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Study on foraging response of Trichogrammatids governed by volatile cues

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Abstract: Volatile cues alter the behaviour of insect natural enemies. Present study focus on extraction, chemical identification of volatile cues present in tomato variety K-21 and their impact assessment on *Trichogramma japonicum* Ashmead, *Trichogramma brasiliensis* Ashmead and *Trichogramma chilonis* Ishii. For this, the targeted variety was grown in laboratory and field condition in same season by following suitable agronomical conditions. Leaf samples were collected in vegetative and flowering phase of growth for allelochemical extraction. Petri dish bioassay studies were carried out to observe the allelochemical effect of volatile cues present in hexane leaf extracts in different concentrations *viz.*, 4,00,000 ppm, 2,00,000 ppm, 1,00,000 ppm, 50,000 ppm and 25,000 ppm on targeted egg parasitoids. Observation indicated a higher foraging response by Trichogrammatids under the influence of volatile cues derived from plants of vegetative stage growing in field condition (VSF) as evidenced by parasitoid activity index (PAI) and percent parasitisation (%PARA) studies. Among the three Trichogrammatids, *T. japonicum* exhibited higher stimulation towards VSF stage of targeted variety. These extracts were also subjected to Gas Liquid Chromatography (GLC) to find out the presence of saturated hydrocarbons. Chromatogram of targeted extracts reveals the presence of favourable as well as unfavourable saturated hydrocarbons ranging from C_{22} to C_{34}; varying in numbers and concentrations. Elevated stimulation in foraging response of egg parasitoids could be ascribed to the presence of favourable volatile cues emitted by K-21.

Key words: Allelochemicals, K-21, natural enemy, parasitisation, parasitoid activity index, pests, tomato, *Trichogramma*, tritrophic interaction.

Introduction

In an agroecosystem, to feed and reproduce, insect pests and their insect natural enemies depend on volatile cues for inter-species communications (El-Wakeil *et al.* 2017; Ripper 1944; Rodriguez-Saona *et al.* 2012; Vet & Dicke 1992). The quantity and quality of blend emitted from same source may be different in varied conditions (Degen *et al.* 2004; Mccormick *et al.* 2014). These cues act as a key component in governing tritrophic interaction mechanism in an ecosystem (Dicke & Loon 2000; Hossain *et al.* 2017; Price *et al.* 1980; Yasumatsu 1971). Volatile cues, especially allelochemicals in a crop ecosystem are becoming a popular approach among professional and progressive farmers (Kelly *et al.* 2014; Van Lenteren 2000). It is reported that the foraging efficiency of insect natural enemies can be enhanced by applying allelochemicals present in plant extracts (Chailleux *et al.* 2013; Giunti *et al.* 2015; Wilson & Woods 2016).

Tomato is an important commercial vegetable crop grown worldwide. Abiotic and biotic stresses like heat, cold, heavy metal, salinity and insect infestation have become major limitations for tomato growth and yield (Amin *et al.* 2017; Singh *et*

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al. 2013). Tomato variety K-21 is a high yielding hybrid and has been a subject of research in various studies related to heavy metal stress, salinity stress and insect pest infestation. Many studies have reported K-21 as a tolerant variety for different abiotic stresses and recommended it over other cultivars (Fariduddin *et al.* 2012; Hayat *et al.* 2011). But heavy insect pest infestation in tomato is still a major concern and makes this high yielding variety a preferred choice for plant-insect interaction studies (Matsuda *et al.* 2001; Shakya *et al.* 2015).

Egg parasitoids of genus *Trichogramma* (Hymenoptera: Trichogrammatidae) has been studied to be polyphagous, most potential, widely distributed and biologically diverse natural enemies found globally (Puneeth & Vijayan 2014; Tang *et al.* 2017). Proposed study focuses on role of volatile cues present in allelochemical extract of tomato variety K-21, their positive traits against insect pest infestation and ability to attract insect natural enemies for indirect defence mechanism. Current approach of interaction between particular *Trichogramma spp.* and plant derived volatile allelochemical cues will be helpful to condemn the use of hazardous chemicals. It can be utilized as a promising tool to manage insect pest population upto a threshold level in an agroecosystem without disturbing the trophic levels.

