Indian Journal of Entomology 85(Special Issue) May 2023: 1-6 eRef. No. NWRABNRG01 DoI. No.: 10.55446/IJE.2023.1150

BEHAVIOURAL AND ELECTROPHYSIOLOGICAL RESPONSE OF *TRICHOGRAMMA ACHAEA* TO OVIPOSITION INDUCED VOLATILES IN TOMATO

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ABSTRACT

A laboratory experiment was conducted at ICAR-National Bureau of Agricultural Insect Resources, Bengaluru to study the behavioural response of *Trichogramma achaea* towards healthy, conspecifics (*Tuta absoluta* oviposited on *Tuta absoluta* infested plants) and heterospecifics (*Tuta absoluta* oviposited on *Liriomyza trifolii* infested plants) tomato plants by choice and no-choice parasitization assays and olfactometer studies. Headspace volatiles were collected from tomato plants oviposited by conspecifics, heterospecifics and healthy plants, analysed using gas chromatography-mass spectrometry (GC-MS) where 14, 17 and 8 volatile organic compounds were detected respectively. Electrophysiological compounds and antennal response of *T. achaea* were identified using gas chromatography-electro-antennographic detector (GC-EAD). EAD active compounds of *T. achaea* were 3-Hexanol, α-phellandrene, trans-β- ocimene, pentacosane and γ-terpinene.

Key words: *Trichogramma achaea*, conspecifics, heterospecifics, choice and no-choice parasitization assay, olfactometer studie, EAD active compound, *Tuta absoluta*, volatile organic compounds, electrophysiological compounds, antennal response

Tomato is one of the major vegetable crops grown in South India. In nature plants are infested by various herbivores which in turn produce semiochemicals. These semiochemicals play a direct or an indirect role in plant defence mechanisms. Oviposition of insects is the major concern from where the damage process starts. Volatiles that are induced upon oviposition are known as "oviposition induced plant volatiles" (OIPV's) which may act as synomones. These volatiles help in attracting natural enemies and switch on defence mechanisms before the pest starts damaging the plant. South American Tomato Leaf Miner, *Tuta absoluta* (Meyrick, 1917) and American Tomato Leaf Miner, *Liriomyza trifolii* (Burgess) are the most common pests of tomato. *Trichogramma achaea* Nagaraja & Nagarkatti was known to be one of the most promising egg parasitoid of *T. absoluta*. However very little information is available on the host seeking behaviour and electrophysiologically active compounds of *T. achaea* on OIPV's of tomato plants. This study helps in understanding the foraging behaviour as well as ways to implement these identified semiochemicals in effective management practices.

MATERIALS AND METHODS

Tomato leaf miner, *T. absoluta* (along with infested leaves) were collected from the polyhouse at ICAR-NBAIR, Yelahanka campus, Bengaluru and also from farmers' fields around Malur and Kolar districts of Karnataka. The freshly collected infested leaves of tomato along with *T. absoluta* larvae were placed in polythene covers (45 cm × 30.5 cm), secured with rubber bands and brought to the laboratory. In the laboratory, the larvae along with infested leaves were placed in plastic containers lined with tissue papers to avoid moisture accumulation. The containers were covered with muslin cloth and tied with rubber bands to avoid larval escape. The containers were kept in ambient conditions (27± 1°C, 75± 2 % RH and 14L:10D h photoperiod) until emergence of adults. Adult moths emerging from the containers were collected and released into net cages (1×1×1 m). The freshly emerged moths were provided with honey solution (0.5%) on cotton wads ad libitum. For supporting egg laying, 40-50 days old healthy tomato plants (Sahoo TO-3251, Syngenta India Limited) grown in polybags

(6" diameter) were placed in a cage on a regular basis. From these infested plants the pre-pupae were collected and placed in insect culture cage (90×50×50 cm) for further adult emergence under laboratory conditions as mentioned above. Similarly serpentine leaf miner (*L. trifolii*) infested tomato leaves were also collected from experimental fields and farmers' fields and maintained in the laboratory. The infested leaves were placed in plastic containers lined with blotting paper to avoid moisture accumulation. These containers were kept at ambient conditions until adult emergence (27±1°C, 75±2% RH and 14L:10D h photoperiod). The freshly emerged leaf miner adults were provided with honey solution (0.5%) on cotton wads ad libitum. *T. achaea* culture was procured from the mass rearing laboratory of ICAR-NBAIR as and when required during experimentation.

