



Electrophysiological and behavioral response of *Goniozus nephantidis* Muesbeck, to plant and host volatiles

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Abstract

Experience of parasitoids with host and host related products have effects on their behavior. Learning can be induced by conditioning parasitoids at various stages i.e. pre imaginal or imaginal (after eclosion). Here we studied the volatiles from damaged, undamaged coconut leaflets and *Opisina arenosella* larval frass that cause electrophysiological and behavioural response in the bethylid parasitoid *Goniozus nephantidis*. The possibility of using the host frass for conditioning the parasitoids was explored. The damaged coconut leaflets released cyclopentanone, butanol, and alpha pinene and these compounds were absent in undamaged leaflets. Z-3 hexenol, a green leaf volatile was trapped from both damaged and undamaged plants. Alpha pinene released from undamaged leaflets caused the highest electroantennogram (EAG) peak amplitude (0.330mV) in female antennae. This was followed by cyclopentanone (0.30mV), hexanone (0.29mV) and hexanal (0.29mV) which were all at par. *G. nephantidis* reared on *Corcyra cephalonica* conditioned with the larval frass of *O. arenosella* when provided a choice preferred to parasitize *O. arenosella* as compared to *C. cephalonica*. Conditioned parasitoids when released in the field caused 59 per cent reduction in larval population per leaflet as compared to 29 per cent reduction by unconditioned parasitoids two months after release. Conditioning of *G. nephantidis* with odors of *O. arenosella* will aid to enhance the host searching ability of the parasitoids that were reared on *C. cephalonica*.

Keywords: Electrophysiology, *Goniozus nephantidis*, olfactory conditioning, *Opisina arenosella*

Introduction

Coconut black headed caterpillar, *Opisina arenosella* Walk. is a serious pest on coconut. The larvae feed on the parenchymatous tissues on the undersurface of the leaflets and construct galleries of silk and excreta (Nirula *et al.*, 1951). Under favorable conditions the pest multiplies rapidly and devastates the leaf lamina. In the year following severe outbreak there is a crop loss of 45.4 % in terms of nut yield. In addition infested palms show reduction in bunch and leaf production to the tune of 21.0 and 13.8 %, respectively. The pest-attacked palms regained their normal yield potential by the fourth year after heavy pest infestation (Chandrika Mohan *et al.*, 2010). Occurrence of this pest is severe in Kerala, Karnataka, Tamil Nadu, Andhra Pradesh and Pondicherry. Their occurrence is also reported from Orissa, West Bengal, Maharashtra and Gujarat (Venkatesan *et al.*, 2003). During outbreaks this pest is

managed by root feeding of monocrotophos @ 10 mg palm / spraying dichlorvos @ 0.02 % (Nair *et al.*, 1997). The residual toxicity coupled with the difficulties experienced in application of pesticides in target areas that are 10-12 m above the ground level caused the technology to be poorly adopted by the farming community and this prompted the farmers to rely on biological methods to manage this pest in a sustainable manner.

Among the bioagents recommended for the management of *O. arenosella*, the gregarious ectoparasitoid, *Goniozus nephantidis* Muesbeck promising one (George *et al.*, 1977). The field-collected parasitoids are mass multiplied on alternate lab host *Corcyra cephalonica* (Stainton) (Ramadevi *et al.*, 1988) and then released in the field to target *O. arenosella* which they had no previous experience in the recent past. Though an artificial diet for rearing *O. arenosella* is

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been developed (Murthy *et al.*, 2003), the cumbersome process and the cost involved make it less popular among the users. Hence, alternate host *C. cephalonica* is widely used in parasite breeding station to multiply *G. nephantidis*. Continuous mass multiplying of *G. nephantidis* on lab host may reduce its host searching ability in the field.

The behavior of natural enemies is influenced by the elements from various trophic levels. The chemical cues from the host mediate the attraction of the parasitoids towards it. In case of larval parasitoids they are attracted by perceiving the cues from target stage and in addition they use the kairamone from host products to locate the target larval stage (Vinson, 1981). Attraction towards host products is important in *O. arenosella* larvae as they are concealed in frass. The specific cues used in host location by parasitoids can be used in olfactory conditioning of parasitoids, so as to facilitate their learning to host cues even if they were reared on alternate lab hosts.