Materials and Methods

Establishment of culture

Culture of C*orcyra cephalonica* Stainton (Lepidoptera: Pyralidae) were established in Pest Control Laboratory, Amity University Uttar Pradesh, NOIDA (U.P.), India as described by Sreekumar & Paul (2000).

Pure breed lines of Trichogrammatids (*Trichogramma chilonis* Ishii, N.A. No. NBAII-MP-TRI-13; *Trichogramma japonicum* Ashmead, N.A. No. NBAII-MP-TRI-65; *Trichogramma brasiliensis* Ashmead, N.A. No. NBAII-MP-TRI-70) were procured from National Bureau of Agricultural Insect Resources (NBAIR), Bangalore, India. Cultures of these *Trichogramma spp.* were maintained in Pest Control Laboratory, Amity University Uttar Pradesh, NOIDA (U.P.), India by following the protocol of Paul *et al.* (2002).

Allelochemical extraction

Tomato variety K-21 was grown by following agronomical practices in laboratory and field condition in same season. 30 g leaf sample was collected for extract preparation in vegetative stage of growth from plants grown in laboratory condition (VSL) as well as in field condition (VSF). Similarly, in flowering stage of growth, samples were collected from laboratory and field grown plants and referred as FSL and FSF, respectively. From all the leaf samples, hexane extracts were prepared as per method opted by Archna *et al.* (2009). From the allelochemical extract stocks, aliquots were used for preparing 4,00,000 ppm, 2,00,000 ppm, 1,00,000 ppm, 50,000 ppm and 25,000 ppm concentrations for bioassays and GLC studies.

Bioassays

Petri dish bioassays were carried out by placing six Tricho cards (30 eggs card^{-1}) in each 150 mm × 15 mm petri dishes. Five Tricho cards were treated with 30 µl of desired concentration of plant extract whereas sixth card was applied with 30 µl HPLC grade hexane as control (Srivastava *et al.* 2017).

Statistical analysis

Data obtained from interaction of various concentrations of allelochemical cues with different Trichogrammatids were tabulated as parasitoid activity index (PAI) and parasitisation rate (PARA). PARA values were converted into percent parasitisation rate (% PARA). For data analysis, three factors *viz.,* plant growth stages, *Trichogramma spp.* and concentrations of allelochemical cues were selected. Data were subjected to Three Way Analysis of Variance (ANOVA) using Windostat software version 8.5. Original data means (OM) were calculated. The original data was transformed to obtain Square-root transformation means (SRTM) of PAI and Arc sine/Angular transformation means (ATM) of %PARA. Difference between SRTM/ATM of a Trichogrammatid at individual concentrations and its control was accounted as difference of transformed means (DoTM). Comparison of the control treatment with each of the five allelochemical extract treatments was conducted using least significant difference (LSD)/critical difference (CD) test procedure. Results have been analysed using transformed values and their differences.

GLC Analysis

Hexane extract samples were analyzed for the presence of saturated hydrocarbons by Gas Liquid Chromatography (GLC) on Shimadzu GC-2010 fitted with flame ionization detector (FID) and capillary column (Rtx 5 MS, 25 m). Column temperature was 120 °C to 280 °C at the rate of 10 °C min^{-1} and column maximum temperature was 330 °C. Injector temperature was 260 °C and detector temperature 270 °C. Nitrogen was used as a carrier gas with total flow rate of 16.3 ml min^{-1}. Injection volume was 2 µl. The unknown saturated hydrocarbons were identified by comparing with C_{21}–C_{40} alkane standard obtained from Sigma Aldrich, USA. For calculating the concentration of saturated alkanes present in the plant extracts, the following formula was used (Paul *et al.* 2002):

$$C_u = (A_u \ / \ A_k)^* C_k$$

Where C and A denote concentration and area, respectively; u and k denote unknown and known saturated alkanes, respectively.