Tomato seeds (Sahoo TO-3251, Syngenta India Limited) were sown in seedling trays and were placed in net cages to avoid pest infestations. The host plants were maintained in polybags after transplanting following standard agronomic practices without any pesticide application. To avoid pest infestation, regular water sprays were given at frequent intervals and maintained in net cages. The selected host plants viz., Tomato (*Solanum lycopersicum*) healthy (uninfested plants), conspecific (*Tuta absoluta* oviposited on *Tuta absoluta* infested) plants, heterospecific (*Tuta absoluta* oviposited on *Liriomyza trifolii* infested) plants were maintained in the poly bags (6" diameter) in polyhouse.

Laboratory experiment of choice and no-choice assays were conducted to know the parasitization potential of *T. achaea* during 2018-2019. Fresh eggs (30 numbers) of *T. absoluta* were maintained (excess eggs were removed) on healthy, conspecific and heterospecific infested tomato plants of 45-day old using camel hair brush. These plants were placed in net cages and were replicated five times. *T. achaea* (10 numbers) collected into the test tube with the help of an aspirator were released into net cages for parasitization. Parasitized eggs were identified by change of colour from pale yellow to black. Based on number of parasitized eggs, parasitization (%) was calculated.

Olfactometer studies were conducted to test the response of *T. achaea* to the volatile compounds of selected host plants. Female parasitoids were subjected to the following tests (dual choice assays) in Y-arm olfactometer: healthy plants v/s conspecifics; healthy plants v/s heterospecifics; conspecifics v/s heterospecifics. An olfactometer with a 1 cm internal diameter, 15 cm main length and 15 cm long side arms was used. Air was pumped through the activated charcoal filter and humidified by passing it through a bottle with distilled water before being directed into the two arms of the olfactometer. The airflow was adjusted to 30 ml/ min. The olfactory stimuli were obtained by impregnating 10 µl of volatiles on to separate filter papers strips (Whatmann No.1, 5 cm length × 0.75 cm width). The solvent was allowed to evaporate for 1 min before placing the filter papers inside the arms of the olfactometer. *T. achaea* adults (30 numbers) were released at the entrance of the main arm and left for five minutes to make a choice. In all bioassays, after each run, the olfactometer was rotated by 90° to avoid any directional bias. The whole setup was covered with a muslin cloth to avoid light. After five replicates, the olfactometer was thoroughly washed with methanol and oven dried at 120°C (Milonas et al., 2019).

OIPV's were provoked by releasing ten *T. absoluta* females into cages containing healthy tomato plants, conspecifics and heterospecifics separately. Healthy plants were used as control. Five plants were used in each treatment. Volatiles were collected from healthy (uninfested), conspecific infested plants and heterospecific infested tomato plants. A single potted tomato plant was placed inside an autoclave bag where the soil part was covered with aluminium foil to avoid volatiles from roots and soil. Gentle stream of air was blown through an activated charcoal cartridge @ 30 ml/ min. It was allowed to pass over the samples held in an autoclave bag. The odour laden air was trapped in a glass tube containing Porapak-Q adsorbent (Supelco) 30 mg (50-80 mesh) with glass wool on either side as stoppers. The collection was made for 6 hrs. The trapped volatiles in the adsorbent were eluted with HPLC grade Hexane (400 µL) and condensed to 200 µL by passing a gentle stream of ultra-high pure nitrogen. Sample vials with extracts were stored in -20°C until analysis by gas chromatography coupled mass spectrometry (GC-MS). In each volatile collection, the system was cleaned to prevent contamination. The adsorbent vial was first cleaned 5 times with dichloromethane, then cleaned 5 times with methanol and finally 3 times again with hexane. To ensure that the system becomes free of contaminants before volatile collection, blank-run was obtained each time. The blank collection was eluted as mentioned above and tested in GC-MS. Collected volatile samples were chemically identified using GC-MS at Chemical Ecology laboratory, ICAR-NBAIR, Bengaluru. GC-MS analyses were

performed on a 7890A gas chromatograph system (GC) (Agilent technologies) interfaced with a 5975C mass spectrometer detector (MSD) (Agilent technologies) with triple axis detector (electron impact ionization, 70 eV) through a HP-5 MS phenyl siloxane capillary column (30 m × 0.25 mm) (J&W Scientific, Folsom, California). The temperature of the column and oven were maintained at 40°C for 1 min. and then increased @ 20°C/ min to 280°C and held at 300°C for 10 min. The injector and column temperatures were 250°C. The total run was for 36 min. The mass spectrometry data library was NIST. 17 and MS search 2.0 software was used in the analysis. Internal standard Bromodecane 100 ng was used. One micro litre of concentrated volatile was injected into the machine using a Hamilton micro syringe.

