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Research Article

Behavioural response of specific larval endoparasitoid, *Apanteles machaeralis* (Wilkinson) to volatile cues from its host insect, *Diaphania indica* (Saunders) and the host plant (*Cucumis sativus* L.)

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ABSTRACT: *Apanteles machaeralis* (Wilkinson), a braconid specialist larval endoparasitoid of *Diaphania indica* occurs naturally causing significant levels of parasitism. The present study explores the response of the *A. machaeralis* to odour cues from *D. indica* damaged cucumber plant as well as host larvae. Different odour treatments namely, *D. indica* larval body volatiles (T_1), volatiles from larval excreta (T_2), volatiles from larvae + excreta (T_3), volatiles from *D. indica* body wash (T_4), volatiles from the healthy, mechanically damaged and *D. indica* infested cucumber plants (HIPVs) were collected using headspace analysis and the response of female *A. machaeralis* was studied using olfactometer assays. Results of the study conclusively indicated that *A. machaeralis* is highly attracted to host larval body wash as parasitic wasps spent significantly more time made more entries into the treated region in single as well as dual choice assays. The electoantennographic response (EAG) further supported the olfactometer bioassays. The GC-MS analysis revealed significant differences in the volatile emissions of different treatments studied. The utilization of host insect body cues and HIPVs in the host recognition by the specialist endoparasitoid *A. machaeralis* is discussed in detail.

KEY WORDS: Cucumber moth, HIPVs, olfactometer assays, specialist parasitoid, volatiles

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INTRODUCTION

Plants produce a significant number of volatile plumes (Herbivore Induced Plant Volatiles, HIPVs), when damaged by herbivores. These organic compounds have heterogeneous behavioural functions across multi-trophic levels (Turlings *et al.,* 1990; De Moraes *et al*., 1998; Pare and Tumlinson, 1999; Mumm and Hilker, 2005; Kamala Jayanthi *et al.,* 2020). These HIPVs are specific to each plant species as well as herbivores and are perceived by neighbouring plants (Steidle and Schöller, 1997; Sullivan and Berisford, 2004), conspecific/ heterospecific herbivores associated with the crop (Mumm and Hilker, 2005; Wei and Kang, 2006; Ngumbi and Fadamiro, 2012) and their natural enemies (Wei *et al.,* 2007). HIPVs often guide natural enemies and help them locate their prey thereby serving as long range radar (Rutledge, 1996). Location of host habitat and host insect is crucial for natural enemies as it impacts their offspring's fitness and survival (Schnee *et al*., 2006; van Dam *et al*., 2010). In the complex odour network the natural enemies must fine tune their olfactory system to its finest to discriminate and track down the host's habitat location (= long range signals) and the host's micro-habitat location (= short range signals). Once they locate the host's habitat, they may depend on volatile cues (= kairomones) produced by the host insect itself. Thus, at short distances, volatile cues produced by the host insect serve as an arrestant for natural enemies (Meiners *et al*., 2002; Rains *et al.,* 2004; Harris *et al*., 2012). Specialist parasitoids whose degree of host specificity is much narrower compared to generalists must depend on specialized volatile cues from suitable host larvae and their host plant to locate them and thus they have been considered as good models for insect olfaction studies (Meinersv *et al.,* 2002; Rains *et al.,* 2004; Harris *et al.,* 2012).

Braconid, *Apanteles machaeralis* (Wilkinson) is a major specialist larval endoparasitoid of Cucumber moth, *Diaphinia indica* (Saunders), which is a serious pest of several cucurbitaceous crops. The female *A. machaeralis* oviposits inside the *D. indica* larva and the emerging parasitoid larvae

feed on the host's larval body tissues causing its death. The host location ability of *A. machaeralis* seems to be highly specialized as the female parasitoidis able to detect the presence of a host larva, even when surrounded by a very complex odor background (Kigathi *et al.,* 2009; de Rijk *et al.,* 2016). Whether *A. macheralis* depends on the olfactory cues emanating from the *D. indica* infested host plant or host insect (=*D. indica* larvae) in the host location processes has not been investigated till date. The present study was aimed to identify the source of olfactory cues that the female parasitoid, *A. macheralis* uses for locating its *D. indica* larval host in infested cucumber plant, *Cucumis sativus* L. Volatiles from *D. indica* infested cucumber plant, healthy cucumber plant, mechanically damaged cucumber host plant, *D. indica* larvae, *D. indica* larval faeces served as odour sources to evaluate the behavioural response of *A. macheralis* females.