Results

Analysis based on orientation response

Results indicated that out of 60 mean PAI values (values of interactions of three Trichogrammatids with five concentrations of allelochemical cues obtained from four stages of K-21 variety), 40 exhibited a higher response than the mean PAI of their control. *T. japonicum* exhibited a highly significant orientation response in form of DoTM towards VSL at 50,000 ppm and 25,000 ppm individual concentrations (1.90 and 2.17) as well as towards VSF at 2,00,000 ppm, 50,000 ppm and 25,000 ppm individual concentrations (2.09, 2.68 and 1.93, respectively). *T. brasiliensis* showed a significantly high orientation response towards VSL at 4,00,000 ppm individual concentration (2.06). In all the tested stages, no significant orientation response was observed by *T. chilonis*.

The overall mean PAI of five individual concentrations was also found to be higher than their control mean PAI in few interactions. *T. chilonis* exhibited a higher response *i.e.*, 3.60 each towards VSF and FSL; response of *T. japonicum* was 3.61, 2.90 and 2.66 towards VSL, VSF and FSF, respectively whereas *T. brasiliensis* exhibited a higher overall mean response *i.e.*, 2.22, 1.31 and 1.18 towards VSL, VSF and FSF, respectively (Table 1).

To establish the most effective growth stage of the selected tomato plant K-21 to attract Trichogrammatids for indirect defence mechanism, final mean values of overall mean PAIs at targeted growth stages were calculated and their difference from mean PAI of control was depicted using a histogram. It clearly indicated that the selected plant allelochemicals elicited varied orientation responses in Trichogrammatids at different stages of the growth. VSF was found to be the most effective (0.71) followed by FSF (0.25) and VSL (0.06). FSL induced orientation responses were lower than their control mean (Fig. 1).

Response of three Trichogrammatids evidenced by mean PAI towards four growth stages indicated *T. japonicum* (0.66) as the most effective natural enemy to be attracted towards K-21 allelochemicals followed by *T. brasiliensis* (0.29). *T. chilonis* exhibited least orientation response (Fig. 2).

On comparing the five concentrations of allelochemical cues for orientation response, 25,000 ppm and 50,000 ppm extract concentrations were found to be the most preferred concentrations by targeted Trichogrammatids.

Impact on mean percent parasitisation rate

Results indicated that out of 60 mean %PARA values (values of interactions of three Trichogrammatids with five concentrations of allelechemical cues obtained from four stages of K-21 variety), 35 exhibited a higher response than the mean %PARA of their control. *T. japonicum* exhibited a highly significant parasitism response in form of DoTM towards VSF at 2,00,000 ppm, 50,000 ppm and 25,000 ppm individual concentrations (25.23, 29.80 and 23.82, respectively) as well as significant response towards VSL at 50,000 ppm and 25,000 ppm individual concentrations (17.97 and 22.36). *T. brasiliensis* showed a significantly high response towards VSL at 4,00,000 ppm individual concentration (27.07). In all the tested stages, no significant parasitisation response was observed by *T. chilonis*.

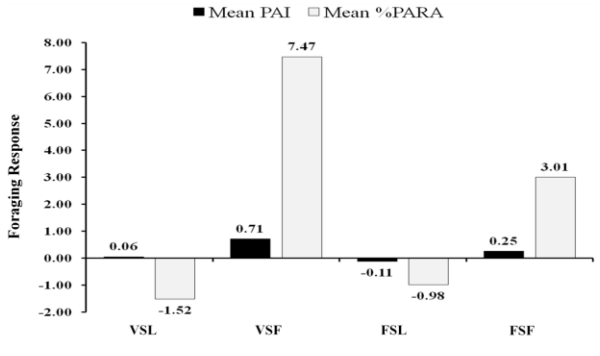

Fig. 1. Impact of various growth stages of variety K-21 on foraging response of three Trichogrammatids. Values of PAI/ %PARA on bars in histogram for targeted growth stages = Average of overall mean PAIs/ %PARAs due to a particular growth stage – Average of mean PAIs/ %PARAs of control for that growth stage.

