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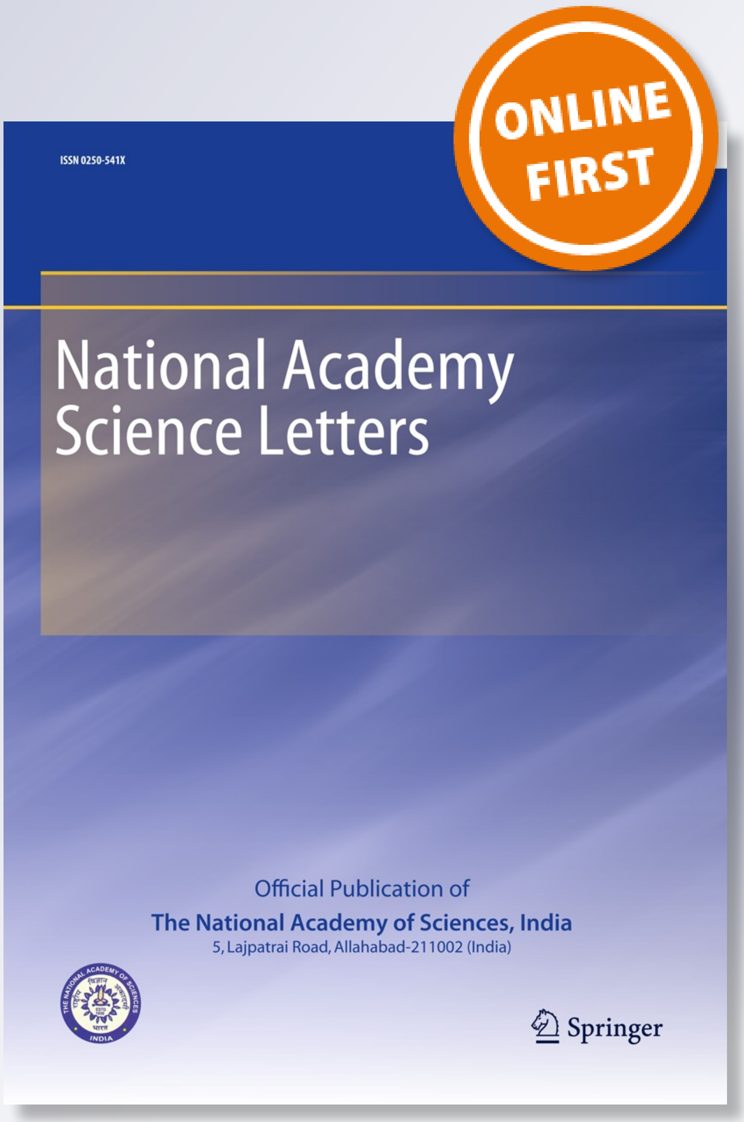

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RESEARCH ARTICLE

Response of *Exorista sorbillans* to Volatiles of Host Plants of *Antheraea assama*, Westwood

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Abstract Plants release green leaf volatiles as constitutive or induced defense and that parasitoids might use these chemicals as cues for searching their herbivorous host has become a subject of intense research in the recent years. In addition to its contribution to enhance knowledge regarding tritrophic interactions, identification of the chemical cues are likely to give leads in development of chemical traps for management of parasitoids. *Antheraea assama*, the producer of golden yellow muga silk is parasitized by a dipteran fly, *Exorista sorbillans* and the seed broods of the silk worm suffer a great loss due to the parasitization. This work was carried out to study the response of the endoparasitoid *E. sorbillans* (Diptera: Tachinidae) to fed sualo (*Litsea polyantha*) leaves and certain pure green leaf volatile compounds. The study was done by subjecting adult female flies to different odour sources using olfactometer. Parameters considered were latency, rest, walking, grooming and proboscis extension period. Sualo leaves fed for different period of time, C6 aldehydes, monoterpene hydrocarbons, oxygenated monoterpenes and certain sesquiterpenes enhanced fly activity significantly. Certain compounds showed increased activity of the flies with increase in concentration of the compounds. The potential candidate compounds for *E. sorbillans* management are highlighted.