MATERIALS AND METHODS

Host insect

Larvae of cucumber moth, *D. indica* were collected from the experimental fields of ICAR-Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka, India.

Parasitoid

Diaphinia indica larvae which were parasitized by *Apanteles machaeralis* were collected from the IIHR experimental fields and brought to the laboratory. The parasitized larvae along with the parasitoid cocoons were kept in a plastic container (45 x 50 cm) and were maintained at ambient conditions (12L:12D, 27 ± 1°C, 75% relative humidity) until the emergence of the adult wasps. The emerged adult wasps (♂ and ♀) were provided with 10% honey on cotton swab *ad libitum* and allowed to mate. Later, the mated females were separated and were used for behavioural assays.

Host plant

The selected host plant, *C. sativus* was maintained in grow polybags (6 x 8") without any pesticide application. To avoid insect pest infestation, regular water sprays were given at frequent intervals.

Host plants infested with *Diaphinia indica* larvae

For the establishment of infested cucumber plants, mixed instars of *D. indica* larvae (n=40) were released on healthy *C. sativus* plants with the help of a camel hair brush and were covered with a white transparent polythene cover to avoid larval escape. Holes were made on the cover using a needle to allow aeration. The larvae were allowed to feed on the plants continuously for 24 h, and later the larvae were removed from the plants. The plants which were fed by *D. indica* larvae were used for the collection of plant volatiles (=HIPVs) through air entrainment.

Host plants with mechanical damage

To simulate mechanical damage, the leaves of healthy *C. sativus* plants were damaged using scissors. Such damaged plants were used to collect plant volatiles through air entrainment.

Host plant volatiles

Plant volatiles from healthy, *D. indica* infested as well as mechanically damaged *C. sativus* plants were collected through air entrainment as per the procedure described by Jayanthi *et al.,* (2012). The test plants were draped with autoclaved polythene bags (41 x 32.5 cm) with both outlet and inlet ports inside the cover. The cover was tightly tied at the base of the plant using rubber bungs and silica wool to prevent air passage. Volatiles were collected onto Porapak Q (50 mg, 60/80 mesh; Supelco, Sigma Aldrich, St Louis, USA) which was placed in a glass tube (5 mm dia.) and inserted into the outlet collection port, placed in the cover. Further, pumps drew air (800 mL/min) through these tubes. Air that was purified by passage through an activated charcoal filter was pumped into the cover through the inlet port (400 mL/min). All connections were made with PTFE tubing with brass ferrules and fittings (Swagelok, India) and sealed with PTFE tape. Porapak Q tubes were heated at 100°C for 2 h under a stream of purified nitrogen to remove contaminants. Volatiles were collected from different treatments for 24 h and the Porapak Q columns were eluted with 750 µl of redistilled diethyl ether with an internal standard (5µg/µL of ethylbenzoate, 99.9% pure, Sigma Aldrich, U.S.A.; Anfora *et al.,* 2009). The collected volatile samples were stored in a freezer (−20°C) until further use.

Host larval odours

A total of four different types of host insect odours (*D. indica* larval body odours (T_1), *D. indica* excreta odours (T_2), *D. indica* larvae + excreta odours (T_3), *D. indica* larval body wash (T_4) were collected for the study. Of which, the first three host insect odours namely T_1, T_2, T_3 were collected through air entrainment from different treatments namely *D. indica* larvae (n= 125), *D. indica* larval excreta (4.60g) and *D. indica* larvae + excreta (n = 125, 4.60g respectively) as per the procedure described by Jayanthi *et al.,* (2012). All the three treatments were placed individually inside a cylindrical glass vessel and closed with a lid having collection and inlet ports at the top. The flange on the open end of the glass vessel was clipped to make the vessel air tight. Volatiles were collected on Porapak Q as explained above. Before the volatile collection, glassware was washed with liquid detergent, rinsed with distilled water and acetone and was then dried in an oven at 180°C for 2 h. The Porapak Q

columns used for the collection of volatiles were eluted with redistilled diethyl ether and the volatile samples were stored in a freezer (−20°C) until further use.