Learning / experience would help the parasitoids to use the stimuli to enhance the probability in finding their suitable host (Bleeker, 2002). Experience with host and host related products have effects on the parasitoids behavior (Turlings *et al.*, 1989; Vet *et al.*, 1995). Experience or learning will increase the parasitoids response specifically to the cues they have experienced (preference induction). Learning can be induced by olfactory conditioning of parasitoids at various stages i.e. pre imaginal or imaginal (after eclosion). In case of imaginal conditioning they use the information acquired immediately upon emergence (Kester and Barbosa, 1991). Hence, a study was taken up to characterize the electrophysiological and behavioural response of *G. nephantidis* to coconut and its host volatiles and their possible use in olfactory conditioning of the parasitoid.

Materials and Methods

The study was conducted at Central Plantation Crops Research Institute, Kasaragod, India. *G. nephantidis* culture maintained on *C. cephalonica* (Lepidoptera: Pyralidae) and *O. arenosella* were used for the study. The parasitoids were cultured by placing the adult female wasp and larvae of *C. cephalonica* (35 mg) / *O. arenosella* in a vial of 2.5 x 10 cm plugged with cotton. Drops of 50 % honey (in water) were placed on the wax coated paper and it served as food. All the cultures were maintained at 25°C and 65 - 70 % RH with a constant dim light illumination.

Olfactory assay of *G. nephantidis*

A 'Y' tube Olfactometer was used to study the orientation of adult parasitoids (Starved and fed) to food (Honey) and host products. '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 reared on *O. arenosella* 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 640x480 resolution at the rate of 30 frames per second and stored in MPEG format. The MPEG video clips were converted into AVI format in 'truecolor' 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 ANOVA and the means compared by DMRT.

Volatile trapping

The volatiles of undamaged coconut leaf, larval damaged leaf and larval frass were collected using the dynamic head space sampling technique. An air entrainment apparatus fabricated at Entomology section in CPCRI, Kasaragod was used. The matrix to be sampled was placed in a glass chamber of 30 cm long and 5 cm wide. The volatiles were collected from coconut leaves undamaged by insects, coconut leaf lets damaged by

coconut black headed caterpillar, *O. arenosella* and fresh larval frass of *O. arenosella*. Gentle stream of air was sucked through activated charcoal cartridge @ 30 ml / min. It was allowed to pass over the samples held in a glass chamber. The odor laden air was trapped in glass tube containing Poropak Q absorbent (Supelco) 30 mg (50 - 80 mesh) with glass wool on either side as stoppers. The collection was made for six hours. The trapped volatiles in the adsorbent were eluted with HPLC grade dichloromethane (400 µl) and condensed to 200 µl by passing gentle stream of ultra high pure nitrogen. Sample vials with extracts were stored in - 20^o C until analysis using GC MS.

Sample analysis

From the collections, 1 µl of the sample was analyzed on a Varian GC MS at Natural Products laboratory at Indian Institute of Chemical Technology, Hyderabad. All samples were analyzed in a fused silica capillary column. The temperature of column and oven were maintained at 50^o C for 1 min. and then increased @ 5^o C to 130^o C and held at 130^o C for 1 min and then increased @ 10^o C / min to final temperature of 220^o C and maintained at 220^o C for 10 min. The injector and column temp were 250^o C. The total run was for 37 min. The volatile constituents were identified by comparison of mass spectra with the NIST library.

Electrophysiology recordings from antennae of *G. nephantidis*

EAG responses of *G. nephantidis* adults (male and female) were made using commercially available electroantennographic system (Syntech, Hilversum, The Netherlands) consisting of a dual electrode probe for antenna fixation, a CS-05 stimulus controller and an IDAC 232 box for data acquisition. 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.

The volatile solutions (5 µl) were applied on filter paper strips of 3 cm length and 5 mm dia. and were placed into the microtip pipettes (Tarsons 100 - 1000 µl) connected to stimulus controller by silicone rubber tube. After 10 seconds the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the antenna by injecting the vapour 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 puffs was 120 s.

