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Table : 5, Figs : 4

Effects of allelochemicals from leachates of larvae of *Leucinodes orbonalis* Guenee and leaves of Brinjal, Chilli and Tomato on the foraging behaviour potential of Trichogrammatids

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ABSTRACT

We studied the effects of hexane eluted allelochemicals pool (i) from larvae of *Leucinodes orbonalis* Guenee and (ii) leaves of brinjal (Pusa Hybrid 5) (*Solanum melongena* L.), chilli (WS-632) (*Capsicum annum* L.) and tomato (NS-563) (*Solanum lycopersicum* L.) during vegetative stage grown in laboratory (VSL) and field (VSF) on the foraging behaviour of three Trichogrammatids. The foraging behaviour of *Trichogramma pretiosum* Riley was stimulated by leachates of *L. orbonalis*, brinjal (VSF) and chilli (VSL and VSF), whereas that of *Trichogramma japonicum* Ashmead was influenced by brinjal (VSL) and tomato (VSL) leachates. The compounds present in the leachates were identified by gas chromatography-mass spectrometry (GC-MS) and were: Heptacosane (C_{27}) in *L. orbonalis*, brinjal (VSL and VSF) and chilli (VSL and VSF), Tricosane (C_{23}) in *L. orbonalis*, brinjal (VSL and VSF) and chilli (VSL) and Docosane (C_{22}) in brinjal (VSL), chilli (VSL and VSF) and tomato (VSL), the most preferred leachates by Trichogrammatids. Thus, these compounds could provide effective biocontrol of Lepidopteran insects in Solanaceous crops by stimulating the foraging behaviour of *T. pretiosum*.

Key words: Alkanes, brinjal, *Capsicum annum*, chilli, *Leucinodes orbonalis*, natural enemies, parasitization, parasitoid activity index, pest, *Solanum lycopersicum*, *Solanum melongena*, tomato, *Trichogramma*

INTRODUCTION

Solanaceae is important family of vegetable crops, but its members are heavily infested by Lepidopterous pests. *Leucinodes orbonalis* Guenee (Lepidoptera: Pyralidae) is major shoot and fruit borer of this family, especially of brinjal crop (28,57) and causes 60-80 % damage (38,40). Its control is great challenge for the vegetable farmers because of its feeding habits. Non-judicious application of chemical pesticides in crops to control pests has created several problems including the development of resistance and resurgence in key pests (20,59). The harmful inorganic compounds in pesticides also kills the insect natural enemies, pollinators and other agro-dependent organisms (3,9,21,48,56,60). To overcome such consequences, Integrated Pest Management (IPM) has now been accepted as alternative, eco-friendly and self-sustainable approach (5,14). Low foraging behaviour of natural enemies, mainly of Trichogrammatids (Hymenoptera: Trichogrammatidae), is major hindrance in IPM (1,17,54).

The foraging behaviour of Trichogrammatids is highly influenced by non-nutritional volatile organic compounds (VOCs) emitted by host plants and host insects

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(31,61,62,63). These cues act in interspecific manner and are called allelochemicals (2,7,13,16). There are 14-classes of allelochemicals *viz*., alkanes, alkenes, alkynes, fatty acids and their derivatives, terpenoids and phenylpropanoid aromatic compounds (49). The alkanes are abundant in the epicuticle of plant and insect body surfaces, which play indirect role in plant defense by recruiting insect natural enemies and in governing tritrophic interactions (22,23,34,53,67). This observation has been verified by scanning electron microscopy (SEM) and electroantennography (EAG) techniques (51,52).

Hence, this study aimed to find the influence of allelochemicals derived from (i) leachate of larval body wash of *L. orbonalis* and (ii) leaf leachates of brinjal (*Solanum melongena* L.), chilli (*Capsicum annum* L.) and tomato (*Solanum lycopersicum* L.) on (iii) behavioural changes in attractiveness and parasitization potential of 3-Trichogrammatids *i.e.*, *Trichogramma chilonis* Ishii, *Trichogramma japonicum* Ashmead and *Trichogramma pretiosum* Riley and (iv) to identify the chemical compounds in these crude leachates.

MATERIALS AND METHODS

Researches were done during 2018-19 under controlled conditions in Biological Control Laboratory and in Organic Agriculture Field of Amity University, NOIDA, India (28º 54'N latitude and 77º 33'E longitude, 212 m above mean sea level, mean rainfall: 728 mm/year annual, minimum temperature : 11 ºC and maximum temperature : 42 ºC).

Insect culture: Isofemale lines of 3-Trichogrammatids, *T. chilonis* (N.A. No. NBAII-MP-TRI-13), *T. japonicum* (N.A. No. NBAII-MP-TRI-65) and *T. pretiosum* (N.A. No. NBAII-MP-TRI-70) were procured from National Bureau of Agricultural Insect Resources, Bangalore, India. For further multiplication, Trichogrammatids were reared in our laboratory on C*orcyra cephalonica* Stainton (Lepidopter : Pyralidae) eggs (27).

Plant varieties: One hundred seeds each of brinjal (Pusa Hybrid 5), chilli (WS-632) and tomato (NS-563) were sown on 22 February, 2018 in plastic trays (8.5 cm x 45.3 cm x 47.8 cm) using sterile soilrite as growing medium in laboratory conditions [25 °C± 1, relative humidity : 70 % ± 5, photoperiod : 12 h (L/D) and light intensity 2000 lux]. Two weeks old seedlings of test crops were transplanted at 1 seedling/pot in 10-pots (63 cm x 63 cm x 50 cm) containing manured soil (60 % sandy loam + 40 % farm manure). Five pots were kept in laboratory under similar condition as mentioned above and remaining 5-pots were placed in Organic Agriculture Field, Amity University, NOIDA. Apical leaf samples from each plant were collected from time to time as per the experimental requirement.

Leaf leachates: Thirty g leaf samples from each potted crop variety were collected during the vegetative stage from both laboratory and Organic Agriculture Field. Leaves were washed with double distilled water to remove dust. These were air dried in shade at room temperature. To prepare leaf leachate, 30 g leaves were soaked in 300 mL hexane for 24 h. The leachates were filtered through Whatman no.1 filter paper. Three g anhydrous sodium sulphate was added in the filtrate and incubated at laboratory condition (25 °C± 1, relative humidity: 70 % ± 5) for 24 h. The filtrate was passed through silica gel (60-120 mesh) column. The filtrate was distilled at 60-70 ºC and the residues left at the bottom of round bottom flasks were dissolved in hexane (10 mL x 3) and collected in glass vial. Solvent was fully evaporated and the residues left were dissolved in 3 mL hexane. Out of this, 2 mL leachate was stored at -20 ºC. Rest 1 mL leachate was diluted in hexane to prepare 25 mL of 100 % working concentration (*i.e.,* C1) that was later used for gas chromatography-mass spectrometry (GC-MS) profiling and for bioassays. Aliquot of C1 was serially diluted with hexane to prepare lower concentrations: 50 % (C2), 25 % (C3), 12.5 % (C4) and 6.25 % (C5). Hexane served as control (C6) in the bioassay studies (4).