To obtain *D. indica* larval body wash (T_4), a total of 125 larvae of *D. indica* were placed in 50 mL beaker and 5 mL of *n*- hexane (99.9%) was added and left for 5-10 min. The *n*-hexane filtrate was collected and into which 2 g of sodium sulphate was added to remove the moisture traces. The final filtrate was reduced to 500µl using a slow stream of nitrogen and an internal standard of 5µg/µL of ethyl benzoate was added to the extract for chemical quantification. This filtrate which served as *D. indica* larval body wash (T_4) was stored in a freezer (−20°C) until further use.

Electroantennographic bioassay (EAG)

Electroantennogram (EAG) recordings were made as described by Cork *et al.,* (1990) using 2-4 days old mated *A. machaeralis* females. In this bioassay, empty air and honey were used as negative and positive controls respectively. The olfactory stimuli were obtained by impregnating 10µl of odour samples from host plant and host larvae of *D. indica* (T_1-T_7) onto separate filter paper strips (Whatman No.1, 6 cm length x 0.5 cm breadth). The solvent was then allowed to evaporate for 1 min before placing the filter papers inside the glass pasture pipettes (10 cm length and 6 mm outer diam.). Stimulation of antennal preparation was carried out by means of controlled airflow (300 mL/min) through the pipette with the filter paper. By injecting a puff of purified air (0.5 sec), odour stimulation was administered, amplified and recorded using Autospike software (Syntech EAG Model IDAC-4, Intelligent Data Acquisition Controller). To measure stimulus–response, the test stimuli were successively given along with interspersed control stimulation. Between stimulus presentations, purified air was blown over the antennal preparation for at least 30 sec. The EAG Probe was set at a sampling rate of 100 with a filter rate of 0-32 Hz. The responses (amplitudes) to the host plant volatiles are expressed as mean of all recorded antennal depolarizations.

Antenna was changed for each replication and a total of seven replicates were carried out for each stimulus. Antennal response for the test volatiles of different treatments were recorded based on the downward deflection signal (in mV) of gravid female antenna for all host plants and host larvae volatiles and the data were subjected to one-way ANOVA using Graph pad prism (version 7.03)

Gas chromatography coupled mass spectrometry analysis (GC-MS)

Chemical composition of Porapak Q elutes of different treatments *viz.,* larval body volatiles of *D. indica* (T_1), volatiles from larval excreta (T_2), volatiles from larvae + excreta (T_3), larval body wash (T_4), volatiles of *D. indica* infested cucumber plant (T_5), volatiles from healthy cucumber (T_6), volatiles from mechanical damaged cucumber plant (T_7) were analysed by using GC-MS Agilent 7890B GC system equipped with Mass Spectrophotometry (Agilent 5977 MSD).A capillary column Agilent J & W (HP-5 MS UI) of 30 m length, 0.250 mm Diameter and 0.25 µm film thickness was used to examine the samples. The thermal programme was initially set at 60ºC for 1 min later ramped at 15ºC/min up to 240ºC and held for 2 min the flow rate of 1mL/min with helium as the carrier gas. MS full scan mode (70 eV) and AMU ranged from 40 to 450. One micro litre of the sample was injected in split less mode ratio (40 mL/min) with injection temperature of 270ºC. Total volatile emissions were calculated by the sum of all GC-FID peak areas in the chromatogram and individual compounds were quantified as relative per cent area. The compounds were identified by GC retention time, mass spectrum and KOVATS index (C7 to C30 homologous series of *n*-alkenes as standard, Sigma-Aldrich; Kovats, 1965) using NIST 14 software. Identified compounds were authenticated by co-injecting standard synthetic compounds along with the samples (Jayanthi *et al*., 2012).

Olfactometer bioassays

To study the behavioural responses of female *A. machaeralis* to different test volatiles (T_1-T_7), olfactometer bioassays were carried out using a circular Perspex four-arm olfactometer [120 mm dia.], placed inside a cage (0.62m length x 0.62m wide x 0.62m height), illuminated from above by diffused, uniform lighting using a fluorescent bulb (15W) and surrounded by black light proof walls to prevent influence of any external visual stimuli as per the procedure described by Jayanthi *et al.,* (2012). The experiments were conducted at ambient room temperature (27±1°C). Prior to the experiment, all glassware was washed with liquid detergent, rinsed with acetone and distilled water and baked in an oven overnight at 180°C. Perspex components were washed with Teepol solution, rinsed with 80% ethanol and distilled water, and were left to air-dry. The bottom of the apparatus was lined with filter paper (Whatman No 1, 12 cm dia.) and air was drawn through the four arms towards the centre at 350 mL min^{-1}.