Keywords *Exorista sorbillans* · Green leaf volatiles · Monoterpenes

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Introduction

Insects use peripheral sense organs in detecting cues derived from the environment to localize the host arena. Majority of studies regarding mechanism of host location in parasitoid insects have focused on Hymenopterans [1, 2] and to a limited extent on Dipterans [3, 4]. In both the groups of parasitoids olfactory mechanism has received greater attention which probably is a stronger contributor in decision making with respect to host finding. They perceive odours emanated from hosts which help them to localize, recognize and accept the specific host either for food or for oviposition. These odorous chemicals are systemically released in very large amount in response to herbivory [5]. That these volatile chemicals emitted by host plant due to herbivory are used by the third trophic level was first shown in predatory mite [6]. Since then in a number of systems the phenomenon has been shown. Natural enemies probably get adapted to detect and use those herbivore induced plant volatiles as cues during foraging [7, 5].

Tachinid dipterans are diverse. The *Exorista* spp. is a tachinid that parasitizes silk worms and many other lepidopterans [8]. *Exorista sorbillans* is a major parasitoid of *Antheraea assama*, the producer of the golden yellow muga silk and is reported not only from India but also from Bangladesh, Pakistan, Japan, China, South Korea, Thailand and Vietnam. Although certain works have been carried out regarding cues involved in host location and selection by the generalist parasitoid *Exorista mella*, the mechanism involved in silk worm parasitization by *Exorista* spp. is poorly understood. Knowledge regarding the sources of volatile chemical cues perceived by the flies and their behavioural response to the cues will help in understanding the mechanism of host location for parasitization. The objective of the present study was to assess the probable

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olfactory cues which might be used by *E. sorbillans* for parasitization of *A. assama*.

Materials and Methods

Insect

Maggots of *E. sorbillans* were collected from sericulture farmers of Upper Assam (Jorhat, Sivsagar, Dibrugarh and Tinsukia) and were maintained in laboratory condition 22–300 °C). The newly emerged adult Uzi flies were kept in wooden boxes covered with net (32 × 41 × 26 cm). Each terrarium was provided with separate petri dishes containing cotton soaked with water and sugar cubes sprinkled with yeast-bacto powder. Males and females were kept together in a common chamber to ensure mating. Just after mating the females were separated and kept separately in different chambers for experiments.

Odour Source

(i) 100 fifth instar larvae of *A. assama* were released in a twig of 3 years old sualo plant. Leaves from the fed twig were collected after feeding for 6, 24, 72, and 96 h and were used as odour source for studying the response of the Uzi flies to fed leaves.

(ii) Fifteen standard volatile compounds were used as odour source at 1, 10, 50, and 100 ppm concentration. The compounds were purchased from Sigma-Aldrich (Table 1) and the concentrations were prepared in dichloromethane.

Behaviour Assay

For studying response of the mated female the experiment was conducted in an all glass olfactometer consisting of two chambers connected through an arm, one for keeping the odour source (odour source chamber) and the other (odour receiving chamber) for keeping the fly (Fig. 1). Humidified, charcoal filtered air was drawn through the olfactometer by a pump connected to a flowmeter. In the experiment for studying behavioural response to fed sualo leaves, the leaves were kept in odour source chamber and the fly was introduced to the odour receiving chamber. In the experiment with standard compounds Whatmann filter paper was impregnated with 1 ml of test solution and was introduced into the odour source chamber. Water soaked filter paper was kept in the odour receiving chamber into which the experimental insect was introduced. Air was allowed to flow at 25 ml/s over the odour source to be received by the experimental fly. For control odour, air was passed through odour free chamber in case of experiment using sualo leaves as odour source. For control odour in case of standard compounds as odour source, a filter paper soaked with 1 ml solvent was kept in odour chamber over which air was flown. Behavioural response was recorded using stopwatch for 5 min. The parameters for study were period of latency (The time between release of the insect and its first movement), rest and activity (grooming, jumping, jerking, oviposition, flying and proboscis extension) were recorded and analyzed according to the method of Stireman [9] with modification. Number of insects used were 30. Non responding insects were discarded.

Statistical Analysis

All data were analyzed using the software packages SPSS-13 and MINITAB-15. One-way analysis of variance at 95 % confidence level was used to calculate the significance difference of different responses of Uzi flies between the control and the different hours of fed sualo treatments. Correlation coefficient was calculated to determine the correlations between the different concentrations of compounds and the responses of the Uzi flies.