Larval body wash: $2^{nd}/3^{rd}$ instar larval stage of *L. orbonalis* (N.A. No. NBAIR-IS-CRA-01) were procured from National Bureau of Agricultural Insect Resources, Bangalore, India and reared in our laboratory for further development. Active $4^{th}/5^{th}$ instar larvae were used to prepare the larval body wash leachate in hexane. Larvae were kept at -20 ºC for immobilization for 10 min. Five g of larvae were weighed and placed in stoppered bottle. To it, 100 mL hexane was added and incubated in water bath shaker at 28 ºC, 200 rpm for 2 h and subsequently at 50 ºC, 200 rpm for 20 min. The larval body wash was filtered through Whatman no.1 filter paper. 0.5 g anhydrous sodium sulphate was added in the filtrate and incubated at room temperature for 24 h. The filtrate was passed through silica gel (60-120 mesh) column. The filtrate was distilled at 60-70 ºC and the residues left at the bottom of the round bottom flask were dissolved in hexane (10 mL x 3) and collected in glass vial. Solvent was fully evaporated and the residue left was directly reconstituted in 12.5 mL hexane to prepare 100 % working concentration (*i.e.,* C1) that was later used for gas chromatography-mass spectrometry (GC-MS) profiling and carrying out bioassays (36). Dilution factors for concentration preparation were same as in case of leaf leachate preparation.

Bioassays: The *C. cephalonica* eggs were collected and washed with hexane to remove allelochemicals from the surface of host eggs. Tricho cards were prepared by pasting 30 eggs on 2 x 2 cm^2 paper. Experimental arena comprised of 6-petri dishes (150 mm × 15 mm), each containing 6-Tricho cards arranged randomly and equidistantly towards the periphery of petri dishes. In each petri dish, 5-Tricho cards were treated with 30 µL of larval/leaf leachate as per treatments and the sixth card (control) was treated with 30 µL hexane. Ten healthy females of selected *Trichogramma spp.* were released in the centre of each petri dish and were allowed to move towards the Tricho cards. The bioassay was done at 26 °C± 2, 65 %± 5 RH and 160 lux light intensity. Orientation response of egg parasitoids was recorded every 5 min. till 45 min. After bioassays, Tricho cards were incubated at 26 °C± 2 and 65 %± 5 RH. On 5^{th} day, parasitization (PARA) was observed by counting the number of blackened eggs on each Tricho card (55).

Gas Chromatography-Mass Spectrometry: To identify hydrocarbon compounds, GC-MS analysis was done using the gas chromatography (Agilent 7890A) coupled to an Agilent 5975C inert mass selective detector with Triple-Axis Detector (single quadrupole mass spectrometer). Separation was attained using a HP-5MS column (30 m length × 0.25 mm internal diameter × 0.25 µm film thickness) coated with a non-polar phase of 5 % phenyl methyl silox. The GC column oven initial temperature was 50 °C that was held for 2 min with a ramp of 8 °C/min from 50 °C to 250 °C followed with a ramp of 10 °C/min from 250 °C to 280 °C and the final temperature was held for 28 min. Helium (99.99 %) was the carrier gas with 1 mL/min constant flow rate. 2 µL of the sample from each leachate was injected in split mode (split ratio 10:1) with an inlet temperature 260 °C and MS interface temperature 280 °C. The MS was run in scan mode from 40 Da to 650 Da, with the source and quadrupole set at 230 °C and 150 °C, respectively. The MS was operated in electron impact (EI) positive ion mode (70 eV). Tentative identification of the derived allelochemicals were achieved by comparing their elution order and mass spectra with those from Wiley mass spectral library (W9N11) (46).

Statistical analysis: Orientation responses from petri dish bioassays with 3-Trichogrammatids was tabulated and parasitoid activity index (PAI) was calculated. Parasitization (PARA) data were converted into percent parasitization (% PARA). Heterogeneous variances in raw data were subjected to normalization through transformation (Square-Root and Arc Sine) using One Way Analysis of Variance (ANOVA) (Windostat software version 8.5). Least Significant Difference (LSD) Test at 5 % level of significance was applied on ANOVA results to know the significant difference between responses at individual concentration over control. Subsequently, unpaired Student's *t*-test analysis using GraphPad (www.graphpad.com) was done to obtain two-tailed *p*-value of responses with significant difference of individual concentrations with that of control (18). In each interaction for parasitoid activity index (PAI) and percent parasitization (% PARA), % stimulation (+) / inhibition (-) over control were also calculated and represented in the text (PAI, % X_{PAI}; % PARA, % $X_{\%\ PARA}$) (39).

$$\%\ X_{PAI\ /\ \%\ PARA} = [(A_{PAI\ /\ \%\ PARA} - B_{PAI\ /\ \%\ PARA}) / B_{PAI\ /\ \%\ PARA}] * 100$$

% $X_{PAI\ /\ \%\ PARA}$ = % Stimulation (+) / Inhibition (-) over control

$A_{PAI\ /\ \%\ PARA}$ = Individual Mean or Overall Mean of PAI or % PARA

$B_{PAI\ /\ \%\ PARA}$ = Control Mean of PAI or % PARA

RESULTS AND DISCUSSIONS

Allelochemicals cues from leachates of *L. orbonalis* larval body wash and leaves of brinjal, chilli and tomato grown in pots showed different foraging behaviour of *T. chilonis*, *T. japonicum* and *T. pretiosum*.

***L. orbonalis*:** Interactions of Trichogrammatids with five concentrations of the larval body wash of *L. orbonalis* showed high foraging response (1.20± 0.07, +36.4 %; 7.58± 0.69, +86.7 %) of *T. pretiosum* over control (0.88± 0.11; 4.06± 0.00). Its parasitization response was statistically significant ($p < 0.05$). The foraging response of *T. chilonis* at individual concentrations was significant at C1 (1.68± 0.06, $p = 0.0063$, +25.4 %; 17.86± 0.58, $p = 0.0003$, +48.8 %). *T. pretiosum* showed significant foraging behaviour at C3 (1.72± 0.11, $p = 0.0003$, +95.5 %; 12.00± 0.94, $p = 0.0001$, +195.6 %) whereas, significant parasitism also occurred at C4 (9.44± 1.08, $p = 0.0005$, +132.5 %) (Table 1, Figure 1a & 1b). Herbivore-emitted volatiles alter the behaviour of parasitoids (15,37,54). In this study, we observed the elevated response of *T. pretiosum* and *T. chilonis*. *T. pretiosum* is very useful in parasitizing the eggs of *L. orbonalis* (42,47). The parasitizing efficiency of *T. chilonis* on *L. orbonalis* eggs was < *T. pretiosum* (41). However, *T. pretiosum* and *T. chilonis* are bioagents to manage *L. orbonalis* in brinjal crop fields (10,25). These are similar to our findings. *T. japonicum* provides good control of *L. orbonalis* infestation (50), however, we did not find any changes in the efficiency of *T. japonicum* to cues emitted by *L. orbonalis*.

Table 1. Foraging potential of three Trichogrammatids to *L. orbonalis* larval body wash.