Individual adult female parasitoids were introduced into the central chamber through a hole on the top of the olfactometer. Each parasitoid was given 2 min to acclimatize in the olfactometer, after which the experiment was run for 15 min for each replicate.

Two series of bioassays namely single choice and dual

choice assays were carried out to study the behavioural response of female *A. machaeralis* to different odour samples. In single choice assay, all odour samples (T_1-T_7) were tested against solvent control (diethyl ether/hexane as the case may be). Each replicate involved one treated arm and the remaining three arms served as controls. In dual choice assay, the odour samples that elicited significant response in female *A. machaeralis* parasitoid were compared in different combinations (T_1 vs T_4; T_1 vs T_5; T_4 vs T_5). Here, each replicate involved two treated arms and the remaining two served as control arms (solvent). The test samples (10 µl) were applied to a filter paper and the solvent was allowed to evaporate prior to placement. Filter paper strips with solvent (10 µl) served as control. Ten (n = 10) replicates were carried out for each assay.

Observations on the time spent and the number of entries made into each arm were recorded using Olfa software (F. Nazzi, Udine, Italy). Single choice and dual choice assays data were subjected to *t*- test and one way ANOVA respectively using Graph pad prism (v7.03).

RESULTS AND DISCUSSION

Single choice assays with different odour treatments along with the solvent control against *Apanteles machaeralis* females revealed that the specialist parasitoids were significantly attracted to T_1 (*Diaphania indica* larval body volatiles), T_4(larval body wash) and T_5 (HIPVs from *D. indica* infested Cucumber plant) as they spent significantly more time (min) in the treated regions over control. In case of T_1, *A. machearlis* females spent significantly more amount of time in the treated region over control [Time spent (min): Mean ± S.E, 2.75 ± 0.37, control = 1.89 ± 0.18, P = 0.02]. However, no significant difference was noticed among the number of entries made into the treated as well as control regions [Entries (number): Mean ± S.E, 3.80 ± 0.84, control = 4.53 ± 0.27, P= NS). In case of T_4, the female parasitoids spent significantly more time and made more entries in the treated region compared to the control [$T_{4:}$ time spent (min): Mean ± S.E, 3.36 ± 0.40, control = 2.86 ± 0.13, P = 0.001; Entries (number): Mean ± S.E; 16.60 ± 1.48, control = 11.77 ± 1.58, P = 0.003]. Similar to T_1, in case of T_5 female parasitoids spent significantly more amount of time in the treated region over control but no significant difference was found with respect to the number of entries made [T_5: time spent (min): Mean ± S.E, 3.17 ± 0.58, control = 2.46 ± 0.32, P= 0.004; entries (number): Mean ± S.E, 2.90 ± 0.46, control = 2.23 ± 0.20, P = 0.76].

The parasitoids did not respond to the volatiles of the remaining treatments *viz.,* T_2 (larval excreta), T_3(larvae + excreta) T_6 (healthy cucumber plant) and T_7 (mechanically damaged cucumber plant) [$T_{2:}$ time spent (min): Mean ± S.E, 2.63 ± 0.56, control = 1.99 ± 0.27, P = 0.360; entries (number): Mean ± S.E, 6.40 ± 1.37, control = 5. 56 ± 1.20, P = 0.10; T_3: time spent (min): Mean ± S.E, 2.17 ± 0.41, control = 2.19 ± 0.19, P = 0.95; entries (number): Mean ± S.E, 6.60 ± 1.63, control = 7.03 ± 1.31, P =0.75; T_6: time spent (min): Mean ± S.E, 1.57±0.38, control = 2.23±0.20, P = 0.17; entries (number)Mean ± S.E: 6.70 ±1.14, control = 7.53 ±0.67, P = 0.59; T_7 time spent(min): Mean ± S.E, 1.69±0.47, control =2.54 ±0.34, P =0.27: entries (number): Mean ± S.E, 7.60 ± 1.49, control = 8.47 ± 0.99, P = 0.33].