Results and Discussion

When the mated Uzi fly was exposed to sualo leaves fed for different hours as shown in Fig. 2, the activity period significantly enhanced over control. In contrast, the latency period was significantly longer in response to control. After every activity the insect used to remain in standstill posture which was considered as rest period. The total period of rest however did not vary significantly. The results indicated a probability that the responses of the flies were elicited by the odours emanated from the fed leaves and hence those odorous chemicals might be considered as cues used by *E. sorbillans* for host finding during foraging. This result agrees with studies on related tachinid *Exorista japonica* and *E. mella* which prefer wide range of hosts but are attracted to odours of plants infested with hosts [10, 11] and also general 'green leaf volatiles' [9]. The activity period in this experiment included walking and grooming behaviour. While the reason for increased grooming is difficult to be defined, increased walking behaviour may suggest increased searching behaviour for host elicited by odour emanated from the fed leaves. Proboscis extension is a reflex behaviour widely studied in bees where antennal stimulation with sugary water causes the proboscis to stick out for feeding. As insects use green leaf volatiles as cues for food finding, increased period of extension of proboscis in Uzi fly in response to odorous chemicals released by host plant might also reflect antennal stimulation for

Table 1 List pure volatile compounds used (Sigma-Aldrich)

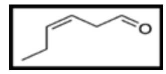
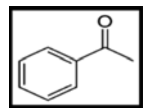
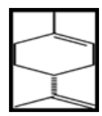
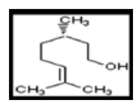
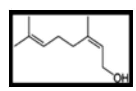
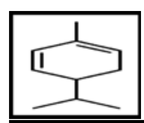
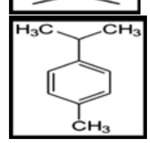
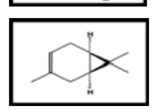
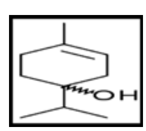
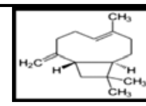
S.N.	Compound name	Purity (%)	Structure	Chemical group
1	Hexanal	98		Aldehydes
2	Decanal	99		
3	CIS-3-hexenal	99		
4	Acetophenone	99.99		Aromatic ketone
5	Undecane	99		Alkane hydrocarbon
6	D-Limonene	99		Monoterpenes
7	β-Citronellol	98		
8	Nerol	99.99		
9	R(−)-α-phellandrene	99.99		
10	*P*-cymene	99		
11	Delta carene	99		
12	Terpinene-4-ol	99.99		

Table 1 continued

S.N.	Compound name	Purity (%)	Structure	Chemical group
13	β-Caryophyllene	98		Sesquiterpenes
14	α-Humulene	99.99		
15	Farnesol	99		

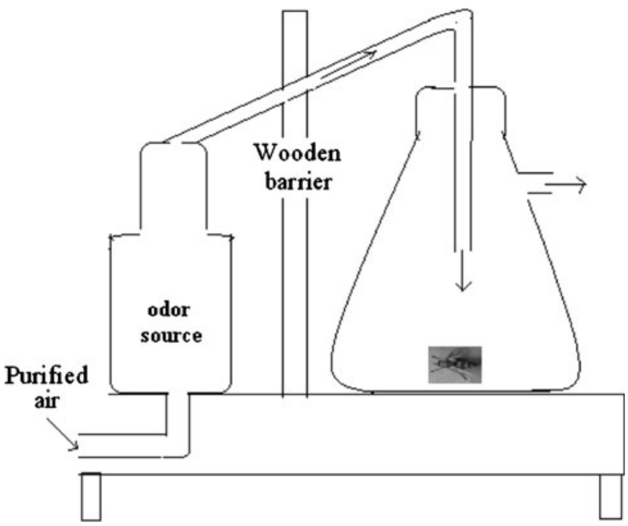
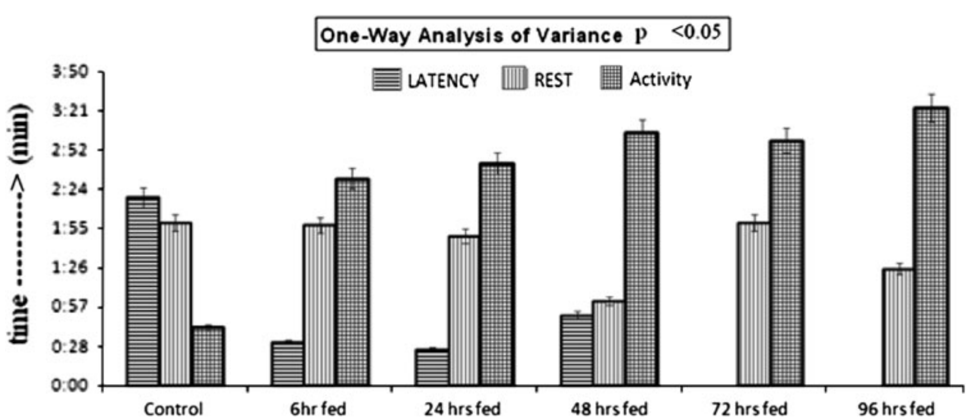


Fig. 1 Olfactometer

feeding. But the subject needs further detailed experimentation.