Chemicals tested for electroantennographic responses were selected on the basis of a bibliographic search for the compounds eliciting antennal response to hymenopteran parasitoids from various chemical groups. Chemicals were obtained from Merck (India) Ltd. and used without further purification.

Antennal response to various compounds was recorded from ten adults with three replications per antenna. For data analysis the EAG responses were corrected for solvent and other background effects by subtracting the averaged EAG responses of the solvent responses recorded before and after each sample as described previously. Hexanal was used as standard and responses to other compounds were expressed as percent of the amplitude to the standard.

Imaginal conditioning of *G. nephantidis*

In order to assess the learning in *G. nephantidis*, twenty adult females (5 days after eclosion) reared on *C. cephalonica* larvae were placed on a conditioning apparatus developed in our laboratory. The apparatus consists of two glass tubes (Borosil, length 10 cm., internal diameter 2 cm.) fitted tightly together by a polycarbonate tube (Length 3 cm, internal diameter 2.2 cm.). The contents in the glass tubes were separated by a nylon mesh with a gap (1 mm = 40 mesh) in the middle between the two mesh. There is an adjustable screw holes (2 nos.) to permit the flow of air. The parasitoids were kept in the tube on one side and *O. arenosella* along with damaged leaflets and frass were placed in the opposite side. The adult wasps were conditioned for 72 h. They were provided 50 % honey as food during the conditioning period. Response of conditioned *G. nephantidis* was assessed by choice method in Y tube as described earlier.

Efficacy of the conditioned *G. nephantidis*

A field trial was conducted in farmer's coconut garden in Kasaragod during 2006-07 to assess the efficacy of the conditioned parasitoids. Palms were West Coast Tall with age of more than 30 years. The selected coconut garden was free from pesticide application. Initial field observation and pretreatment sampling revealed more than 80 per cent of the palms were affected by *O. arenosella* as it was an outbreak in Kasaragod district during 2006-07. Majority of the pest was in late larval stage. The treatment consisted of conditioned and unconditioned parasitoids (to host odor) and a control where no parasitoids were released. For olfactory conditioning, the parasitoids were exposed to odor of larval frass and larval damaged leaves in the apparatus

mentioned earlier. The unconditioned parasitoids were reared on *C. cephalonica* and the adult parasitoids that emerged from *C. cephalonica* that did not have exposure to *O. arenosella* larvae or its frass. The dose for the release of conditioned and unconditioned parasitoids were fixed @ 20 parasitoids per palm. Four releases were made at fortnightly intervals during January to February 2006. Each treatment was imposed on 10 palms/replicate. Three replicates were maintained. The method and time of release of parasitoid was as per the technique suggested by Venkatesan *et al.* (2003). To estimate the efficacy of the conditioned and unconditioned parasitoids, pre and post release counts on the larval and pupal population was taken on ten leaflets per palm in ten palms. Observations were taken once in two months. The data was subjected to one way ANOVA and comparison of mean was carried out using DMRT.

Results and Discussion

Olfactory behavioral assay of *G. nephantidis* reared on *O. arenosella*

The olfactory response of starved and fed *G. nephantidis* was assayed in Y maze. The orientation of the parasitoid to the arm having the odor was estimated by capturing the image of the parasitoids choosing the arms.

The results indicate that the starved parasitoids were attracted more to honey, while the parasitoids that were fed, oriented towards the host or host products. In the case of fed parasitoids 63 per cent of the parasitoids oriented to the arm with *O. arenosella* hemolymph followed by 35 per cent to *O. arenosella* frass (Table 1).

Table 1. Olfactory response of *G. nephantidis* by choice assay

Treatment	% Olfactory response of <i>G. nephantidis</i>	
	Fed	Starved
Honey	19 ^c	32 ^a
<i>O. arenosella</i> larvae	30 ^c	19 ^{cd}
<i>C. cephalonica</i> larvae	26 ^d	17 ^c
<i>O. arenosella</i> frass	35 ^b	13 ^e
<i>O. arenosella</i> hemolymph	63 ^a	26 ^b

Mean followed by same alphabet do not differ significantly by DMRT

In the case of starved parasitoids they were more attracted towards food source (32 %) than the larval products. Sugars are the main energy source for adult parasitoids. Adult parasitoids having access to carbohydrate rich food have greater longevity and fecundity (Jervis *et al.*, 1996). The sugar deprivation period is a crucial point as a number of empirical studies show that it is this sugar deprivation period in parasitoids that prompts the female parasitoids to switch from host

searching to food searching (Wackers, 2001). The increased orientation of starved parasitoids to honey more than to its host indicates that the starved parasitoids released in the field would tend to spend much of their time in foraging for food rather than searching the host.