Table 1. Effect of tomato variety K-21 volatile cues on Parasitoid Activity Index of three Trichogrammatids. TCS, *T. chilonis*; TJ, *T. japonicum*; TB, *T. brasiliensis*; ppm, parts per million; #, overall mean PAI at five individual concentrations (excluding hexane); [ ], Square-root transformation means of original PAI data; If DoTM for mean PAI is greater than LSD/CD, the interaction is said to be significant (*) or highly significant (**).

Growth stage	Natural enemy	Data type	Mean PAI at individual concentrations						Overall # Mean
			4,00,000 ppm	2,00,000 ppm	1,00,000 ppm	50,000 ppm	25,000 ppm	Hexane (Control)	
VSL	TCS	OM	3.50	9.00	6.33	5.83	2.33	16.00	5.40
		SRTM	[1.57]	[2.64]	[2.25]	[2.21]	[1.51]	[3.73]	[2.04]
		DoTM	-2.16	-1.09	-1.48	-1.51	-2.21		
	TJ	OM	9.17	4.33	18.33	22.67	24.17	8.00	15.73
		SRTM	[2.55]	[2.08]	[3.89]	[4.64]	[4.91]	[2.74]	[3.61]
		DoTM	-0.19	-0.65	1.16	1.90**	2.17**		
	TB	OM	14.83	5.17	3.17	9.17	5.50	1.83	7.57
		SRTM	[3.30]	[2.00]	[1.50]	[2.50]	[1.80]	[1.24]	[2.22]
		DoTM	2.06**	0.76	0.26	1.26	0.56		
VSF	TCS	OM	12.00	14.17	9.00	15.67	16.67	13.17	13.50
		SRTM	[3.32]	[3.75]	[3.07]	[3.93]	[3.93]	[3.40]	[3.60]
		DoTM	-0.08	0.36	-0.33	0.53	0.54		
	TJ	OM	5.33	12.00	4.17	17.17	12.33	1.83	10.20
		SRTM	[2.14]	[3.36]	[1.88]	[3.95]	[3.20]	[1.27]	[2.90]
		DoTM	0.87	2.09**	0.61	2.68**	1.93**		
	TB	OM	1.50	4.17	2.33	1.33	2.17	1.00	2.30
		SRTM	[1.16]	[1.64]	[1.32]	[1.08]	[1.35]	[1.01]	[1.31]
		DoTM	0.15	0.63	0.31	0.06	0.33		
FSL	TCS	OM	11.67	12.00	16.00	13.67	15.50	11.50	13.77
		SRTM	[3.32]	[3.26]	[3.91]	[3.58]	[3.92]	[3.19]	[3.60]
		DoTM	0.13	0.08	0.73	0.39	0.73		
	TJ	OM	4.83	4.33	2.67	5.00	9.00	7.50	5.17
		SRTM	[2.11]	[1.84]	[1.58]	[1.97]	[2.52]	[2.47]	[2.00]
		DoTM	-0.36	-0.63	-0.89	-0.50	0.05		
	TB	OM	11.33	1.67	7.17	6.67	2.00	5.50	5.77
		SRTM	[2.65]	[1.31]	[2.43]	[2.42]	[1.36]	[2.32]	[2.03]
		DoTM	0.33	-1.02	0.11	0.10	-0.97		
FSF	TCS	OM	1.67	2.00	1.33	2.67	2.00	2.00	1.93
		SRTM	[1.42]	[1.57]	[1.31]	[1.71]	[1.45]	[1.53]	[1.49]
		DoTM	-0.11	0.04	-0.22	0.18	-0.08		
	TJ	OM	6.50	14.83	8.33	2.17	12.17	4.33	8.80
		SRTM	[2.31]	[3.26]	[2.90]	[1.40]	[3.42]	[2.05]	[2.66]
		DoTM	0.27	1.21	0.85	-0.65	1.37		
	TB	OM	1.33	1.83	1.33	0.33	0.67	0.67	1.10
		SRTM	[1.31]	[1.42]	[1.26]	[0.88]	[1.03]	[0.99]	[1.18]
		DoTM	0.32	0.43	0.27	-0.11	0.04		
Mean		OM	6.97	7.13	6.68	8.53	8.71	6.11	7.60
		SRTM	[2.26]	[2.34]	[2.27]	[2.52]	[2.53]	[2.16]	[2.39]
$F_{30, 360} = 1.62$, $P < 0.05$			SE(m)		SE(d)		LSD/CD at 5%		LSD/CD at 1%
			0.52		0.73		1.43*		1.88**

