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Semiochemicals from the aggregation site of home invading nuisance pest, *Luprops tristis* (Coleoptera: Tenebrionidae)

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ABSTRACT: *Luprops tristis* (Mupli beetle) is noted for the magnitude of nuisance caused by home invasion in millions prior to monsoon rains and subsequent dormancy inside residential buildings in rubber plantation belts of Kerala state of India, for the last three decades. Return of the new generation beetles into the same shelters used by parent generation strongly suggests the involvement of semiochemicals based at aggregation sites and identification of such semiochemicals may offer alternative management strategies for this nuisance species. In the present study, air borne volatiles from the aggregation site during different phases of dormancy were collected, identified, bioassayed for behavioural response and possible role of identified volatile compounds in the selection of aggregation sites and maintenance of aggregation has been discussed.

KEYWORDS: Mupli beetle, *Luprops tristis*, home invasion, aggregation site, semiochemicals

INTRODUCTION

Litter dwelling detritivore beetle *Luprops tristis* (Fabricius) (Tenebrionidae: Lagriinae: Lupropini), is a serious home invading nuisance pest in the rubber plantation belts of Kerala state of India, for the last three decades. Their massive seasonal invasion in to residential buildings prior to monsoon rains and prolonged presence inside the houses for eight months (Sabu *et al*., 2008) makes them the most dreaded nuisance pest of the region. *L. tristis* breeds and feeds in rubber litter, thick litter stands present in rubber plantations during pre-summer and summer period are the ideal breeding and feeding ground and its biology is synchronised

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with the phenology of annual leaf shedding and leaf sprouting of rubber trees (Sabu and Vinod, 2009a; b). Presence of thousands of hectares of rubber plantations along the western slopes of the South Western Ghats in Kerala, with an astonishing population of *L. tristis* concealed in lower litter layers makes conventional insecticidal chemical based control methods practically impossible.

Since home invasion of the beetle takes place during rainy season, affected people are left with little choice but to kill the home invaded beetles by indoor application of various insecticides. Despite three decades of their wide spread presence in the region, no efficient strategies for controlling the population build up of *L. tristis* have been developed and there is a need to develop environmentally benign control tactics (Aswathi and Sabu, 2011). All across the region, the new generation beetles have been selecting the same sites for aggregation even after the treatment of chemical insecticides in shelters (Sabu *et al*., 2008). It lead to the proposal that the semiochemicals released by the aggregated beetles could be involved in the attraction of new generation beetles to the shelters used by the previous generation, and identification of the semiochemicals prevailing in the aggregation sites may enable development of a pheromone-mediated management programme to control the menace.

MATERIALS AND METHODS

Home invasion of *L. tristis* occurs by April/ May period and the aggregation and dormancy last for eight months. Dormancy phase has been divided into four phases namely initial, mid, last and post-dormancy and the semiochemicals present in the aggregation site were collected during each phase.

Collection of volatile compounds from aggregation sites

Air-borne volatiles were collected using activated charcoal as adsorbent from a rock cave close to a rubber plantation at Kattipara, in Kozhikode district of Kerala state during 2009-2010 period. Selected rock cave is an aggregation site for the beetles for the past 15 years. Adsorbent material was purified following Millar and Sims (1998) and placed in a circular earthen vessel at the site of aggregation. Adsorbent material was retrieved after 72 hours, eluted in n-Hexane (HPLC grade, Fischer scientific), the extract was filtered through Glass-Fibre filter paper (Whatman), evaporated in to 2 ml under room temperature and stored in a freezer at -20º C.

GC-MS Analysis and Identification of volatile compounds

Compounds in the Hexane extract were analyzed with a gas chromatograph (GC) coupled to a mass spectrometer (MS) (Hewlett Packard 5890 series II and Hewlett Packard 5971 series-Mass selective detector) and fitted with a silica capillary column (Agilent, model HP5-MS). Data was acquired under the following GC conditions: Injection: 1µl; running time: 45 minutes; inlet temperature: 250°C, carrier gas: helium at 51 $cm.s^{-1}$, split ratio 13:1, transfer-line Temperature:

280°C, initial temperature: 40°C, initial time: 2 min, rate: 10°C.min^{-1}, final temperature: 260°C. Component identification was made on the basis of mass spectral fragmentation pattern, retention time and comparison with authentic constituent's mass spectral and retention time that matches with Wiley library.