In either cases of male or female, the larval hemolymph was more attractive to the parasitoids. This was followed by larval frass in the case of fed insects. The feeding of the body fluids of the parasitoids prior to egg laying on them has been reported earlier (Subaharan *et al.*, 2007). Among the larval host, *O. arenosella* was more attractive than *C. cephalonica*. The response of the parasitoids in the behavioural assay was confirmed by electrophysiological response of the antennae to the odors.

Olfactory preference of *G. nephantidis*

The Y tube choice assay described earlier was used but with slight modifications. Unlike the earlier assay where one arm was control here the combinations were assessed. Five different stimulus combinations were tested 1) air vs larval damaged coconut leaf, 2) air vs frass 3) air vs undamaged coconut leaf 4) undamaged coconut leaf vs frass 5) damaged coconut leaf vs frass. Preference in dual choice Y tube assay were analysed with a G test against an expected ratio of 50 : 50.

In Y tube we examined the responses of female *G. nephantidis* to odors from the host products, damaged and undamaged coconut leaf lets. When given a choice between clean air and host products they preferred the arm with frass (G test 33.91) as compared to damaged leaflet followed by undamaged leaf. When presented with frass and damaged and undamaged leaflet they still exhibited more preference towards the arm having the frass odor (G test 56.25) (Fig.1). This indicates the role of kairomones in the frass substrate that aid in attracting the parasitoids. The increased response to frass warrants the need to identify the profile compounds in the frass that would attract *G. nephantidis*. Generally cues derived directly from the host are difficult to detect from a distance, as herbivores have evolved strategies to avoid detection. Instead, more easily detectable indirect cues derived from other stages of the host, like feces or from the plant it feeds on is used by the parasitoid (Vinson, 1981).

Electrophysiological response of *G. nephantidis*

The mean antennal response of hexanal (standard) was 0.3 mv. Among the compounds assayed, females responded more to hydrocarbons (dodecane, tridecane, hexadecane and heptadecane), whilst the males responded more to alcohols (Ethanol, propanol, butanol, hexanol, heptanol, octanol, nonanol and decanol).

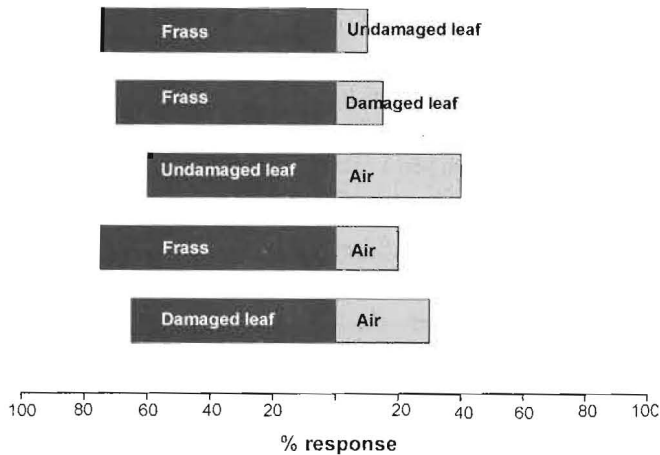


Fig.1. Response of *G. nephantidis* to odors from host products and coconut leaflets. Graphical representation on the left is the response form arm 1 and on the right is arm 2

Response to alcohols

Among the alcohols assayed the normalized EAG response was maximum to hexanol in case of females (49.6 ± 0.8). Hexanol is an herbivore induced volatile. The herbivore induced volatiles are used as cues by the parasitoids to reach their host. The antennal response to ethanol, propanol, butanol, pentanol and octanol were at par. In males ethanol (53.05 ± 0.57) caused maximum response. In either cases of male and female, the compounds with higher carbon chain length *viz.*, nonanol and decanol caused minimum response as they are relatively less volatile (Table 2).