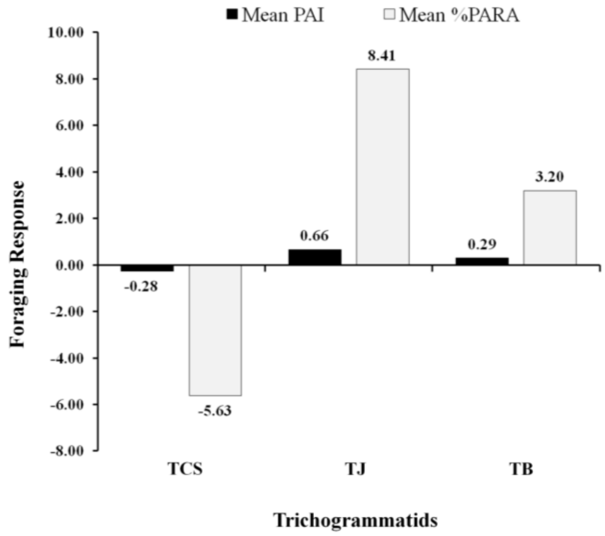

Fig. 2. Cumulative foraging response of three Trichogrammatids for variety K-21 emitted allelochemical cues. Values of PAI/ %PARA on bars in histogram for targeted Trichogrammatids = Average of overall mean PAIs/ %PARAs of an individual *Trichogramma spp.* from all the growth stages – Average of mean PAIs/ %PARAs of control for that *Trichogramma spp.* from all the growth stages.

The overall mean %PARA of five individual concentrations was also found to be higher than their control mean %PARA in few interactions. *T. japonicum* exhibited a higher response *i.e.*, 35.36, 30.83 and 21.25 towards VSL, VSF and FSF, respectively; response of *T. chilonis* was 25.83 and 5.81 towards FSL and FSF whereas *T. brasiliensis* exhibited a higher overall mean response *i.e.*, 23.22, 11.49 and 6.23 towards VSL, VSF and FSF, respectively (Table 2).

To confirm for the most effective growth stage of K-21, final mean values of overall mean %PARAs were calculated and their difference from mean %PARA of control was depicted in Fig. 1. It clearly shows that the selected variety emitted volatile allelochemical cues from different stages of growth reflect varied parasitisation response of Trichogrammatids. VSF was found to be the most effective (7.47) followed by FSF (3.01). VSL and FSL induced parasitisation responses were lower than their control means.

Response of three Trichogrammatids evidenced by mean %PARA towards four growth stages indicated *T. japonicum* (8.41) as the most effective natural enemy to exhibit enhanced parasitism stimulated by K-21 allelochemicals followed by *T. brasiliensis* (3.20). *T. chilonis* exhibited least parasitisation response (Fig. 2).

On comparing the five concentrations of allelochemical cues for parasitism response, 50,000 ppm extract concentrations were found to be the most preferred concentrations by targeted Trichogrammatids.

GLC profile of the four hexane extracts

GLC profile of the targeted hexane extracts revealed the presence of alkanes ranging from docosane (C_{22}) to tetratriacontane (C_{34}). Octacosane (C_{28}), nonacosane (C_{29}), triacontane (C_{30}), hentriacontane (C_{31}), dotriacontane (C_{32}) and tritriacontane (C_{33}) were present in all the four growth stages. Hentriacontane (C_{31}) and tritriacontane (C_{33}) were found to be exceptionally high in concentration as compared to other alkanes in VSF and FSF conditions (Table 3).

