15 + 0.009 [c]

Means followed by same alphabet are not significantly different by DMRT.

EAG responses do not necessarily reflect the central integrations of peripheral stimuli and therefore do not indicate the resulting behavioral responses (Baehrecke *et al.*, 1989). However, there are reports of correlation of electrophysiological data with behavioral studies with minor differences (Baehrecke *et al.*, 1989; Li and Dickens, 1992; Vaughn *et al.,* 1996). In the present investigation, the electrophysiological antennal response of male and female *B. brevicornis* to host volatiles as well as to the volatile blend from the leaf material mechanically damaged/ herbivore-damaged leaf, showed a higher olfactory receptivity of females as compared to males. In contrast to our findings the similarity in electrophysiological responses between sexes of parasitic Hymenoptera has been reported (Lecomte and Pouzai, 1985; Salom *et al.*, 1992; Li and Dickens, 1992), which suggests that several of the compounds may be used together by both sexes in host habitat/or host community locations.

Table 2: Dose mortality relationship LD values

Chemical	LD_{20}	LD_{50}
Malathion	0.65	1.81
Imidacloprid	0.45	0.91
Carbaryl	0.54	1.15

LD- lethal dose

The insecticides evaluated to fix LD_{50} values had linear regression of the dose mortality relationship. The ranking of insecticides in the order of increasing toxicity to parasitoid was imidacloprid> carbaryl > malathion. The sub lethal dose (LD_{20}) of malathion, imidacloprid and carbaryl were 0.65, 0.45 and 0.54 ppm respectively (Table 2). Parasitoids when exposed to sub lethal dose of insecticides had altered orientation to the host volatiles. The parasitoids from control group had higher response to damaged leaflet and frass volatiles as compared to fresh air (G = 30.53, 1 d.f., P=< 0.000). There was no significant difference in odor choice (air vs damaged leaflets + frass) in the parasitoids exposed to imidacloprid (0.45 ppm) and carbaryl (0.54 ppm) (G = 0.11, 1 d.f., P= 0.75; G=0.09, 1 d.f., P=0.75 respectively). In either cases < 50 % parasitoids responded to the test while majority of the parasitoids remained still without moving (Table 3). The detection of chemical stimuli and its association to adapted behavior is entirely dependent on nervous transmissions. Toxic effects of many insecticides cause perturbation of nervous transmissions, and this cause interference with kairomone perception, depending on the type of insecticide and the level of exposition (Komez *et al.*, 2001).

Table 3: Effect of sublethal dose of insecticide on *B. brevicornis* orientation

Insecticide (Sublethal dose)	Per cent response of female *B. brevicornis* to arms containing		G test
	Air	Damaged coconut leaf let + Frass	
Carbaryl 0.54 ppm	14.99	13.33	ns
Imidacloprid 0.45 ppm	13.31	11.66	ns
Malathion 0.65 ppm	18.33	23.33	*
Control	13.31	58.33	**

Sublethal dose behavior relationship on patch residence time of parasitoids

The LD_{20} values of insecticides were considered as sub lethal dose for assaying the behavior of adults. Parasitoid exposed to sub lethal dose of insecticides when evaluated for their residence time in kairamone patch revealed that the control group had a residence time of over 150 sec. on the larval frass patch followed by malathion at 50 sec. Parasitoids exposed to imidacloprid and carbaryl did not spend over 10 sec in the frass patch. In majority of the cases they were immobile (Fig.3).

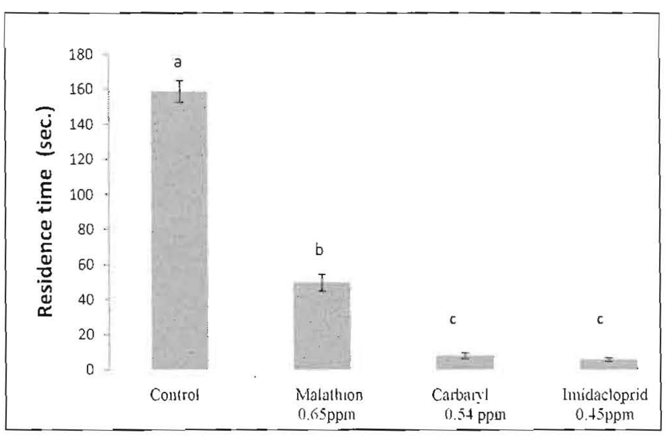

Fig. 3: Effect of sub lethal dose on patch residence by *B. brevicornis*. Means followed by same alphabet are not significantly different by DMRT.

Studies suggest that highly variable and potentially strong sub lethal effects are the general feature of the impact of synthetic organic pesticide on parasitoids. The behavior of the parasitoid exposed to insecticide was modified, but the arrestment by kairomones was still effective as seen in case of parasitoids exposed to sub lethal dose of malathion (Table 3). The modified behavior of the females exposed especially to insecticides can be explained by the mode of action of the insecticide. Chlorpyrifos interferes with the behavioral response of *L. boulardi* to kairomone of its host (Komez *et al.*, 2001)

The study on the feeding status on olfactory response of *B. brevicornis* to host volatiles clearly establishes that the parasitoids have to be fed on emergence prior to field release as this would enhance their searching ability. Though sublethal dose of insecticides of imidacloprid, malathion and carbaryl doesn't cause mortality, they cause disturbance in peripheral sensory physiology of *B. brevicornis* which alters their olfactory response to host volatiles. Information gained on sub lethal dose will help to time the safe interval for release of *B. brevicornis* in pesticide treated fields.

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