Bioassay for behavioural response

An olfactometer made up of glass with a central chamber and two side chambers connected with glass tubes of length 12 cm and internal diameter1.75cm was employed. A power running aerator system with a regulator was attached to the olfactometer and the air speed in the system was adjusted to 2.5 L min^{-1}, and it was previously humidified and filtered on active charcoal. Commercially available standards for the identified potential compounds were purchased (Alfa Aesar) and n-hexane was used as solvent. Tests, each lasting 15 minutes, were repeated for 5 times using pre-dormancy males and females separately. Chemical standards of identified compounds were placed at the end of one of the arms, using glass fiber filter paper (2x2 cm) impregnated with 5.0 µL of the solution, while the same volume of hexane was used as a control at the end of the other arm. Only insects that reached the arms of the olfactometer and remained near the odour source were considered. Data obtained with insects that reached control and test odour sources were normally distributed and parametric statistics were used for comparison of the data. Variations in number of beetles reaching test chamber and control chamber in each experiment were analysed with one-way ANOVA test. For all analyses, significance was determined at $P<0.05$. Minitab Statistical software version 16 was used for all statistical analysis.

RESULTS

GC-MS profile of the compounds identified from the aggregation sites of *L.tristis* during various phases of aggregation and following post dormancy departure were provided (Fig 1.). Altogether 12 compounds with known semiochemical function in insects were recorded with eight compounds during various phases of dormancy and six compounds after post dormancy return of beetles (Table 1.)

Seven volatile compounds namely, Hexadecane, Pentadecane, Tetradecane, Heptadecane, Decane, Dotriacontane and Hexacosane were identified during the initial phase of dormancy, one volatile fraction representing Pentatriacontane during the mid phase and 6 volatile fractions representing Dotriacontane, 1-Tridecene, 1-Heptadecene, Tridecane, 1-Pentadecene and Hexadecane were recorded after the post dormancy return of the beetles to the rubber plantation litter. No volatile fractions representing compounds with previous report of semiochemical function in insects were recorded during the last phase of dormancy.

Behavioral responses were tested using olfactometer for Hexadecane and Dotriacontane, the major volatile fractions during the early and the post dormancy phase. Both the sexes did not show any behavioural response towards Hexadecane (Test= 2.50 ± 0.97, Control= 2.20 ± 1.48,

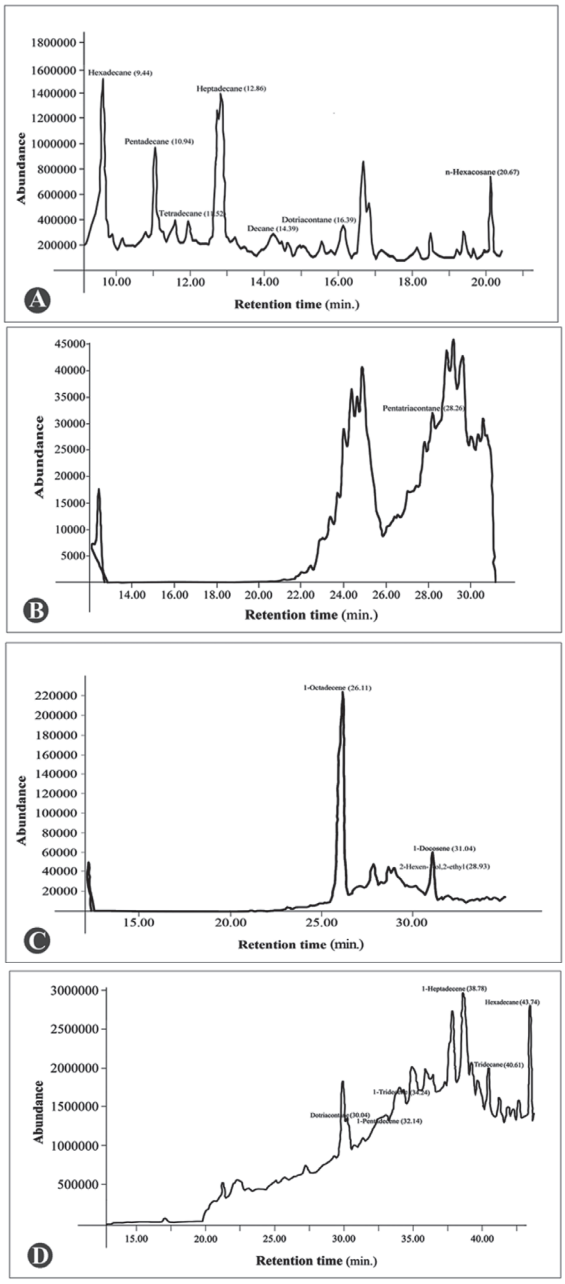

Fig. 1: GC MS profile of volatile samples collected from aggregation site of *Luprops tristis* during different phase of aggregation and dormancy. A-Initial phase,B-midphase, C-final phase and D-after dormancy.

Table 1. List of potential pheromone components identified from the aggregation site of *L. tristis* during different phases of aggregation with supporting literature (1-Initial phase; 2- Mid phase; 3- Last phase and 4 - Post-dormancy.