GC and GC–MS analysis of volatiles released by primary host plants of *A. assama* reported elsewhere [12, 13]. revealed that the plants released a good number of volatile chemicals (C6 alcohols, aldehydes, esters and terpenoids) upon herbivory by larvae of *A. assama*. On the basis of these earlier studies we selected fifteen volatile compounds to be used as odour source. As shown in Fig. 3 the period of latency decreased in comparison to control in response to all chemical compounds. However in case of *P*-cymene and beta-caryophyllene the period increased with increase in concentration of the compound till 50 ppm only and decreased at 100 ppm concentration. In case of farnesol although at initial concentration (1 ppm) the latency period increased, with increase of dose (10–100 ppm) the period decreased. There was significant negative correlation between concentration and latency period in response to hexanal, *P*-cymene, delta-carene, undecane, acetophenone, terpinene-4-ol, farnesol, decanal, nerol and β-caryophyllene (Table 2). The period of rest decreased in comparison to control in case of all chemical compounds and significant negative correlation was observed in response to acetophenone, terpenene-4-ol, farnesol, α-humulene, β-citronellol, nerol and β-caryophyllene. In contrast, the period of activity increased in response to all compounds. Highly significant positive correlation between activity period and concentration was observed in response to acetophenone, terpenene-4-ol, farnesol, α-humulene and β-caryophyllene. Literature reveals that volatile compounds of similar