Coupled gas chromatography-electroantennography detection (GC-EAD) analyses for collected odour samples were performed on a 7890A GC-MS system (Agilent technologies) through flame ionization detector (FID). The split injector with the split ratio of 50:1 was used and HP 5% phenyl methyl siloxane capillary column (30 m length: 0.25 mm inner diameter: 0.25 µm film thickness) was used for the separation of compounds and a FID (250°C) which was ignited using high purity hydrogen gas and reference gas zero air (99.999%) (White and Chamber, 1989). Carrier gas was Helium (99.99% purity) (0.5 kg/ cm^2) and injection temperature was 250°C and split mode was used. The GC temperature program started at 40°C for 5 min, increased to 280°C at the rate of 10°C/ min, and held for 20 min. The effluent emerging from the column were split using Agilent splitter with makeup gas and the effluents were sent to the (electroantennography detection) EAD and FID (1:1) through a glass wool column of 0.25 mm thickness for the response of antenna to the effluents. One µL of the sample was injected into the injector and traces were recorded using the Agilent Chem Station. Glass capillaries filled with 0.1 N NaCl were used as electrodes. Silver wires were used for electrical contact. The base of the abdomen of the female wasp was mounted on the reference electrode and the top of the antenna was placed in the recording electrode. Electrodes were put in the appropriate holder and connected to the probe. The mounted insect was placed 0.5 cm from the end of the glass tube. Five successful GC-EAD recordings with different female antennae were performed. The traces from FID and EAD were plotted and the matching peaks along with their retention times were measured and recorded in a computer attached to the EAG system as per (Anastasaki et al., 2018). Chi-Square test was used for the analysis of Y-tube olfactometer studies using SPSS version 25.

RESULTS AND DISCUSSION

In no-choice assays, observations on parasitization (%) of *T. achaea* on *T. absoluta* eggs from selected host plants viz., healthy, conspecific infested and heterospecific infested plants revealed more parasitization (%) as compared to healthy plants (69.33%) followed by conspecific infested (63.33%) plants. Parasitization was very low on heterospecific infested plants (36.00%) (Table 1). There was a significant difference between healthy and heterospecific infested plants; conspecific infested and heterospecific infested plants, whereas parasitization was not statistically different in healthy plants and conspecific infested plants. Interestingly, similar results were observed under choice-assays also (Table 2). Highest parasitization (%) was observed on healthy plants (66.66%) followed by conspecific infested (64.00%) and heterospecific infested plants (27.33%). *T. achaea* were not allured towards the heterospecific infested plants, as the EAD active compounds responsible for attraction of the parasitoids were found to be lacking.

Olfactometer studies of dual choice assays revealed that the parasitoids were attracted to both healthy and conspecific infested tomato plants which was statistically not significant (χ^2=0.36, df =1, p= 0.54); significant difference was observed between healthy and heterospecific infested plants (χ^2=3.55, df =1, p= 0.05). Females of *T. achaea* chose conspecific oviposited plants over heterospecific oviposited plants and the

Table 1. Mean parasitization (%) of *T. achaea* against *T. absoluta* eggs in No-Choice assays

Treatment	Mean	Std. error	Std. deviation	Mean parasitization (%)
Healthy	20.8000[a]	1.20000	2.68328	69.33
Conspecifics	19.0000[a]	1.37840	3.08221	63.33
Heterospecifics	10.8000[b]	1.24097	2.77489	36.00

Table 2. Mean parasitization (%) of *T. achaea* against *T. absoluta* eggs in Choice assays

Treatment	Mean	Std. error	Std. deviation	Mean parasitization (%)
Healthy	20.0000[a]	1.64317	3.67423	66.66
Conspecifics	19.2000[a]	1.71464	3.83406	64.00
Heterospecifics	8.2000[b]	1.80000	4.02492	27.33