Sl. No.	Compound	CAS	Function
1.	Hexadecane [1,4]	544-76-3	Aggregation pheromone component Tenebrionidae (Keville and Kannowski, 1975)
2.	1- Heptadecene [4]	6765-39-5	Aggregation pheromone component Tenebrionidae (Keville and Kannowski, 1975)
3.	1-Pentadecene[4]	13360-61-7	Aggregation pheromone component Tenebrionidae (Arnaud *et al*., 2002)
4.	Dotriacontane [1,4]	544-85-4	Cuticular hydrocarbonTenebrionidae (Lockey, 1982; Lockey, 1984)
5.	Hexacosane [1]	630-01-3	Cuticular hydrocarbonTenebrionidae (Lockey, 1982; Lockey, 1984)
6.	1-Tridecene [4]	2437-56-1	Male sex pheromoneTenebrionidae (Geiselhardt *et al.*, 2008)
7.	Pentadecane [1]	629-62-9	Allomone component (Defence substance) Carabidae (Evans, 1988)
8.	Tetradecane [1]	629-59-4	Allomone component(Defence substance) Carabidae (Eisner *et al.*, 1977)
9.	Heptadecane [1]	629-78-7	Allomone component (Defence substance) Carabidae (Eisner *et al.*, 1977; Evans, 1988; Attygalle *et al.*, 1992)
10.	Decane[1]	124-18-5	Allomone component (Defence substance) Carabidae (Evans, 1988)
11.	Tridecane[4]	629-50-5	Allomone component (Defence substance) Carabidae (Eisner *et al.*, 1977)
12.	Pentatriacontane [2]	630-07-9	Cuticular Hydrocarbon Psyllidae (Guédot *et al.*, 2009)

p>0.05 for males and Test= 2.40 ± 1.18, Control= 2.40 ± 0.97, p>0.05 for females) and Dotriacontane (Test= 1.5 ± 1.08, Control= 1.7 ± 1.70, p>0.05 for males and Test= 2.0 ± 1.15, Control= 1.80 ± 1.32, p>0.05 for females).

DISCUSSION

Study revealed the presence of 10 compounds with aggregation property and defensive functions and a distinct seasonality in the occurrence of compounds with 7 volatile fractions in the initial phase, 6 after the post dormancy return of beetles, one in the midphase and no fractions in last phase of dormancy. Out of the 10 major compounds identified, no compound was present during all the four phases of dormancy indicating variations in the semiochemicals during different phases of dormancy. Compounds present during initial and post dormancy phase were mainly aggregation pheromone components namely, Hexadecane, 1-Heptadecene (Keville and Kannowski, 1975), 1-Pentadecene (Arnaud *et al*., 2002), 1-Tridecene (Geiselhardt *et al*., 2008) and cuticular hydrocarbons, Dotriacontane and Hexacosane (Lockey, 1982; Lockey, 1984). Functions of the two cuticular hydrocarbons present at initial phase and one (Dotriacontane) at post dormancy phase is unknown. Since cuticular hydrocarbons associated with more volatile compounds function as a pheromone in many aspects of social life (Walter *et al*., 1993), as a spacing pheromone (Howard and Blomquist, 1982), and as an aggregation pheromone (Rivault et al., 1998), it is highly likely that these two cuticular hydrocarbons, (Dotriacontane and Hexacosane) also play a major role in the aggregation behaviour of L.tristis. Pentadecane (Evans, 1988), Tetradecane (Eisner *et al*., 1977), Decane (Attygalle *et al*., 1992) and Heptadecane (Evans, 1988), which are reported as allomone components with defensive function in Carabids would be released to repel the predators that might prey upon the aggregated beetles. Compounds with aggregation property, defensive function and spacing property were present in the initial phase indicating that these are released to ensure maintenance of aggregation in the site. It is likely that the compounds with aggregation property (Hexadecane) present during the initial and post dormancy phase contributes towards attraction of beetles to the sites and maintenance of the aggregation in the shelter which remained unoccupied by beetles for 3-4 months and cuticular hydrocarbons in spacing out the beetles in the aggregation site.

Pentatriacontane, the only compound identified during the mid phase of dormancy was reported earlier as cuticular hydrocarbon in Psyllidae (Guédot *et al*., 2009) and no major compounds were recorded during the mid and last phase of aggregation and dormancy. Lack of compounds with aggregation and defensive property during mid phase and towards the end of dormancy suggests that after the establishment of aggregation and dormancy, such compounds are not released after initial phase. Presence of similar compounds again after the post dormancy retarding phase shows that release of such compounds occur before the departure of post-dormancy beetles from the aggregation shelters and such compounds left behind may be the cues for the next generation beetles to locate the shelters which have been used by their parent generation. Presence of Dotriacontane and Hexadecane as major fractions in the early phase and after the post dormancy retarding phase indicates that these two compounds have

play a major role in maintaining the aggregation and recognising the aggregation sites by the new generation beetles. However, absence of behavioural response towards the two major fractions, Dotriacontane and Hexadecane indicates that L.tristis aggregations are not solely managed by individual volatile chemicals like aggregation pheromones as reported in many other tenebrionids, but it involves interaction or synergistic effects of more than one compound which may or may not be previously reported as insect semiochemicals.

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