Fig. 2 Behavioural response of *E. sorbillans* to fed sualo leaves



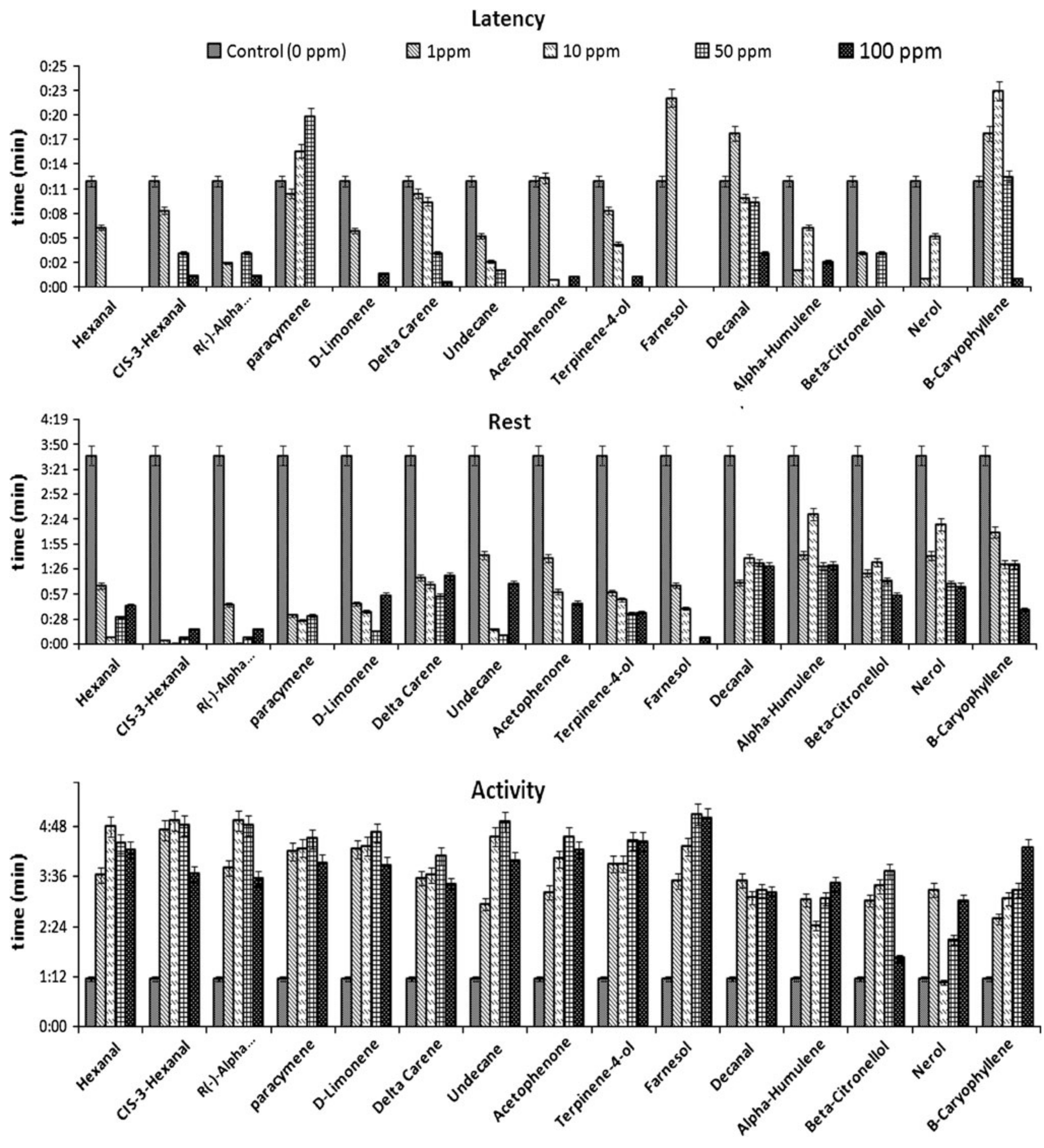


Fig. 3 Comparison of behavioural responses of *E. sorbillans* to different concentrations of the standard volatile compounds

chemical structure are emitted by plants of a wide variety when damaged by insects and mites [14]. Based on this structural similarity of emitted compounds, activation of common biosynthetic pathways in wide range of plant families have been suggested for being used by parasitoids and predators [15]. The three common pathways recognized are lipoxygenase leading to production of 'green leaf volatiles', isoprenoid derived terpenoid synthetic pathway leading to production of mono- and sesquiterpene and shikimic acid pathway leading to production of methyl salicylate and indole [16]. From the results of the present study it may be concluded that the fly, *E. sorbillans* responds to products of both lipoxenase pathway (hexanal, decanal, acetophenone) and terpenoid pathway (oxygenated

Table 2 Table showing correlation between concentrations and different responses of the flies

Compounds name		Correlation coefficient
Hexanal	Concentration and latency	−0.579
	Concentration and rest	0.030
	Concentration and activity	0.128
CIS-3-hexanal	Concentration and latency	−0.465
	Concentration and rest	0.953
	Concentration and activity	−0.858
R(−)-α-phellandrene	Concentration and latency	0.088
	Concentration and rest	−0.292
	Concentration and activity	−0.411
P-cymene	Concentration and latency	−0.615
	Concentration and rest	−0.855
	Concentration and activity	−0.492
D-Limonene	Concentration and latency	−0.452
	Concentration and rest	0.240
	Concentration and activity	−0.493
Delta carene	Concentration and latency	−0.989
	Concentration and rest	0.111
	Concentration and activity	−0.167
Undecane	Concentration and latency	−0.905
	Concentration and rest	−0.050
	Concentration and activity	0.275
Acetophenone	Concentration and latency	−0.519
	Concentration and rest	−0.517
	Concentration and activity	0.636
Terpinene-4-ol	Concentration and latency	−0.798
	Concentration and rest	−0.860
	Concentration and activity	0.868
Farnesol	Concentration and latency	−0.579
	Concentration and rest	−0.809
	Concentration and activity	0.805
Decanal	Concentration and latency	−0.864
	Concentration and rest	0.309
	Concentration and activity	−0.326
α-Humulene	Concentration and latency	−0.278
	Concentration and rest	−0.591
	Concentration and activity	0.744
β-Citronellol	Concentration and latency	−0.377
	Concentration and rest	−0.913
	Concentration and activity	−0.699
Nerol	Concentration and latency	−0.567
	Concentration and rest	−0.776
	Concentration and activity	0.297
β-Caryophyllene	Concentration and latency	−0.951
	Concentration and rest	−0.918
	Concentration and activity	0.965

monoterpene, monoterpene hydrocarbon and sesquiterpenes). Considering the response in relation to all the parameters the aromatic ketone Acetophenone, the oxygenated terpenes namely terpinene-4-ol and sesquiterpene namely farnesol and β-caryophyllene may be considered to have elicited the best response. Earlier, wide range of volatile compounds were reported to be involved in the olfactory orientation of the fly through electroantennogram studies [12]. which is probably due to the ability of the fly to parasitize different lepidopteran hosts feeding on different plants. The chemicals identified to be important in olfactory orientation and subsequent behavioural response of the insect as a whole may be used in development of chemical trap for effective management of the parasitoid. Further studies using different other chemicals and their blends in both laboratory and field condition is expected to provide meaningful leads for the purpose of management.

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