Table 2. Antennal response of female and male *G. nephantidis* to alcohols

S.No.	Compound	Female antennae response (%)	Male antennae response (%)
1	Ethanol	42.0 ± 1.1 ^{cd}	53.05 ± 0.57 ^f
2	Propanol	44.6 ± 1.4 ^d	46.66 ± 0.88 ^c
3	Butanol	43 ± 1.1 ^{cd}	42.6 ± 1.4 ^d
4	Pentanol	42 ± 1.0 ^{cd}	42.0 ± 1.5 ^{cd}
5	Hexanol	49.6 ± 0.8 ^e	44.0 ± 0.5 ^d
6	Heptanol	41 ± 1.1 ^c	39.66 ± 0.33 ^{bc}
7	Octanol	42 ± 0.5 ^{cd}	42.66 ± 0.33 ^d
8	Nonanol	29 ± 1.0 ^a	37.3 ± 1.2 ^{ab}
9	Decanol	35.3 ± 1.2 ^b	36.66 ± 0.6 ^a

Mean followed by same alphabet do not differ significantly by DMRT

In either cases of female and male dodecane caused the lowest antennal response 0.5 ± 0.28 and 23 ± 0.57 % respectively. Maximum response was effected by tridecane (46.6 ± 0.88) in female and hexadecane (42.66 ± 1.4) in male. Hydrocarbons are commonly found on the host surface (Table 3).

Table 3. Antennal response of female and male *G. nephantidis* to hydrocarbons

S.No.	Compound	Female antennae response (%)	Male antennae response (%)
1	Dodecane	0.5 ± 0.28 ^a	23.0 ± 0.57 ^a
2	Tridecane	46.6 ± 0.8 ^d	29.33 ± 1.45 ^b
3	Pentadecane	41.66 ± 0.8 ^c	29.33 ± 0.33 ^b
4	Hexadecane	33.6 ± 0.33 ^b	42.66 ± 1.4 ^d
5	Heptadecane	36 ± 1.1 ^b	35.66 ± 0.3 ^c

Mean followed by same alphabet do not differ significantly by DMRT

Response to aldehyde and terpenoids

Among the aldehydes, propanal caused maximum response in females ($66 \pm 0.5\%$) and octanal caused highest response (53 ± 0.5 %) in males. Among the terpenoids iso eugenol caused maximum response in males ($37.3 \pm 0.5\%$) and females (36.3 ± 0.66) (Tables 4 and 5).

Table 4. Antennal response of female and male *G. nephantidis* to aldehydes

S.No.	Compound	Female antennae response (%)	Male antennae response (%)
1	Propanal	66 ± 0.5 ^d	0 ^a
2	Heptanal	37 ± 1.15 ^c	0 ^a
3	Octanal	28 ± 0.5 ^a	53.0 ± 0.5 ^d
4	Nonanal	28 ± 1.1 ^a	36.0 ± 0.5 ^c
5	Decanal	31.66 ± 0.8 ^b	33.6 ± 0.8 ^b

Mean followed by same alphabet do not differ significantly by DMRT

Table 5. Antennal response of female and male *G. nephantidis* to terpenoid

S.No.	Compound	Female antennae response (%)	Male antennae response (%)
1	Eugenol	25 ± 0.5 ^a	37.0 ± 0.5 ^b
2	Iso eugenol	36.33 ± 0.66 ^c	37.3 ± 0.6 ^b
3	Linalool	31.33 ± 0.88 ^b	29.3 ± 0.6 ^a

Mean followed by same alphabet do not differ significantly by DMRT

Antennal EAG response to host and its products

The EAG response of male antennae to volatiles of host and its product was lower than that of females. The normalized EAG response was maximum to larval hemolymph in both females (225 %) and males (170 %). The increased attraction of parasitoids to larval hemolymph as observed by olfactory behavioral assay was confirmed in electrophysiological response of the antennae. The antennal response of larval frass was higher in females (80 %) as compared to males (40 %) (Fig. 2). Both male and female antennae of *G. nephantidis* exhibited higher response to odors from *Opisina* larvae damaged leaf than the mechanically damaged leaf. This clearly establishes the role played by host odors and