Natural Enemy	Leachate Concentrations					Mean #	C6 (Control)	$CD_{.05}$
	C1 (100 %)	C2 (50 %)	C3 (25 %)	C4 (12.5 %)	C5 (6.25 %)			
Parasitoid Activity Index (PAI)								
T. chilonis	1.68± 0.06**	1.17± 0.16	1.40± 0.08	1.52± 0.06	1.17± 0.16	1.39± 0.06	1.34± 0.08	0.33
T. japonicum	1.05± 0.11	1.40± 0.08	1.77± 0.06	1.17± 0.16	2.80± 0.06**	1.64± 0.12	2.44± 0.07	0.29
T. pretiosum	1.34± 0.08**	0.79± 0.09	1.72± 0.11***	1.20± 0.11	0.97± 0.12	1.20± 0.07	0.88± 0.11	0.30
Percent Parasitization (% PARA)								
T. chilonis	17.86± 0.58***	4.06± 0.00	8.37± 1.36	9.44± 1.08	4.06± 0.00	8.76± 1.00	12.00± 0.94	2.39
T. japonicum	5.13± 1.08	9.44± 1.08	9.44± 1.08	7.29± 1.45	16.94± 3.54	9.65± 1.10	23.65± 0.45	5.54
T. pretiosum	5.13± 1.08	5.13± 1.08	12.00± 0.94***	9.44± 1.08***	6.21± 1.36	7.58± 0.69*	4.06± 0.00	3.25

{PAI/% PARA values of C1 to C6 are mean of six replicates; #: Average of the mean PAI/% PARA at individual leachate concentrations (C1 to C5); ¥: Overall mean – Control mean; $SE_{(m)}$: Standard error of the mean; $SE_{(d)}$: Standard error of the mean difference; $CD_{.05}$: Critical difference at 5 % significance level; *t*-probability at 5 % significance level ($t_{.05}$) = 2.06; Statistical significance of differences between leachate and control: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (LSD test and Student's *t*-test); The values after '±' indicates standard error}

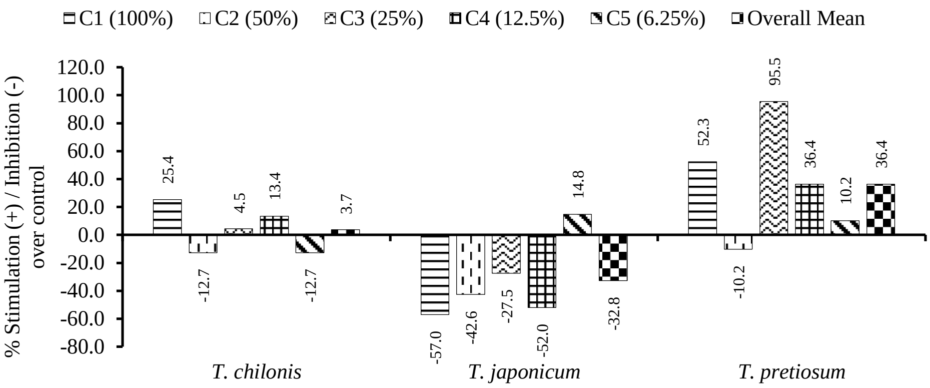

Figure 1a. Effects of *L. orbonalis* larval body wash on parasitoid activity index of three Trichogrammatids.

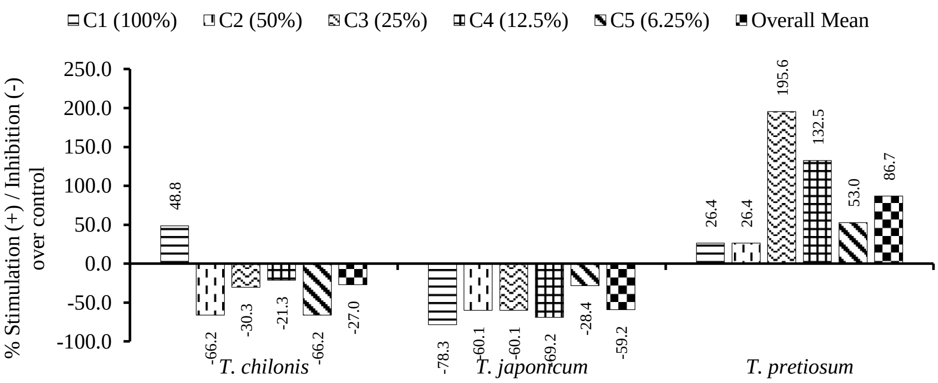

Figure 1b. Effects of *L. orbonalis* larval body wash on parasitization (%) of three Trichogrammatids.

Brinjal: Allelochemicals emitted from VSL stage of brinjal variety 'Pusa Hybrid 5' showed great attraction to *T. japonicum* (3.98± 0.05, p = 0.0001, +48.5 %; 42.99± 1.11, p = 0.0001, +59.5 %) than other two egg parasitoids. *T. japonicum* showed significant foraging response to all individual concentrations. Furthermore, the foraging behaviour of *T. pretiosum* was significant at C2 concentration (3.03± 0.04, p = 0.0001, +20.2 %; 38.58± 0.41, p = 0.0001, +21.3 %).

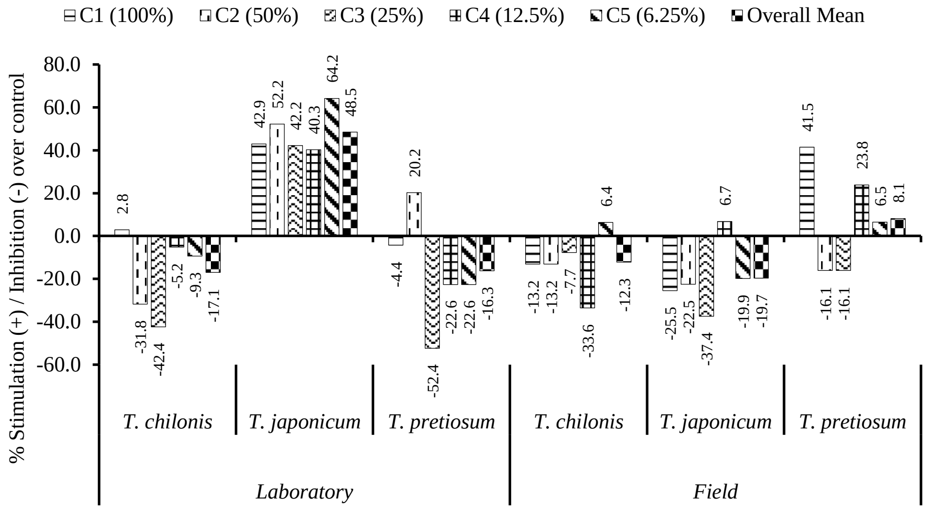

Figure 2a. Effects of brinjal variety 'Pusa Hybrid 5' on parasitoid activity index of three Trichogrammatids.

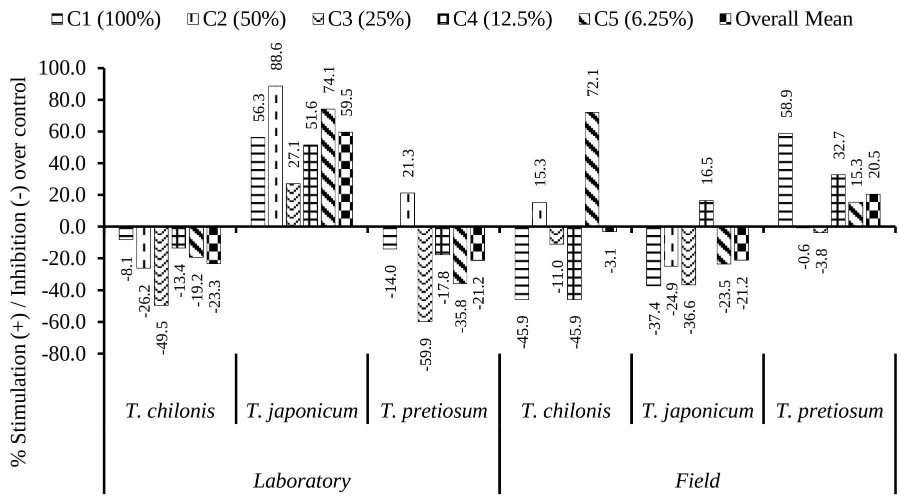

Figure 2b. Effects of brinjal variety 'Pusa Hybrid 5' on parasitization (%) of three Trichogrammatids.