The step-wise dual choice assays between T_1 vs T_4, T_4 vs T_5, and T_1 vs T_5 revealed that in the first combination, the female parasitoids significantly attracted to T_4 over T_1 by spending more time and by making more entries into the treated region [time spent (min): Mean ± S.E, T_4= 3.26 ± 0.34, T_1= 2.27 ± 0.32, control 1= 1.83 ± 0.31, control 2 = 1.80+ 0.34, P = 0.004; no of entries (number): Mean ± S.E, T_4= 13.0 ± 1.90, T_1= 9.10 ± 1.70, control 1= 8.25 ± 0.84, control 2 = 7.96+ 0.28, P = 0.003]. In the second combination (T_4vs T_5), the female parasitoids spent significantly more amount of time into the T_4 region compared to T_5, however, no significant difference was found for the number of entries made by female parasitoids[time spent (min): Mean ± S.E, T_4= 3.30 ± 0.26, T_5= 2.01 ± 0.35,control 1= 2.28 ± 0.34, control 2 = 2.13+ 0.22, P = 0.003; no of entries: T_4 = 6.60± 1.19, T_5= 7.50 ± 1.28, control 1= 5.67 ± 0.94, control 2 = 5.01+0.86, P = 0.55]. In the third combination (T_1vs T_5), parasitoids did not differentiate the treatments either for time spent or number of entries [time spent (min): Mean ± S.E, T_1= 1.57 ± 0.38, T_5 = 2.23 ± 0.20, control 1= 2.04 ± 0.36, control 2 = 2.13+0.26, P = 0.75; entries: T_1= 9.80 ± 1.67, T_5 = 6.50 ± 1.49, control 1= 5.30 ±0.81, control 2 = 4.57+ 0.91, P = 0.40]. The results of the study conclusively indicate that the specific endoparasitoid *A. machaeralis* is highly attracted to host larval body wash (T_4) in terms of both amount of time spent and no of entries made into the treated region in single choice as well as dual choice assays. However, in case of dual choice assays, though the parasitoids spent significantly more time in T_4 over other treatments, they could not differentiate between the treatments T_4 and T_5 for number of entries (Fig. 1).

Electroantennographic response of *A. macheralis* to different volatile treatments (T_1-T_7) revealed that parasitoid female antenna showed significantly high response to larval body wash (T_4: 1.176 ±0.257 mV; mean amplitude ±S.E, P =0.001) followed by larval body volatile (T_1: 0.825 ±0.128 mV; mean amplitude ±S.E, P =0.002), and infested cucumber plant (T_5: 0.737 ± 0.204 mV; mean amplitude ± S.E, P =0.04). The remaining treatments did not elicit significant antennal response in *A. macheralis* [mean amplitude ± S.E,

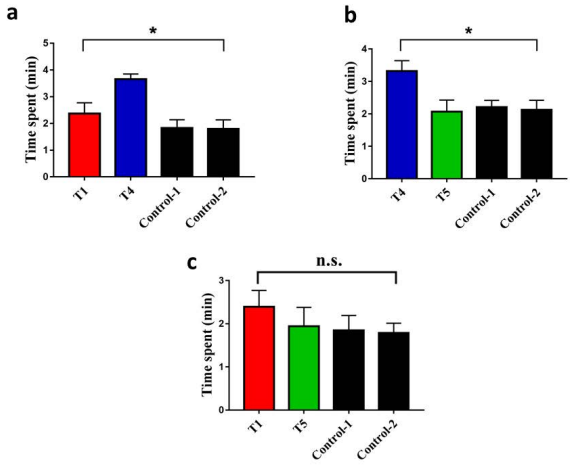

Fig. 1. Dual choice assays showing the response of *Apanteles machaeralis* parasitoid females to (a) T_1 Vs T_4 [Larval body volatiles Vs Larval body wash] (b) T_4 Vs T_5 [Larval body wash Vs Infested cucumber volatiles] (c) T_1 Vs T_5 [Larval body volatiles Vs Infested cucumber volatiles]; *P = 0.004 (a); P =0.003 (b); n.s. indicates non-significance

T_2: excreta, 0.387 ±0.1427 mV; T_3: larvae with excreta, 0.5878 ± 0.1514 mV; T_6: healthy cucumber plant, 0.5478 ± 0.1099 mV; T_7: mechanically injured cucumber plant, 0.7044 ± 0.1255 mV]. These results indicated that *A. machaeralis* female highly preferred larval body cues and infested host plant volatiles (Fig. 2).