Discussion

In agronomical niche, host preference differs for diverse Trichogrammatids (Begum & Anis 2014; Yousuf *et al.* 2015). Utilization of Trichogrammatids as potential egg parasitoids is popular against lepidopterous pests (Jalali *et al.* 2016; Rahman *et al.* 2016). In the present study, behavioural response of three *Trichogramma spp.* was assessed in laboratory. Study also indicated that volatile cues of K-21 are able to alter the foraging behaviour of Trichogrammatids. Foraging response of *T. japonicum* was significantly higher than that of *T. brasiliensis*. *T. chilonis* was found to exhibit least response. Aganon & Adhikari (2004) in their finding advocated the use of *T. japonicum* in controlling *Helicoverpa armigera* infestation. Vijayakumar *et al.* (2015) observed the enhancement of parasitism of *Helicoverpa armigera* eggs by utilizing three Trichogrammatids.

The study on the hexane extracts from VSL, VSF, FSL and FSF of K-21 clearly demonstrated that allelochemicals emitted from different growth stages of the same plant have differential attractiveness and parasitisation response as indicated by mean PAI and mean %PARA of the three Trichogrammatids. This may be attributed by the variation in the number and concentration of individual allelochemicals in hydrocarbon pool of the hexane extracts. VSF was found to emit the most influential allelochemicals, followed by FSF. Differences in the number and concentration of plant volatiles isolated at different stages of growth have also been reported by Oliveira *et al.* (2005) and

Table 2. Effect of tomato variety K-21 volatile cues on Percent Parasitisation of three Trichogrammatids. TCS, *T. chilonis*; TJ, *T. japonicum*; TB, *T. brasiliensis*; ppm, parts per million; $, overall mean %PARA at five individual concentrations (excluding hexane); [ ], Arc sine/Angular transformation means of original %PARA data; If DoTM for mean %PARA is greater than LSD/CD, the interaction is said to be significant (*) or highly significant (**).

Growth stage	Natural enemy	Data type	Mean %PARA at individual concentrations						Overall$ mean
			4,00,000 ppm	2,00,000 ppm	1,00,000 ppm	50,000 ppm	25,000 ppm	Hexane (Control)	
VSL	TCS	OM	11.67	29.44	19.44	17.22	7.78	53.89	17.11
		ATM	[14.54]	[28.95]	[22.21]	[19.99]	[13.35]	[46.11]	[19.81]
		DoTM	-31.57	-17.15	-23.90	-26.12	-32.75		
	TJ	OM	24.44	10.56	46.67	47.78	55.56	22.78	37.00
		ATM	[25.72]	[16.82]	[42.18]	[43.86]	[48.24]	[25.89]	[35.36]
		DoTM	-0.16	-9.06	16.29	17.97*	22.36*		
	TB	OM	42.78	22.78	8.33	29.44	15.00	6.11	23.67
		ATM	[38.03]	[23.24]	[10.88]	[27.51]	[16.46]	[10.96]	[23.22]
		DoTM	27.07**	12.29	-0.08	16.55	5.50		
VSF	TCS	OM	53.33	62.22	61.67	78.33	57.22	64.44	62.56
		ATM	[47.21]	[52.83]	[52.03]	[63.08]	[49.54]	[53.24]	[52.94]
		DoTM	-6.03	-0.41	-1.20	9.84	-3.70		
	TJ	OM	20.00	37.22	13.33	47.22	37.22	7.22	31.00
		ATM	[22.36]	[37.04]	[17.53]	[41.61]	[35.63]	[11.81]	[30.83]
		DoTM	10.55	25.23**	5.72	29.80**	23.82**		
	TB	OM	2.22	12.22	11.67	6.67	8.89	3.33	8.33
		ATM	[6.95]	[14.73]	[12.84]	[9.92]	[13.01]	[7.81]	[11.49]
		DoTM	-0.86	6.93	5.04	2.11	5.20		
FSL	TCS	OM	18.89	17.78	25.00	21.67	20.56	18.33	20.78
		ATM	[25.11]	[23.01]	[28.66]	[26.02]	[26.36]	[23.51]	[25.83]
		DoTM	1.61	-0.50	5.16	2.51	2.86		
	TJ	OM	12.78	7.78	3.89	7.78	22.78	13.89	11.00
		ATM	[18.98]	[13.71]	[10.76]	[15.13]	[24.48]	[18.37]	[16.61]
		DoTM	0.61	-4.66	-7.61	-3.24	6.11		
	TB	OM	17.22	8.89	12.22	13.89	4.44	12.78	11.33
		ATM	[19.83]	[13.56]	[17.48]	[18.28]	[9.27]	[19.20]	[15.69]
		DoTM	0.64	-5.64	-1.71	-0.91	-9.93		
FSF	TCS	OM	1.11	0.56	0.00	2.22	1.67	0.00	1.11
		ATM	[5.87]	[5.13]	[4.06]	[7.53]	[6.45]	[4.06]	[5.81]
		DoTM	1.82	1.08	0.00	3.47	2.40		
	TJ	OM	15.00	26.67	11.67	5.56	26.11	7.22	17.00
		ATM	[19.33]	[26.27]	[18.68]	[12.45]	[29.52]	[14.34]	[21.25]
		DoTM	4.99	11.93	4.34	-1.89	15.18		
	TB	OM	3.33	3.33	0.00	0.00	1.67	1.11	1.67
		ATM	[7.81]	[8.77]	[4.06]	[4.06]	[6.45]	[5.87]	[6.23]
		DoTM	1.93	2.89	-1.82	-1.82	0.58		
Mean		OM	18.56	19.95	17.82	23.15	21.57	17.59	20.21
		ATM	[20.98]	[22.01]	[20.11]	[24.12]	[23.23]	[20.10]	[22.09]
$F_{30, 360} = 1.61$, $P < 0.05$			SE(m)		SE(d)		LSD/CD at 5%		LSD/CD at 1%
			6.22		8.80		17.30*		22.66**