In the VSF stage, overall mean responses of *T. pretiosum* were slightly higher (2.68± 0.11, +8.1 %; 31.71± 1.15, +20.5 %) than control but was statistically non-significant. At individual concentration level, C1 (3.51± 0.02, *p* = 0.0001, +41.5 %; 41.81± 0.41, *p* = 0.0001, +58.9 %) and C4 (3.07± 0.13, *p* = 0.0019, +23.8 %; 34.92± 0.34, *p* = 0.0001, +32.7 %) allelochemical cues emitted from VSF stage stimulated the foraging behaviour of *T. pretiosum*. Significant parasitization were observed at C5 cues of VSF stage by *T. pretiosum* (30.36± 0.47, *p* = 0.0001, +15.3 %). Foraging behaviour of *T. japonicum* was significantly influenced by C4 concentration of VSF stage (4.93± 0.02, *p* = 0.0001, +6.7 %; 55.76± 0.46, *p* = 0.0001, +16.5 %). Application of C5 concentration at VSF stage evoked the parasitism response in *T. chilonis* (23.20± 0.57, *p* = 0.0001, +72.1 %) (Table 2, Figure 2a & 2b). Brinjal emits VOCs which influences the behaviour of natural enemies (35). Crude foliar leachate of brinjal influences the orientation and parasitization efficiency of *T. chilonis* (30). In field trials, frequent releases of Trichogrammatids along with other IPM tools successfully controlled the insect pest in brinjal crop (50,66).

Chilli: Chilli variety 'WS-632' derived allelochemicals caused effective behavioural changes in foraging response of *T. pretiosum*. The egg parasitoid showed significant parasitoid activity index ($p < 0.001$) to cues emitted from C2, C3, C4 and C5 concentrations of VSL stage. *T. japonicum* demonstrated significant parasitoid activity index and parasitization response towards VSL cues at concentration of C3 (4.56± 0.02, *p* = 0.0001, +25.6 %; 42.77± 0.32, *p* = 0.0001, +9.9 %) and C5 (4.04± 0.02, *p* = 0.0001, +11.3 %; 46.59± 0.31, *p* = 0.0001, +19.8 %). *T. chilonis* showed significant parasitoid activity index at concentration C2 of the VSL cues (4.80± 0.02, *p* = 0.0001, +4.3 %).

Parasitoid activity index of *T. pretiosum* was high in C1, C2, C3 and C4 concentrations of VSF stage (Table 3, Figure 3a & 3b). Volatile cues emitted from chilli increases the parasitic efficiency of *T. chilonis*, *T. evanescens* and *T. ostriniae* (8,24,26,64). Our findings indicated that chilli emitted volatile cues have maximum effects on *T. pretiosum*.

Tomato: Volatile cues from tomato variety 'NS-563' leaf samples had greater effects on orientation potential of Trichogrammatids. However, *T. japonicum* showed significant foraging behaviour to C2, C3 and C4 concentrations of VSL stage than control. C2 concentration in VSL improved the foraging behaviour of *T. chilonis* (3.85± 0.03, *p* = 0.0001, +9.7 %; 45.32± 0.32, *p* = 0.0001, +10.1 %).

In the VSF stage, despite significant foraging response by *T. chilonis* (3.87± 0.03, *p* = 0.0001, +10.3 %; 55.08± 0.34, *p* = 0.0001, +15.1 %) to C3 concentration, the overall parasitization was non-significant (Table 4, Figure 4a & 4b). Tomato allelochemicals enhances the efficacy of Trichogrammatids (11,12,19,43). Ten tomato cultivars leaf leachates enhanced the effectiveness of *T. chilonis* in terms of foraging response (45). In field study, *T. japonicum* had high parasitism of *Helicoverpa armigera* eggs on tomato plants (65). Recent study on tomato variety K-21 allelochemicals also showed higher parasitism responses by *T. japonicum* (55).

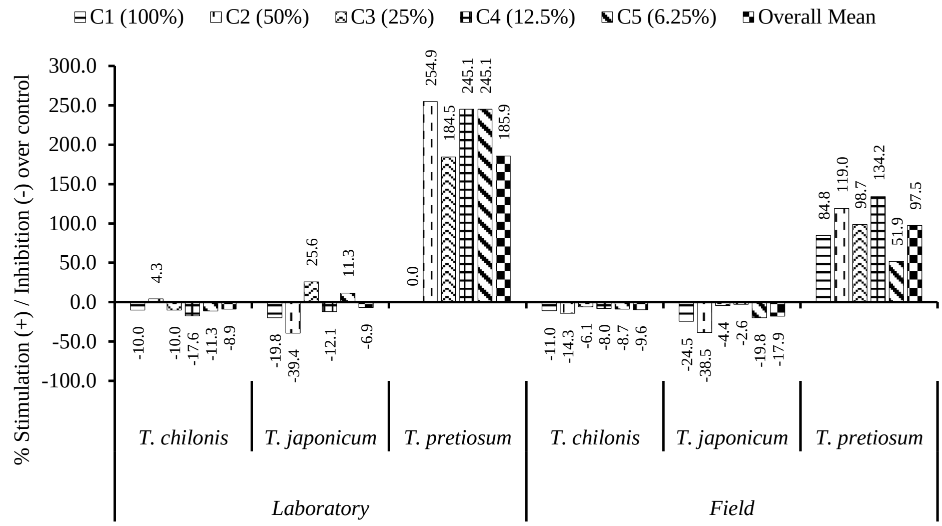

Figure 3a. Effects of chilli variety ‘WS-632’ on parasitoid activity index of three Trichogrammatids.

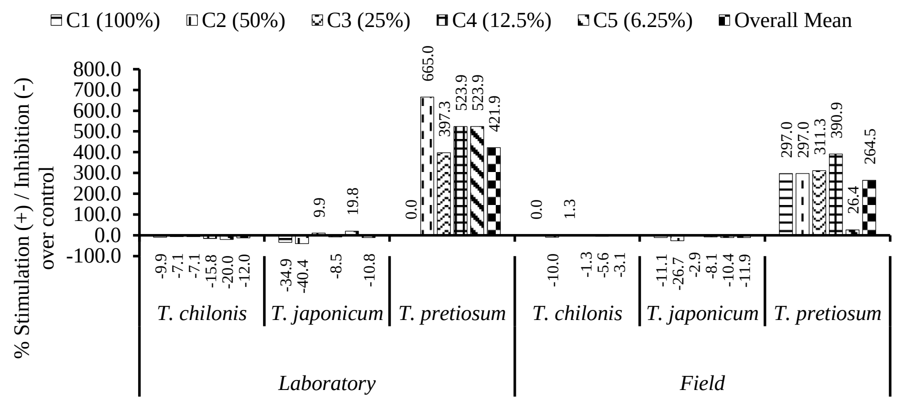

Figure 3b. Effects of chilli variety ‘WS-632’ on parasitization (%) of three Trichogrammatids.