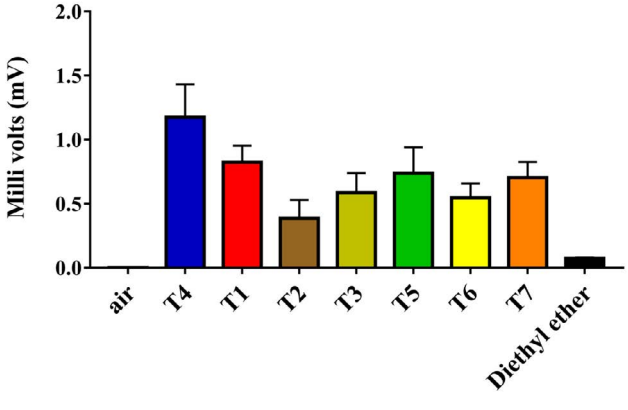

Fig. 2. Electroantennogrphic response of *Apanteles machaeralis* females to T_1 (Larval body volatiles), T_2 (Larval excreta), T_3 (Larva+excreta), T_4 (Larval body wash), T_5 (Infested cucumber plant volatiles), T_6 (Healthy cucumber plant volatiles), T_7 (Mechanically damaged cucumber plant volatiles)

A comparison of volatile compounds of larval body wash (T_4), larval body volatile (T_1) and infested cucumber plant (T_5) through GC-MS analysis revealed significant differences among the functional groups namely terpenoids, esters & acids, aromatic hydrocarbons, phenyl propanoids, alkanes, alkenes, methyl pyridines, indole, alcohols, aldehydes and phenols across the treatments (Supplementary Table 1).

The comparison of GC-MS analysis of larval body wash and larval body volatiles revealed significant qualitative and quantitative differences in the volatile compound emissions (Fig. 3). Significant amounts (> 5µg/mL) of chemical compounds like 3-carene (6.15), (*z*)-ocimene (15.91), 9-methylnonadecane (5.04), dodecanal (5.73), *n*-hexadecanoic acid (5.26), *n*-tetradecanoic acid (6.22), trans-isoeugenol acetate (38.40), isobutyl valerate (8.69), H-Indole, 2-(1,1-dimethyl ethyl)- (6.08), βthujaplicin (8.16), trans-phytol (5.76), *n*-eicosane (19.67), 2,5-di-tert-butylphenol (42.67) were exclusively noticed in larval body wash suggesting that these volatile compounds from *D. indica* larval body, individually or in combination would have served as attractive cues to *A. machaeralis*.

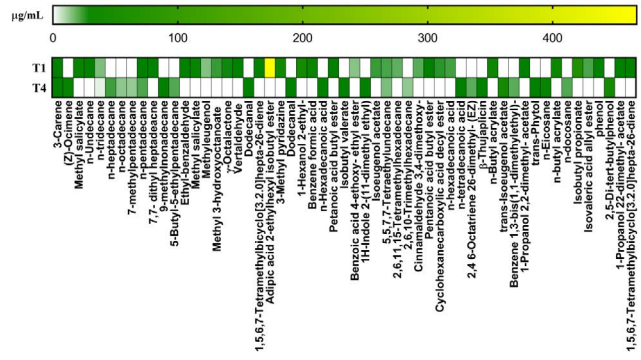

Fig. 3. Heat map showing differences in the volatile compounds of T1 (Larval body volatiles) and T_4 (Larval body wash)

The volatile emissions of damaged cucumber plant differed qualitatively and quantitatively from healthy as well as mechanically damaged cucumber plant volatiles (Fig. 4). In case of HIPV's released from *D. indica* infested cucumber plant, exclusive presence of significant amounts (µg/mL) of *n*-decane (7.02),*n*-hexadecane (6.99),2,4-dimethylundecane (8.00), 4-methyl octane (6.00), 5,8-diethyl dodecane (10.40), 3-methyl pentadecane (76.11), 4 methyl dodecane (172.58 and4-(1-hydroperoxy-2,2-dimethyl-6-methylene-cyclohexyl)-pent-3-en-2-one (8.70) was noticed. Compounds like naphthalene (44.80µg/mL), *n*-octadecane (17.62 µg/mL) and 2,6,10-trimethyl pentadecane (57.73 µg/mL) were emitted exclusively my mechanically damaged cucumber plants (Fig. 4). Compounds like *n*-undecane and 4,5 dimethyl nonane exhibited concentration dependent changes across

the different plant treatments namely healthy (375.63, 249.79µg/mL), *D. indica* infested (513.49, 419.15µg/mL) and mechanically damaged plants (1104.49, 1602.36 µg/mL) respectively. Presence of sesquiterpene compound, farnesene (8.94µg/mL) was noticed only in healthy cucumber plants (Fig. 4).