Table 3. Volatile profile of healthy tomato plants

Sl. No.	Retention time	Name of the compound identified
1.	4.249	2-pPyrrolidinone
2.	7.547	3-hHexanone
3.	8.238	3-hHexanol
4.	9.505	p-xXylene
5.	11.243	(1R)-2,6,6-tTrimethylbicyclo[3.1.1]hept-2-ene
6.	13.149	(+)-4-cCarene
7.	13.253	alpha-pPhellandrene
8.	14.949	dDodecane
9.	16.489	4-methyl-Undecane
10.	21.106	Pentadecane
11.	22.341	2-methyl-Tetradecane
12.	32.141	2,6,10,14-tetramethyl-hHexadecane
13.	33.931	Pentacosane
14.	35.994	2,4-bis(1,1-dimethylethyl)-pPhenol

Table 4. Volatile profile of heterospecifics (*Tuta absoluta* oviposited on *Liriomyza trifolii* infested tomato plants)

Sl. No.	Retention time	Name of the compound identified
1.	3.161	2-pPyrrolidinone
2.	5.391	Cyclohexane
3.	6.155	4-methyl-3-hHexanol
4.	7.222	3-hHexanone
5.	8.594	3-hHexanol
6.	34.727	17-pPentatriacontene
7.	35.669	Dotriacontane
8.	36.392	Tetratriacontane

difference was significant (χ^2=3.84, df =1, p= 0.04). Results revealed that *T. achaea* discriminated between healthy, conspecific and heterospecific oviposited plants. Similar results were reported by (Fatouros et al., 2005). The findings were in contrast with Gontijo et al. (2019) where, *T. achaea* females were not attractive to tomato plants with eggs of *T. absoluta*.

Volatiles collected from the selected plants were analyzed using GC-MS. GC-MS results indicated that there was a lot of variation in the volatile profiles after the plants being oviposited by *T. absoluta* and *L. trifolii* compared to healthy plants. Volatile profiles from healthy plants were represented in Table 3; conspecific infested plants were tabulated in Table 5 and heterospecific infested plants were indicated in Table 4. Studies indicated that healthy plants released nearly 14 volatile organic compounds whereas, conspecific and heterospecific infested plants released 17 and 8 volatile compounds, respectively. The assessment was taken up to know the volatile organic compounds released by plants under various treatments. Certain biochemical analyses need to be carried out to find further details. Chromatogram of healthy plants, conspecific and

Table 5. Volatile profile of conspecifics (*Tuta absoluta* oviposited on *Tuta absoluta* infested tomato plants)

Sl. No.	Retention time	Name of the compound identified
1.	6.016	alpha-pPhellandrene
2.	6.345	o-cCymene
3.	6.414	D-lLimonene
4.	6.524	trans-beta-oOcimene
5.	6.900	gamma-tTerpinene
6.	8.563	4-ethyl-bBenzaldehyde
7.	8.910	Naphthalene
8.	9.776	D-cCarvone
9.	9.898	2-methyl-5-(1-methylethyl)-pPhenol
10.	10.325	1-(4-ethylphenyl)- eEthanone
11.	11.284	Copaene
12.	11.347	3-aAllyl-6-methoxyphenol
13.	11.937	Methyl eugenol
14.	12.295	Caryophyllene
15.	18.123	n-hHexadecanoic acid
16.	19.804	Oleic Acid
17.	21.959	Pentacosane