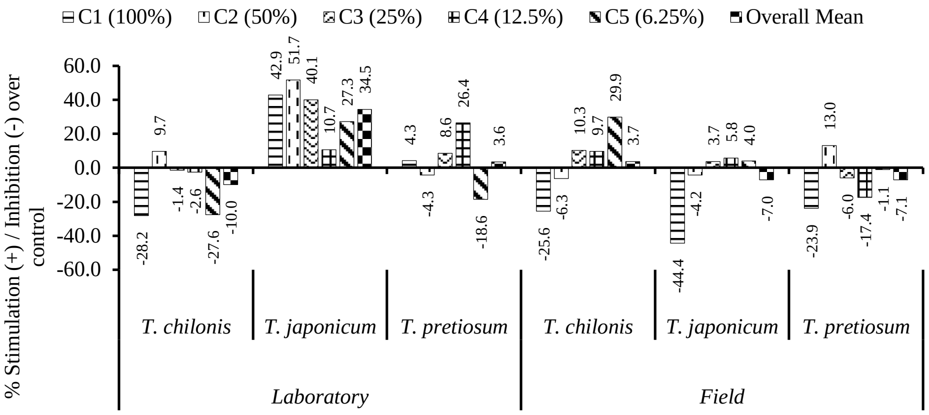

Figure 4a. Effects of tomato variety 'NS-563' on parasitoid activity index of three Trichogrammatids.

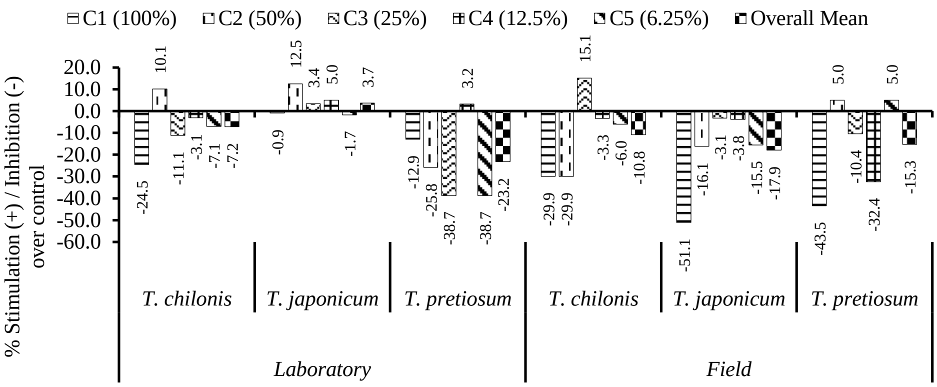

Figure 4b. Effects of tomato variety 'NS-563' on parasitization (%) of three Trichogrammatids.

Our results of the foraging behaviour confirmed that *T. pretiosum* was highly influenced by allelochemicals of *L. orbonalis* larvae, brinjal (VSF) and chilli (VSL and VSF). *T. japonicum* was efficacious to brinjal (VSL) and tomato (VSL), whereas, *T. chilonis* was least effective. Previous studies have also reported the high efficiency of *T. pretiosum* in Solanaceous crops (6,29,32), which is in accord with our results.

Table 2. Foraging potential of three Trichogrammatids to brinjal variety 'Pusa Hybrid 5' during the vegetative phase.

L/F	Natural Enemy	Leachate Concentrations					Mean #	C6 (Control)	$CD_{.05}$
		C1 (100 %)	C2 (50 %)	C3 (25 %)	C4 (12.5 %)	C5 (6.25 %)			
Parasitoid Activity Index (PAI)									
L	*T. chilonis*	3.98± 0.04	2.64± 0.04	2.23± 0.08	3.67± 0.10	3.51± 0.02	3.21± 0.13	3.87± 0.03	0.19
	T. japonicum	3.83± 0.02$^{\gamma}$	4.08± 0.02$^{\gamma}$	3.81± 0.07$^{\gamma}$	3.76± 0.03$^{\gamma}$	4.40± 0.02$^{\gamma}$	3.98± 0.05$^{\gamma}$	2.68± 0.04	0.11
	T. pretiosum	2.41± 0.04	3.03± 0.04$^{\gamma}$	1.20± 0.11	1.95± 0.09	1.95± 0.09	2.11± 0.12	2.52± 0.03	0.23
F	*T. chilonis*	1.91± 0.04	1.91± 0.04	2.03± 0.28	1.46± 0.08	2.34± 0.08	1.93± 0.08	2.20± 0.05	0.40
	T. japonicum	3.44± 0.03	3.58± 0.03	2.89± 0.17	4.93± 0.02$^{\gamma}$	3.70± 0.02	3.71± 0.13	4.62± 0.02	0.23
	T. pretiosum	3.51± 0.02$^{\gamma}$	2.08± 0.04	2.08± 0.04	3.07± 0.13$^{\beta}$	2.64± 0.07	2.68± 0.11	2.48± 0.04	0.21
Percent Parasitization (% PARA)									
L	*T. chilonis*	50.45± 0.32	40.52± 0.41	27.73± 0.52	47.55± 0.41	44.37± 0.40	42.12± 1.48	54.92± 2.38	1.21
	T. japonicum	42.13± 0.43$^{\gamma}$	50.83± 1.83$^{\gamma}$	34.24± 0.46$^{\gamma}$	40.85± 0.32$^{\gamma}$	46.91± 0.50$^{\gamma}$	42.99± 1.11$^{\gamma}$	26.95± 0.39	2.63
	T. pretiosum	27.34± 0.49	38.58± 0.41$^{\gamma}$	12.74± 0.99	26.15± 0.41	20.42± 0.63	25.05± 1.60	31.80± 0.45	1.82
F	*T. chilonis*	7.29± 1.45	15.54± 0.58	12.00± 0.94	7.29± 1.45	23.20± 0.57$^{\gamma}$	13.06± 1.19	13.48± 0.94	3.11
	T. japonicum	29.99± 0.49	35.93± 0.42	30.36± 0.47	55.76± 0.46$^{\gamma}$	36.60± 0.42	37.73± 1.76	47.87± 0.43	1.32
	T. pretiosum	41.81± 0.41$^{\gamma}$	26.15± 0.41	25.33± 0.55	34.92± 0.34$^{\gamma}$	30.36± 0.47$^{\gamma}$	31.71± 1.15	26.32± 1.91	1.28

Table 3. Foraging potential of three Trichogrammatids to chilli variety 'WS-632' during the vegetative phase.

