

Behavioural responses of key parasitoids of *Opisina arenosella* Walker (Lepidoptera: Noctuidae) to the kairomones

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ABSTRACT: Hexane wash of gallery and body of *Opisina arenosella* Walker elicited positive response from the parasitoids namely *Goniozus nephantidis* (Muesebeck), *Brachymeria nephantidis* Gahan and *Elasmus nephantidis* Rohwer, in terms of more number of parasitoids entering the kairomone arm than hexane arm in 'Y' tube olfactometer. Although, numerically, the gallery wash elicited higher response than larval body wash of the host *O. arenosella* to *G. nephantidis*, statistically, the treatments were on par. Chemical analysis of kairomonal substances using GCMS revealed the presence of 13-tetradecenal and myristic acid in the gallery wash, in addition to dodecane, pentadecane, hexadecane, heptadecane, eicosane and tricosane. Larval wash showed terpenoids like alpha-terpinene and alpha humulene.

KEY WORDS: Behavioural responses, *Brachymeria nephantidis*, *Elasmus nephantidis*, *Goniozus nephantidis*, kairomones, *Opisina arenosella*

The productivity of coconut is severely affected by several species of insect pests and among them *Opisina arenosella* Walker is the major pest in coastal Karnataka and Kerala (Abraham, 1994). The effective suppression of the population of this pest through Integrated Pest Management Strategy, involves efficient

use of key parasitoids as a biocontrol component (Anonymous, 1992). Field evaluation of the larval parasitoid, *Goniozus nephantidis* (Muesebeck) (Bethyilidae); prepupal parasitoid, *Elasmus nephantidis* Rohwer (Elasmidae) and pupal parasitoid, *Brachymeria nosatoi* Habu (Chalcididae) revealed significant reduction

in the population of *Opisina arenosella* (Gubbaiah *et al.*, 1989; Kapadia and Mittal, 1994; Sathiamma *et al.*, 1996). The efficiency of these natural enemies varied with change in location, season and crop production system. To enhance the efficiency of potential parasitoids, kairomones are often recommended (Gross *et al.*, 1975; Bakthavatsalam and Singh, 1996). Ghosh and Abdurahiman (1996) observed increased behavioural responses of *Apanteles taragamae* Viereck to the galleries infested by *Opisina arenosella*. However, further studies are lacking. The present laboratory studies were aimed at identifying the kairomones which elicit optimal host seeking response in the larval, pre-pupal and pupal parasitoids of *O. arenosella* so that these infochemicals could be used to enhance the efficiency of the parasitoids in the plantations.

MATERIALS AND METHODS

The cultures of *O. arenosella*, *G. nephantidis*, *B. nephantidis*, and *E. nephantidis* available in the Project Directorate of Biological Control since 1995 were used for the present studies. The larvae of *O. arenosella* were reared on the cut coconut leaves. The culture of *G. nephantidis* was maintained on the laboratory host, *Corcyra cephalonica* Stainton, while other parasitoids were maintained on the late larva/ pupa of *O. arenosella*. The adult parasitoids were maintained on 50 per cent honey solution. The experiments were conducted in the Entomophagous Behaviour Laboratory of Project Directorate of Biological Control

during 1998-99 at room temperature (24-26 °C) and 70 per cent relative humidity. Kairomones were obtained by washing the gallery and body of larva, pre-pupa and pupa of *Opisina arenosella* in hexane as per the standard procedures described earlier (Ananthakrishnan *et al.*, 1991). The concentrations of the kairomones were based on the weight of kairomonal substance in the known quantity of the solvent.

Behavioural response to gallery and body wash

A 'Y' tube olfactometer designed earlier in this laboratory was used to find out the orientation of the adult parasitoids towards the kairomones. The 'Y' tube is made of non-absorbent, transparent, acrylic sheet (3mm thickness) having 20cm long arms and the stem with 3cm inner diameter. Known quantity of the kairomones was poured on to the cotton wigs prepared uniformly using an absorbent cotton and dried in shade to ward off the smell of the solvent. The wigs were kept in one of the arms of the 'Y' tube and the hexane (control) was kept inside the other arm. Five adult parasitoids (depending on the species) were kept on the stem portion of the 'Y' tube and covered with the muslin cloth. Two fans, one on each side of the arm, were placed and a wind flow of 1m/sec was maintained using an electronic regulator. Constant and equal airflow was maintained in both the arms. Visual observations were made on the response of the adult parasitoids to the kairomones. The number of adults entering into the respective arms was counted for 30

minutes. The arms in which kairomones were kept, were interchanged for each replication to eliminate any phototactic effect. Each treatment was replicated ten times. The data were analysed using Wilcoxon Signed Rank Test, comparing number of adults entering into the kairomone arm with that of the hexane arm (Pagano and Gauvreau, 1993). Analysis of variance was done for comparing the kairomones.