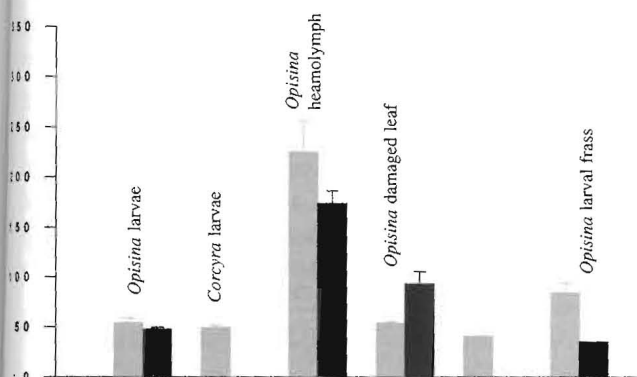


Fig. 2. EAG antennal response to host products. Grey box denotes female and red box denotes male

damaged plant cues that serve as a long range cues for parasitoids. In addition this throws us an opportunity to check if the host saliva is responsible for enhancing the herbivore induced volatiles that acts as cue to attract the parasitoids from long range.

As the behavioural and electrophysiological assay showed that female *G. nephantidis* responded more to host products (frass) followed by damaged and undamaged leaf, it was attempted to collect the volatiles from these matrices and assess their profile and establish their electrophysiological response of the female antennae to those compounds. The volatiles could not be trapped from the hemolymph due to melanization.

Volatile trapping

A total of 22 volatile compounds were released from damaged and undamaged coconut leaf. There was a qualitative difference among the volatiles released between the damaged and undamaged leaf. The larval frass released 11 volatiles. There were five volatiles compounds that were common in all the three matrix. They were 4 methyl 2 pentanol, 1,3 dichloro 2 propane, butanol, 3, 4, 6 trimethyl hexane and 1,3 diethyl benzene.

The damaged leaf released low molecular weight compounds like cyclopentanone, butanol, alpha pinene that were absent in undamaged leaf. These volatiles may be released due to herbivory, but were absent in the larval frass. The use of volatiles emitted from the damaged leaf by parasitoids to identify their host has been reported earlier. The green leaf volatile Z -3 hexanol was trapped from both damaged and undamaged leaf. Z 3 hexanol is perceived by parasitoid *Trichogramma chilonis* (Sen *et al.*, 2005). The hydrocarbons and aldehydes were absent in the larval frass.

EAG response of compounds released by undamaged, damaged coconut leaflet and larval frass

The EAG response of the female antenna of was assessed for the volatiles compounds released from the damaged, undamaged coconut leaf and frass (Fig. 3). The largest peak amplitude was obtained from alpha pinene (0.330 mv) and was significantly different from other compounds. Alpha pinene is not released from undamaged plants but they are released from damaged leaflets and frass. This was followed by cyclopentanone (0.30 mv), hexanone (0.29 mv) and hexanal (0.29 mv). All these compounds were at par in effecting the antennal response of the female parasitoid. The response of green leaf volatiles 1- hexanol (0.24 mv) and Z 3 hexanol (0.11 mv) released by damaged and undamaged leaflets. Terpenoids, linalool, citronellol and R - carvene caused minimum response.

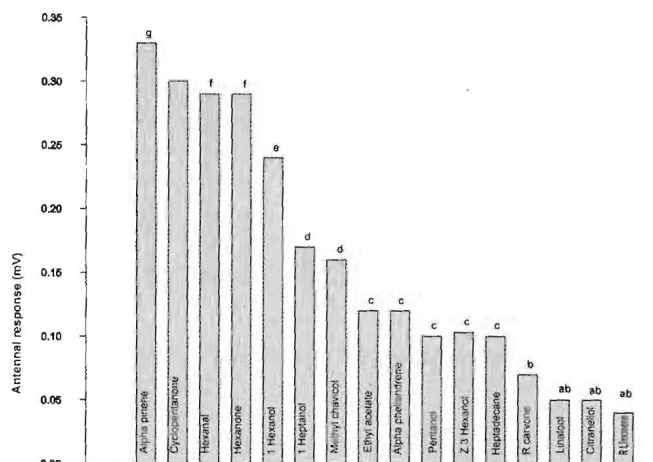


Fig. 3. EAG response to volatiles from damaged and undamaged leaf. Bars followed by same alphabet do not differ significantly by DMRT