L/F	Natural Enemy	Leachate Concentrations					Mean #	C6 (Control)	$CD_{.05}$
		C1 (100 %)	C2 (50 %)	C3 (25 %)	C4 (12.5 %)	C5 (6.25 %)			
Parasitoid Activity Index (PAI)									
L	*T. chilonis*	4.14± 0.03	4.80± 0.02$^{\gamma}$	4.14± 0.03	3.79± 0.02	4.08± 0.02	4.19± 0.06	4.60± 0.02	0.07
	T. japonicum	2.91± 0.09	2.20± 0.05	4.56± 0.02$^{\gamma}$	3.19± 0.03	4.04± 0.02$^{\gamma}$	3.38± 0.16	3.63± 0.03	0.14
	T. pretiosum	0.71± 0.00	2.52± 0.03$^{\gamma}$	2.02± 0.29$^{\gamma}$	2.45± 0.05$^{\gamma}$	2.45± 0.05$^{\gamma}$	2.03± 0.14$^{\gamma}$	0.71± 0.00	0.38
F	*T. chilonis*	3.79± 0.02	3.65± 0.02	4.00± 0.03	3.92± 0.02	3.89± 0.03	3.85± 0.03	4.26± 0.03	0.07
	T. japonicum	3.24± 0.08	2.64± 0.04	4.10± 0.03	4.18± 0.06	3.44± 0.03	3.52± 0.11	4.29± 0.11	0.15
	T. pretiosum	1.46± 0.08$^{\gamma}$	1.73± 0.07$^{\gamma}$	1.57± 0.08$^{\gamma}$	1.85± 0.11$^{\gamma}$	1.20± 0.11$^{\alpha}$	1.56± 0.06$^{\gamma}$	0.79± 0.09	0.27
Percent Parasitization (% PARA)									
L	*T. chilonis*	56.45± 0.34	58.20± 0.45	58.20± 0.45	52.74± 0.51	50.12± 0.41	55.14± 0.62	62.66± 0.49	1.27
	T. japonicum	25.33± 0.55	23.20± 0.57	42.77± 0.32$^{\gamma}$	35.60± 0.33	46.59± 0.32$^{\gamma}$	34.70± 1.73	38.90± 0.33	1.26
	T. pretiosum	4.06± 0.00	31.06± 0.79$^{\gamma}$	20.19± 3.47$^{\gamma}$	25.33± 0.55$^{\gamma}$	25.33± 0.55$^{\gamma}$	21.19± 1.84$^{\gamma}$	4.06± 0.00	4.75
F	*T. chilonis*	51.75± 0.44	46.59± 0.32	52.41± 0.33	51.10± 0.33	48.86± 1.50	50.14± 0.50	51.75± 0.44	2.17
	T. japonicum	38.58± 0.41	31.80± 0.45	42.13± 0.43	39.88± 0.41	38.90± 0.33	38.26± 0.66	43.41± 0.32	1.19
	T. pretiosum	16.12± 0.73$^{\gamma}$	16.12± 0.73$^{\gamma}$	16.70± 0.78$^{\gamma}$	19.93± 0.67$^{\gamma}$	5.13± 1.08	14.80± 0.99$^{\gamma}$	4.06± 0.00	2.36

{L: Potted plants growth location (Laboratory); F: Potted plants growth location (Organic Agriculture Field); PAI/% PARA values of C1 to C6 are mean of six replicates; #: Average of the mean PAI/% PARA at individual leachate concentrations (C1 to C5); $CD_{.05}$: Critical difference at 5 % significance level; *t*-probability at 5 % significance level ($t_{.05}$) = 2.06; Statistical significance of differences between leachate and control: $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ (LSD test and Student's *t*-test); The values after '±' indicates standard error}

Table 4. Foraging potential of three Trichogrammatids to tomato variety 'NS-563' during the vegetative phase.

L/F	Natural Enemy	Leachate Concentrations					Mean #	C6 (Control)	$CD_{.05}$
		C1 (100 %)	C2 (50 %)	C3 (25 %)	C4 (12.5 %)	C5 (6.25 %)			
Parasitoid Activity Index (PAI)									
L	*T. chilonis*	2.52± 0.03	3.85± 0.03$^{\gamma}$	3.46± 0.03	3.42± 0.02	2.54± 0.09	3.16± 0.10	3.51± 0.02	0.14
	T. japonicum	4.56± 0.02$^{\gamma}$	4.84± 0.10$^{\gamma}$	4.47± 0.03$^{\gamma}$	3.53± 0.10$^{\beta}$	4.06± 0.06$^{\gamma}$	4.29± 0.09$^{\gamma}$	3.19± 0.03	0.20
	T. pretiosum	1.46± 0.08	1.34± 0.08	1.52± 0.06	1.77± 0.06$^{\beta}$	1.14± 0.09	1.45± 0.05	1.40± 0.08	0.21
F	*T. chilonis*	2.61± 0.04	3.29± 0.03	3.87± 0.03$^{\gamma}$	3.85± 0.03$^{\gamma}$	4.56± 0.02$^{\gamma}$	3.64± 0.12	3.51± 0.02	0.09
	T. japonicum	2.38± 0.34	4.10± 0.03	4.44± 0.02$^{\gamma}$	4.53± 0.05$^{\beta}$	4.45± 0.02$^{\gamma}$	3.98± 0.15	4.28± 0.02	0.09
	T. pretiosum	1.40± 0.08	2.08± 0.04$^{\gamma}$	1.73± 0.07	1.52± 0.06	1.82± 0.05	1.71± 0.05	1.84± 0.15	0.18
Percent Parasitization (% PARA)									
L	*T. chilonis*	31.08± 0.56	45.32± 0.32$^{\gamma}$	36.60± 0.42	39.88± 0.41	38.25± 0.44	38.22± 0.88	41.17± 0.50	1.27
	T. japonicum	38.25± 0.44	43.41± 0.32$^{\gamma}$	39.88± 0.41$^{\alpha}$	40.52± 0.41$^{\beta}$	37.92± 0.41	40.00± 0.40	38.58± 0.41	1.16
	T. pretiosum	7.29± 1.45	6.21± 1.36	5.13± 1.08	8.64± 1.36	5.13± 1.08	6.43± 0.58	8.37± 1.36	3.71
F	*T. chilonis*	33.55± 0.34	33.55± 0.34	55.08± 0.34$^{\gamma}$	46.27± 0.40	45.00± 1.49	42.69± 1.56	47.87± 0.43	2.15
	T. japonicum	25.33± 0.55	43.41± 0.32	50.12± 0.41	49.80± 0.43	43.73± 0.40	42.48± 1.69	51.75± 0.44	1.25
	T. pretiosum	11.26± 0.74	20.92± 0.50	17.86± 0.58	13.48± 0.94	20.92± 0.50	16.89± 0.78	19.93± 0.67	1.96

{L: Potted plants growth location (Laboratory); F: Potted plants growth location (Organic Agriculture Field); PAI/% PARA values of C1 to C6 are mean of six replicates; #: Average of the mean PAI/% PARA at individual leachate concentrations (C1 to C5); $CD_{.05}$: Critical difference at 5 % significance level; *t*-probability at 5 % significance level ($t_{.05}$) = 2.06; Statistical significance of differences between leachate and control: $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$, $^{\gamma}p < 0.001$ (LSD test and Student's *t*-test); The values after '±' indicates standard error}

Table 5. Gas chromatography-mass spectrometry (GC-MS) analysis of allelochemicals from *L. orbonalis* larval body wash and during the vegetative stage of brinjal, chilli and tomato grown in pots in laboratory and Organic Agriculture Field.