Chemical analysis of the kairomones

The kairomones were analysed using GC (Hewlett Packard Series 6890) and HP-Mass Selective Detector (Series 5973). The kairomones were analysed using the method described by Ananthakrishnan *et al.* (1991). The kairomones, were concentrated from 10ml to 0.1 ml by using Savant Vacuum Concentrator. Out of this, 1µl was injected manually into the column with temperature of 230°C. HP-5MS column having 5 per cent phenyl methyl siloxane (30m X 0.20mm X 0.25µm) was used. The oven temperature was initially set at 40°C with a ramp of 5°C per minute up to 250°C with 10 minutes post run. Each treatment was replicated five times. Standards of the compounds were obtained from M/s Sigma Aldrich and the samples were compared with the standard based on their retention time and mass spectra.

RESULTS AND DISCUSSION

Behavioural responses to gallery and body wash

The adults of all the three parasitoids namely *Goniozus nephantidis*, *Elasmus*

nephantidis and *Brachymeria nephantidis* showed marked differences in their response to kairomones (Table 1). The prepupal parasitoids *Elasmus nephantidis* showed more rapid movement towards the kairomone arm than the other two parasitoids *G. nephantidis* and *B. nephantidis* which followed a slow but steady path towards the kairomone arm. The number of parasitoids that entered the kairomone arm was significantly higher than that entered hexane arm in the case of *G. nephantidis* and *B. nephantidis* as per Wilcoxon Signed Rank Test. However, there is no statistical difference in the number of *E. nephantidis* parasitoids entering the kairomone arm, as more number of parasitoids is non-responsive. Although, numerically the gallery wash (in cases *G. nephantidis*) elicited better response than the body wash of the pest, statistically the treatments were on par (Table 2). In case of *B. nephantidis* numerically pupal wash responded better over gallery wash however, statistically both the treatments were on par.

Gallery wash contains the frass material and faecal matter of the larvae. Frass material was found to be an attractant to species like *Microplitis croceipes* (Cresson) and *Trichogramma* spp. (Gross *et al.*, 1975; Alborn *et al.*, 1995), *Cotesia marginiventris* (Cresson) (Loke and Ashley, 1984; Dmoch *et al.*, 1985), *Eupelmus vuilleti* Crawford (Cortesero *et al.*, 1993), *Macrocentrus linearis* Ness (Din *et al.*, 1986). An allied species of *Goniozus nephantidis*, *Goniozus indicus* Ashmead responded to the volatiles from the frass of larvae of *Eldana saccharina*

Table 1. Behavioural response of parasitoids of *O. arenosella* in 'Y tube olfactometer' to the kairomones

| Species | Treatment | Mean no. of adults in | | | Statistical significance |
|-----------------------|----------------|-----------------------|--------|------|--------------------------|
| | | Kairomone | Hexane | Base | |
| <i>G. nephantidis</i> | Gallery wash | 3.1 | 1.7 | 0.2 | * |
| | Larval wash | 2.8 | 0.9 | 1.3 | * |
| <i>B. nephantidis</i> | Gallery wash | 3.0 | 1.2 | 0.8 | * |
| | Pupal wash | 3.2 | 0.9 | 0.9 | * |
| <i>E. nephantidis</i> | Gallery wash | 14 | 5.5 | 5.5 | N. S. |
| | Pre-pupal wash | 14 | 3.3 | 7.7 | N. S. |

* Significantly different at 5% level under Wilcoxon Signed Ranked Test

N. S. = non significant

Walker (Smith *et al.*, 1994). Ghosh and Abdurahiman (1996) also found that chemical substances from the gallery washings of *Opisina arenosella* served as

prime factor in host searching and oviposition behaviour of another parasitoid, *Apanteles taragamae* Viereck.

Table 2. Comparative response of *G. nephantidis* and *B. nephantidis* adults to the kairomones

| Species | Kairomone | Per cent adults response |
|-----------------------|--------------|--------------------------|
| <i>G. nephantidis</i> | Gallery wash | 62.0 (53.65)* |
| | Larval wash | 55.0 (49.38) |
| <i>B. nephantidis</i> | Gallery wash | 53.0 (46.72) |
| | Pupal wash | 63.0 (55.61) |

*Figures in parentheses are arcsine values.

Between treatments: N. S.; Between species: N. S.