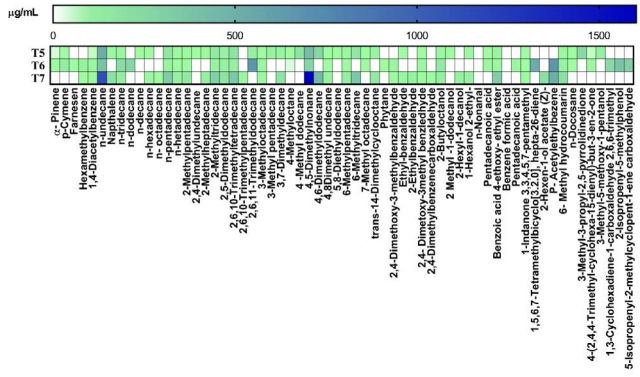

Fig. 4. Heat map showing differences in the volatile compounds of T_5 (Infested cucumber plant volatiles), T_6 (Healthy cucumber plant volatiles) and T_7 (Mechanically damaged cucumber plant volatiles)

The strategy of host location by parasitoids is a systematic process involving series of steps like initial finding of the location of associated plants of the host insects from distance and landing on them, followed by the tracking of specific host insect (herbivore) odours for precise location of its host insect in the final phase of the host location strategy (Rutledge, 1996). In the host-location process of parasitoids, HIPVs play crucial role acting as kairomones /synomones (de Rijk *et al.,* 2016).

In the presents study, the results of single choice four-arm olfactometer assays revealed that female *A. machaeralis* was highly attracted to volatiles from *D. indica* larval body wash, followed by *D. indica* larval body volatiles and *D. indica* infested cucumber plant. Thus, only host insect associated volatile cues (=larval body odours, T_1 and T_4) and HIPVs from the host plant were found to be attractive to female *A. machaeralis* (Fig. 4). Previously, Dweck *et al*., (2010) opined that only host associated volatiles may be attractive or ecologically relevant to parasitoids for the final host location and recognition. Similarly, when choice was given, the females of another specialist parasitoid, *Apanteles taragamae* were attracted to HIPVs from cucumber plant infested with *D. indica* over healthy and mechanically damaged cucumber plants, Nurkoumar *et al.,* (2017).

In the present study, the volatile cues from larval excreta, larva+ excreta, healthy host plant and mechanically damaged host plant did not attract the female *A. machaeralis* indicating *A. machaeralis* locates its host insect mainly through HIPVs as well as host insect (*D. indica*) larval body cues. The EAG response of *A.machaeralis* to all the volatile treatments (T_1-T_7) further supported this where *A.machaeralis* females showed the highest response to larval body wash followed by larval body volatiles and infested cucumber plant volatiles. The results of dual choice assays added further clarity to the parasitoid's preference. Given a choice, the female *A.machaeralis* prefers the odour cues from larval body followed by HIPVs from the cucumber plant. Thus, specific larval endoparasitoid, *A.machaeralis* might use HIPVs from the *D. indica* infested cucumber plant as long-range cues and *D. indica* larval host body cues as short-range cues.

The volatile chemicals from the two treatments of *D. indica* larval body (T_1 and T_4) also differed significantly (Fig. 4). Though in single choice assays both the treatments were found attractive to female parasitoids, later in dual choice assay body wash from *D. indica* larvae (T4) was found to be significantly more attractive. The larval body wash revealed to contain trans-isoeugenol acetate (38.40µg/mL), *n*-eicosane (19.67 µg/mL) and 2,5-di-tert-butylphenol (42.67µg/mL) in significant amounts, suggesting these volatile compounds from *D. indica* larval body, individually or in combination would have served as attractive cues to female *A. machaeralis*.

Several qualitative and quantitative differences in the emissions of volatile compounds were found in *D. indica* infested cucumber plant compared to the healthy and mechanically damaged plants. Sesquiterpenes like naphthalene (44.80µg/mL) was found in significant amounts in HIPVs and completely absent in healthy cucumber plant volatiles. Meents *et al.,* (2019) found naphthalene as one of the components of anti-herbivore defense signals released by sweet potato plants.

The present study emphasizes the role of HIPVs released from the *D. indica* infested cucumber plant as well as *D. indica* larval body cues in the host recognition process of specialist parasitic wasp, *A. machaeralis*. Specialist parasitoids might have innately tuned to HIPVs produced by host plants upon damage by their target herbivore.

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