S. No.	Compound Name	Allelochemicals Cues (%)						
		L. orbonalis	Brinjal		Chilli		Tomato	
			Lab	Field	Lab	Field	Lab	Field
1	(+-)-5-Oxo-2α,7-.alpha,19,20-tetrahydro-16-epipleiocarpamin	-	0.07	-	-	-	-	-
2	(3aS,9aS,9bR)-6,6,9aatrimethyl-transperhydronaphtho[2,1-b	0.48	-	-	-	-	-	-
3	(3S, 11S)-(+)-3,11-Dimethylnonacosan-2-one	-	-	0.51		-	-	-
4	(3SR,4SR)-4-[(RS)-1-Hydroxy-3-bentenyl]-1-(pmethoxyphenyl)	-	-	-	0.45	-	-	-
5	(4R,5R)-cis-4-[Bis(ptolylthio)methyl]-2,2-dimethyl-5-pheny	-	-	0.06	-	-	-	-
6	(4S,5R)-trans-4-[Bis(ptolylthio)methyl]-5-(4-methoxyphenyl	1.12	-	-	-	-	-	-
7	(E)-1-Methoxy-3-iodo-2-nonene	0.26	-	-	-	-	-	-
8	(S)-(E)-(-)-4-Acetoxy-1-phenyl-2-dodecen-1-one	-	-	0.07	0.16	0.51	0.78	0.35
9	1-(2-Hydroxyethoxy) tridecane	1.11	-	-	-	-	-	-
10	1-(Pent-4-ynyl)pyrano[3,4-b]indol-3-one	-	-	-	0.14	-	-	-
11	1,1,3,3,5,5,7,7,9,9,11,11,13,13-Tetradecamethylheptasiloxane	-	-	-	-	0.16	-	-
12	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethylhexasiloxane	0.36	0.08	0.06	0.24	0.19	0.50	-
13	1,1,4,4-Tetraphenyl-3-buten-2-one	-	-	-	0.13	-	-	-
14	1,1-Bis(2'-carboxy-5'-furfuryloxy)methane	0.35	-	-	-	-	-	-
15	1,2,3-Tri (t-Butyl)cyclopropenylium hydrogenedichloride	-	-	0.05	-	-	-	-
16	1,2,3-Tri(t-Butyl)cyclopropenyliumtribromide	0.31	0.09	-	-	-	-	-
17	1,2-Benzenedicarboxylicacid, butyl octyl ester	-	-	-	0.40	-	-	-
18	1,2-Dihydro-1,4-diphenylphthalazine	0.45	0.15	0.10	0.90	0.31	0.48	0.61
19	1,3-Bis(4-chlorobenzyl)-5,6-dihydrobenzo[f]quinazoline	-	-	-	-	4.23	-	-
20	1,3-Dimethyl-2,4-dioxo-6-methyl-8-(p-nitrophenyl)-1,2,3,4-t	-	0.07	-	-	-	-	-
21	13-Dehydroxy-13,14-iminopseudaconine	-	-	0.59	-	-	-	-
22	14α-Cheilanth-13(14)-enic Methyl Ester	-	-	-	-	0.23	-	0.16
23	1-Acetyl-2-phenyl-4,6-diisopropyl-1,2-dihydropyrimidine	-	-	-	0.13	-	-	-
24	1-Amino-1-orthochlorophenyl-2-(2-quinoxalinyl)ethene	-	-	0.07	0.18	-	0.33	-
25	1-Azido-2-(propenyl)benzene	-	-	-	0.31	-	-	-
26	1-Chloro-1-(diphenylphosphoryl)-3-methyl-1,2-pentadiene	-	0.06	-	-	-	-	-
27	1-Dotriacontanol	-	0.06	-	-	-	-	-
28	1-Hexene, 5,5-dimethyl-	-	-	-	-	-	-	0.17
29	2-(1,3-Dioxolan-2-yl)-1-(2-furyl) ethene	0.29	-	-	-	-	-	-
30	2-(2-Amino-4,5-dimethoxyphenyl)-6,7-dimethoxyquinoline	-	0.19	-	-	-	-	-
31	2-(3',5'-Ditrifluoromethylphenyl)-1,1,3,3-tetramethylguanid	-	-	-	-	-	0.28	-
32	2,2',3,3'-Tetrahydroxy-5,5'-dimethyl-6,6'-dinitrobiphenyl t	-	0.07	0.31	-	0.70	-	-
33	2,3,3aα,4α,6,7,11bα,11cα-Octahydro-9,10-dimethoxy-2-methyl-	-	-	-	-	-	-	2.04
34	2,3-Bis(3'-Methoxy-2'-nitrophenylimino)-	-	0.06	-	-	-	-	-

	2Hindole							
35	2,3-Dihydro-1,3,3-trimethyl-2-[N-(methylsulfonyl)imino]-5-p	-	-	-	-	-	-	0.17
36	2,4-Diamino-(E)-6-[2-(3-fluoromethylphenyl)ethenyl]-1,3,5-t	0.27	-	-	-	-	-	-
37	2,6,10-Trimethyl-tridecane	-	-	-	0.31	-	-	-
38	2-Naphthalene-sulfonicacid	-	0.07	-	-	-	-	-
39	3-(Diethoxyphosphorylthio)-3'-ethylthio-4,4',5,5'-tetrameth	-	-	0.15	-	-	-	-
40	3-(Ethoxycarbonyl)-2-phenyl-5-[3'-methyl-4'-(methoxycarbony	-	-	-	-	-	-	0.19
41	3,4-Methylenedioxy-2-trimethylsilylbenzenecarbaldehyde	-	0.08	-	-	-	-	-
42	3,5-Bis(p-Dimethylaminostryl)-2,2-dimethyl-2H-pyrrole 1-Oxi	-	-	-	0.43	-	-	-
43	3-Acetoxy-7α-(acetylthio)-17α-pregna-3,5-diene-21,17-carbol	-	-	-	-	-	0.07	-
44	3-Methyl-1-decen-4-ol	-	-	0.12	-	-	-	-
45	3-Phenoxy-1-[(β-naphthyl)seleno]-2-propanol	0.31	0.07	-	-	-	0.39	-
46	3α,10α-Dihydroxyantherida-6(8),16-diene-7,19-dioicAcid	-	0.06	-	-	-	-	-
47	3α-Hydroxymethyl-2,2-dimethylpenam S,Sdioxide	-	-	-	0.57	-	-	-
48	4-(2',6'-Dichlorophenylmethylene)-1,2,3,4-tetraisoquinoline	0.30	0.08	-	-	-	-	-
49	4-(Dimethylamino)azoestrone 3-methyl ether	0.44	0.07	-	0.17	0.16	-	-
50	4,6-Dimethoxy-3-formyl-2-phenylindole	0.29	-	-	0.16	-	-	-
51	4-Methyldocosane	0.37	-	-	-	-	-	-
52	4-Methylene-1-benzyl-3-phenyl-5,6-dimethyl-1H,3H,6H-pyrimid	-	-	-	0.29	-	-	-
53	4-s-Butyl-3-fluoro-1H-2-benzopyran	-	-	-	-	-	0.64	-
54	5,5''-Diethynyl-2,2':6',2''-terpyridine	1.49	0.21	0.16	1.00	0.26	0.86	39.11
55	5,6-Dihydro-2,3,10,11-tetramethoxyisoquino[1,2-b]quinazolin	-	0.47	0.28	0.28	0.22	0.66	-
56	5-Chloro-3-(3,4-dimethoxyphenyl)-6-methyl-2H-1,4-oxazin-2-o	0.33	0.11	-	-	-	-	0.17
57	6-[(2-Oxocyclohexyl)methyl]-4-oxa-5-azaspiro[2.4]hept-5-ene	0.44	-	-	-	-	-	-
58	6-[N-(4-Fluorophenyl)imino-N'-(4-fluorophenyl)aminomethyl]b	0.30	-	-	0.34	-	-	-
59	6-Carbamoyl-2-(diethylamino)-5methyl-4H-thieno[2,3-d][1,3]o	0.48	0.11	0.08	0.30	0.33	1.13	0.37
60	6-Chloro-4-oxo-4H-1-benzopyran-3-carboxaldehyde 3-Omethyl	-	0.07	-	0.21	0.24	-	-
61	6-Ethyl-5-(4'-trifluoromethylphenyl)pyrimidine-2,4-diamine	0.36	0.07	0.07	0.28	0.20	0.49	0.18
62	7,9-(p-Methoxyphenylidenedioxy)-5-methoxy-2,4,6,8-tetraamet	0.68	-	0.05	-	-	-	-
63	8-Nitro-4-phenyl-1H-1,5-benzodiazepin-2(3H)-one	-	-	-	0.16	-	-	-
64	9[3'-(N,NDimethylamino)propylamino]-2,4-dimethoxyacridine	-	-	-	0.23	-	-	-
65	Allyl Cyclohexyl carbonate	0.49	-	0.27	-	-	-	-
66	Bis(2-ethylhexyl)phthalate	-	-	2.38	8.83	-	43.97	18.79
67	Butane, 2,2,3-trimethyl-	-	0.07	-	-	-	-	-
68	CIS-CARYOPHYLLENE	-	0.23	-	-	-	-	-
69	Cyclodecasiloxane,eicosamethyl-	-	5.14	6.17	-	-	-	-
70	Cycloheptasiloxane,tetradecamethyl-	-	7.66	6.59	-	5.97	-	-
71	Cyclohexasiloxane,dodecamethyl-	-	1.80	1.13	-	1.03	-	-