Larval cuticle and frass elicited strong host searching behaviour and oviposition probing by *Macrocentrus linearis* (Din *et al.*, 1986). Cuticle of host pupae also acted as kairomone for *Brachymeria lasus* Walker (Lee *et al.*, 1989). Pupal wash was also found to evoke response in *Brachymeria intermedia* Nees (Tucker and Leonard, 1977). The pre-pupal integument chemicals diffuse from inside the cocoon to inform the parasitoids about the suitability of the host in case of eulophid, *Dahlbominus fuscipennis* (Rostas *et al.*, 1998).

Kainoh and Tatsuki (1988) are of the opinion that both external and internal kairomones are needed to elicit oviposition response in *Ascogaster reticulatus* Watanabe, the external factor for host location and the internal for oviposition. Kairomones from gallery wash may be used for host location and kairomones from host cuticle may be used for oviposition.

Chemical analysis of the kairomones

Dodecane, pentadecane, hexadecane, heptadecane, eicosane and tricosane are the common compounds present in gallery, larval, pre-pupal and pupal washes of

Opisina arenosella, though their abundance vary in each of the sources (Table 3). 13-tetradecanal and myristic acid are the compounds that are present only in the gallery wash while heneicosane, tridecane and N-docosane are absent (Table 3). Tetradecenal, the pheromone component may have been derived from the ovipositing host insects. Either presence or absence of some compound may induce more attraction to certain kairomonal substances as observed in the present study. Larval wash has additional compounds like alpha terpinene and alpha humulene, the plant derived compounds which may be expressed due to the feeding habits of the larva and not present in the other non feeding stages (Table 3). Plant related volatiles in host frass and larvae appeared to play a major role in successful location of host larvae (Rose *et al.*, 1997). Hexadecane is the major compound in all the extracts. Tetracosane is present in the pupal wash only, while the composition of pentadecane is very high. This may be an important compound for preference of pupae by *Brachymeria nephtantis*. This is the first attempt to identify kairomonal substances from gallery, larval, pre-pupal and pupal wash of *O. arenosella*.

Table 3. Kairomonal substances identified from body and gallery wash of *O. arenosella*

| Compound | Gallery wash (% of total) | Body Wash (% of total) | | |
|---------------------|------------------------------|------------------------|----------|-------|
| | | Larval | Prepupal | Pupal |
| Hexen-1-ol | - | 0.49 | 0.18 | - |
| Vinyl isothiocynate | 1.57 | - | 0.49 | - |
| Alpha-terpinene | - | 0.53 | - | - |
| Para-cymene | - | 0.15 | 0.24 | 0.31 |

| Compound | Gallery wash (% of total) | Body Wash (% of total) | | |
|--------------------|------------------------------|------------------------|----------|-------|
| | | Larval | Prepupal | Pupal |
| Limonene | 0.67 | 0.36 | 0.66 | - |
| Undecane | - | 0.35 | - | - |
| Dodecane | 0.08 | 1.17 | 0.24 | 0.31 |
| Tridecane | - | 1.19 | 2.79 | 0.96 |
| Beta-caryophyllene | 0.27 | - | 0.92 | - |
| Tetradecane | 0.27 | - | 0.50 | 3.92 |
| Nonane | - | 0.07 | - | - |
| Alpha-humulene | - | 0.42 | - | - |
| Pentadecane | 2.48 | 8.99 | 3.31 | 14.09 |
| Hexadecane | 13.49 | 17.40 | 8.93 | 14.86 |
| Heptadecane | 3.71 | 6.85 | 1.94 | 6.63 |
| Octadecane | 0.40 | 3.39 | 1.46 | 6.11 |
| 13-tetradecanal | 6.83 | - | - | - |
| Nonadecane | 0.59 | 2.38 | 0.97 | 4.92 |
| Myristic acid | 9.44 | - | - | - |
| Eicosane | 1.89 | 1.92 | 0.79 | 3.89 |
| Heneicosane | - | 1.37 | 0.43 | 2.54 |
| n-Docosane | - | 1.04 | 0.36 | 1.82 |
| Tricosane | 0.31 | 0.73 | 0.42 | 1.09 |
| Hexacosane | - | 3.11 | 0.61 | - |
| Tetracosane | - | - | - | 1.48 |
| Pentacosane | 0.56 | 0.57 | - | - |

However, the response of the parasitoids to the individual compounds of the kairomone and the combination of the compounds needs further investigation to

develop synthetic kairomonal formulation. The formulations based on the gallery wash could also be used to increase the parasitoid efficiency in the field conditions.

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