Field efficacy of olfactory conditioned *G. nephantidis*

The results indicated that larval population in the palms receiving the treatments ranged from 3.4 to 4.2 larvae / leaflet / palm. The pretreatment observation was taken in first week of January 2006. The parasitoids were released @ 20 per palm. Post treatment observations during March 2006 revealed that the larval population decreased to 59 % in the palms on which the conditioned parasitoids were released, whilst the reduction was to a tune of 26 % in the palms where the unconditioned parasitoids were released. The control plot had the highest larval population (5 per leaflet). Observations in May revealed that there was a significant difference between the larval population in the palms where the conditioned and unconditioned parasitoids were released. Control palms recorded a highest incidence of 4.8 larvae / leaflet.

The larval population declined in the month of July (Fig. 4). The control palms had 2 larvae per leaflet. There larval population was nil in the palms where the conditioned parasitoids were released during the period under observation from September to November 2006. In case of palms in which the unconditioned parasitoids were released there were less than 1 larvae per leaflet. The control palms had more than two larvae per leaflet. Subaharan *et al.* (2005) reported that unconditioned parasitoids had a low level of innate response to *O. arenosella*, but when conditioned to odour of *O. arenosella* they had better host searching ability and it proved the learning ability of *G. nephantidis*.

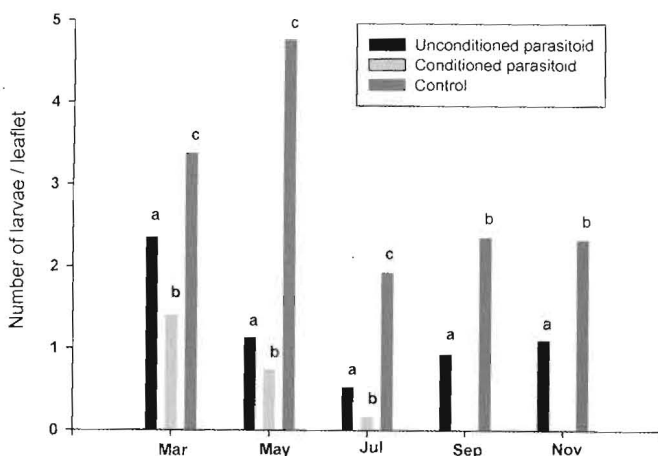


Fig. 4. Field efficacy of conditioned and unconditioned *G. nephantidis* on *O. arenosella* larvae

The pupal population in treated and untreated palms ranged from 1.4 to 1.68 pupae per leaf let per palm. During March there was no pupae in the leaflets where the conditioned parasitoids were released whilst there were 0.6 pupae / leaflet in palms where the unconditioned parasitoids were released. The pupal count was maximum (2.2 pupa/ leaflet) in untreated plots. There was general decline in all the treatments during May but it spiked (2.2 pupa / leaflet) in July in control palms. On a whole the palms where the conditioned parasitoids were released had no pupal population from July onwards and this helped to keep the pest in check.

Mere exposure to the odour cues of *O. arenosella* without any physical contact helps the parasitoid to search the host and parasitize it in the field with ease. The volatile blend emitted after herbivory is a specific indicator of host presence and are attractive to parasitoids. These cues are used in associative learning of the parasitoids which would enable it to encounter the host and there by enhance its fitness (Subaharan *et al.*, 2005). Though the conditioned *G. nephantidis* shows the ability

to learn, the duration of conditioning required for effective learning and the decay rate of the memory in *G. nephantidis* needs to be studied. Further studies using the Gas chromatograph Electroantenna detection (GC EAD) with the trapped volatiles will help to understand the qualitative and quantitative response of the *G. nephantidis* antennae to the volatiles and this would help to develop synthetic blends that can be used for conditioning the parasitoids.

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