72	Cyclononasiloxane,octadecamethyl-	-	6.51	7.47	-	6.70	0.29	-
73	Cyclooctasiloxane,hexadecamethyl-	-	8.18	8.76	-	7.82	0.39	-
74	Di-(2-ethylhexyl)phthalate	-	4.41	-	-	4.47	-	-
75	Di-isodecyl phthalate	-	-	-	0.33	-	-	-
76	Diisooctyl phthalate	26.81	-	-	-	-	-	-
77	Di-n-decylsulfone	-	-	-	-	-	-	1.07
78	Docosane	-	0.06	-	0.51	1.92	8.63	0.83
79	Docosanoic acid, 1,2,3-propanetriyl ester	-	0.08	-	0.17	-	-	-
80	Eicosane	-	0.54	-	-	-	-	-
81	Heptacosane	10.11	1.92	0.73	14.83	1.71	-	2.88
82	Heptadecane, 2-methyl-	-	-	-	0.24	-	-	-
83	Methyl (2R*,3'R*,12bR*)-(+-)-1,2,6,7,12,12bhexahydro-2-(te	-	-	-	-	-	0.07	-
84	Methyl (3S*,4R*)-2,3,4,5-tetrahydro-4-methyl-1,5-dioxo-1H-b	-	-	-	0.71	-	-	-
85	Methyl 5-nitro-2,11-dioxocycloundecane-1-carboxylate	0.48	-	-	-	-	-	-
86	Methyl ester of decyclotrenudine	-	-	-	0.25	-	-	-
87	Methylhexadecylcarbonylhydroxamate	-	-	-	0.20	-	-	-
88	Methylsulfinato[2,3,7,8,12,13,17,18-octaethylporphyrinato]i	-	-	-	0.18	-	-	-
89	N(1)-{4'-[3"-Oxo-4"-(pfluorophenyl)-3",3"a,4",5"-tetrahydr	-	-	-	-	4.97	-	-
90	N,N'-Dicyclohexyl-1-cyano-7-pyrrolidinylperylene-3,4:9,10-t	-	-	-	0.15	-	-	-
91	N1-Acetyl-N2-(1-oxoprop-2-en-1-yl)hydrazide	-	-	-	0.15	-	-	-
92	N-Ethyl-1,3-dithioisoindoline	0.32	0.08	0.11	0.21	-	1.02	-
93	Nonacosane	-	-	-	-	-	-	33.80
94	Nonadecane	-	0.09	1.45	0.81	-	-	-
95	Nonahexacontanoic acid, methyl ester	0.27	-	-	0.31	-	-	-
96	Nonanal	-	0.06	-	-	-	-	-
97	Pentacarbonyl{[(1'R*,1S*,2S*,3R*)-2-[hydroxy(phenyl)methyl]	-	-	-	-	-	-	0.13
98	Pentacosane	-	-	-	11.00	-	-	-
99	Pentadecane	-	0.10	-	0.43	-	-	-
100	Pentane, 2,2,4-trimethyl-	-	-	-	0.17	-	-	0.16
101	Pentane, 3-methyl-	-	-	-	-	-	0.54	-
102	Phenyl 4-[bis(ethoxycarbonyl)but-3-ynyl]-2,3,4-trideoxy-α,L	1.21	0.35	0.97	0.45	0.55	0.95	1.43
103	Propiolic acid	-	-	-	-	-	0.01	-
104	Tetracosamethylcyclododecasiloxane	-	6.69	6.24	-	5.62	-	-
105	Tetracosane	-	1.68	-	-	-	-	-
106	Trans-1-methylene-2-hydroxy-8-methoxycarbonyl-5,9-(2',2'-di	-	-	-	0.17	-	-	-
107	Triacontane	-	-	-	-	-	-	11.49
108	Tricosane	9.99	1.49	6.81	1.16	-	-	-
109	Tri-o-trimethylsilyl, Npentafluoropropionyl derivative	-	-	-	-	-	0.93	-

Field: Organic Agriculture Field; - : Compounds not detected

GC-MS analysis: GC-MS chromatogram analysis of 7-targeted hexane leachates revealed the presence of saturated and unsaturated hydrocarbons, fatty acid derivatives (aldehydes, ketones, alcohols, ethers, esters and carboxylic acids) and heterocyclic aromatic organic compounds. Among these, we focussed on straight chain alkanes, as these are potential allelochemical cues which influences the behaviour of Trichogrammatids (15,36). In our test on pest and crops, there were 10-straight chain alkanes [Pentadecane (C_{15}), Nonadecane (C_{19}), Eicosane (C_{20}), Docosane (C_{22}), Tricosane (C_{23}), Tetracosane (C_{24}), Pentacosane (C_{25}), Heptacosane (C_{27}), Nonacosane (C_{29}) and Triacontane (C_{30})]. The number and concentration of identified alkanes varied in the 7-leachates (Table 5). Higher foraging responses of *T. japonicum* and *T. brasiliensis* were observed to Tricosane (C_{23}) aiming five test Trichogrammatids (58). In *Spodoptera litura* infested chilli crop, Tetradecane (C_{14}), Pentacosane (C_{25}), Hexacosane (C_{26}) and Heptacosane (C_{27}) were identified as active stimulatory components for *T. chilonis* (64). Tricosane (C_{23}) also proved the most favourable cue among 11-saturated hydrocarbons selected as allelochemical source for *T. chilonis* (44). Occurrence of Heptacosane (C_{27}), Tricosane (C_{23}) and Docosane (C_{22}) might be considered as potential allelochemical stimulants for *T. pretiosum*. Previous findings have also recognized the importance of alkanes as potential stimulants for Trichogrammatids (27,33).

CONCLUSIONS

The Heptacosane (C_{27}), Tricosane (C_{23}) and Docosane (C_{22}) emitted from the pest and plant sources are favourable allelochemical cues that influenced the higher foraging behaviour in *T. pretiosum* than *T. japonicum* and *T. chilonis*. There is need to assess the individual concentrations of Heptacosane (C_{27}), Tricosane (C_{23}) and Docosane (C_{22}) and their combinations to prepare stable formulations as stimulants for *T. pretiosum*, to provide an eco-friendly and self-sustainable insect pest control.

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