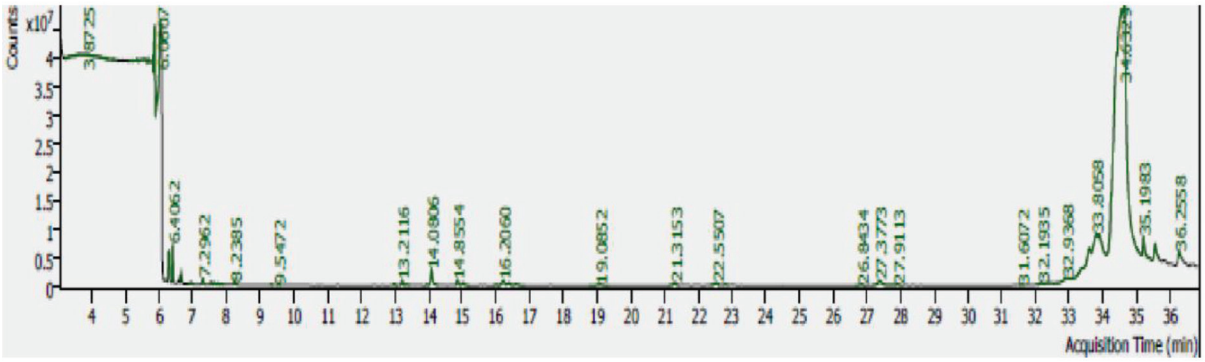

Fig. 1. Chromatogram of healthy tomato plants

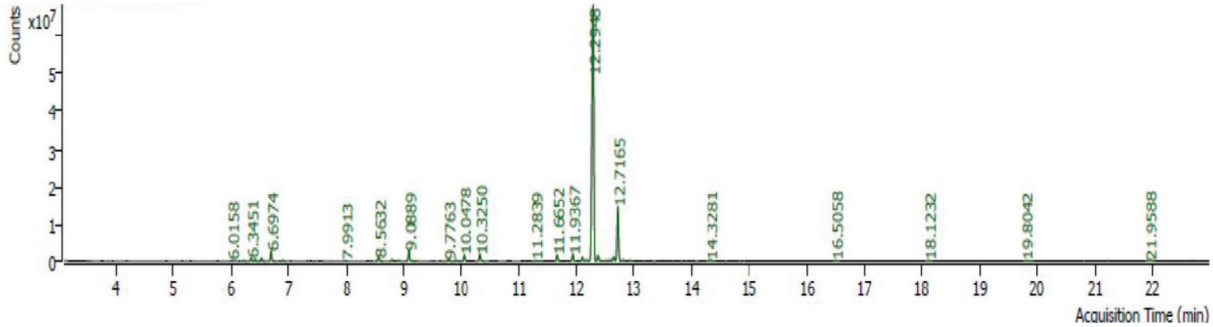

Fig. 2. Chromatogram of *T. absoluta* oviposited tomato plants

heterospecific infested plants are represented in Fig. 1, Fig. 2 and Fig. 3 respectively. The profile reported here is in accordance with Buttery et al. (1987).

Electrophysiologically active compounds in response to *T. achaea* was analyzed using gas chromatography-electro-antennographic detector (GC-EAD). Results indicated that *T. achaea* females responded to volatiles from tomato plants after herbivore attack as well as to healthy plants. EAD active compounds were 3-hexanol, α-phellandrene, trans-β-ocimene, pentacosane and γ-terpinene. However, parasitoids did not respond to the

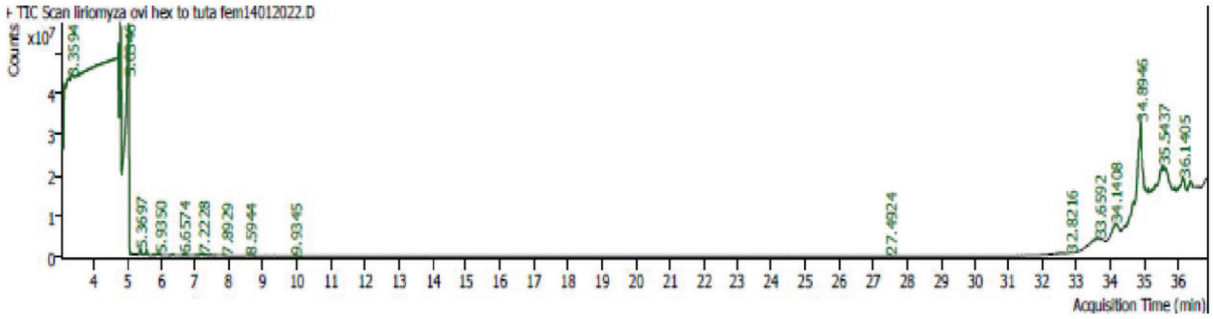

Fig. 3. Chromatogram of *L. trifolii* oviposited tomato plants

compounds like o-cymene, D-limonene, caryophyllene, D-carvone, methyl eugenol, copaene, dodecane, 3-hexanone and (+)-4-carene. Results indicated that the antenna perceive these chemicals which can act as either attractants or repellents. These findings were corroborated with Milonas et al. (2019) who reported 3-(Z)-hexen-1-ol, β-pinene, β-myrcene, nonanal, decanal, γ-terpinene as EAD active compounds.

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(Paper presented: February, 2021;
Peer reviewed, revised and accepted: April, 2022; Online Published: May, 2023)
Online published (Preview) in www.entosocindia.org and indianentomology.org (eRef. No. NWRABNRG01)