Table 3. Gas Liquid Chromatography (GLC) profile of different growth stages of tomato variety K-21. C, carbon; ppm, parts per million; ND, not detected.

Components of alkane standard		Retention time	Concentration (in ppm) of unknown saturated hydrocarbons in tomato variety K-21			
			VSL	VSF	FSL	FSF
C_{22}	Docosane	14.34	ND	0.66	1.19	ND
C_{23}	Tricosane	15.20	ND	ND	1.30	0.56
C_{24}	Tetracosane	16.03	ND	0.43	2.41	ND
C_{25}	Pentacosane	16.83	ND	0.25	4.85	0.63
C_{26}	Hexacosane	17.60	ND	0.31	7.63	ND
C_{27}	Heptacosane	18.35	ND	1.96	10.52	2.20
C_{28}	Octacosane	19.18	0.12	1.71	10.80	0.44
C_{29}	Nonacosane	20.11	0.42	5.37	10.97	5.10
C_{30}	Triacontane	21.21	0.13	1.44	8.15	1.19
C_{31}	Hentriacontane	22.51	4.78	51.60	14.71	38.33
C_{32}	Dotriacontane	24.08	0.56	4.02	5.20	3.90
C_{33}	Tritriacontane	26.00	2.37	14.33	5.81	11.43
C_{34}	Tetratriacontane	28.35	ND	ND	1.36	ND

Mannan *et al.* (2011). Archna *et al.* (2009) compared the leaf extracts of vegetative and flowering phases of nine varieties and one culture of rice and advocated that allelochemicals have varied response on foraging behaviour of *T. japonicum* and *T. chilonis*. Razavi (2011) demonstrated a considerable difference in the hydrocarbon profile of the aerial parts of *Prangos ferulacea* at different stages of growth. Thirty one compounds were identified in the vegetative stage as compared to only seven in flowering stage. Maruthadurai (2011) also stated that different Bt cotton hybrids revealed the presence of varied saturated hydrocarbons in their vegetative and flowering phases of growth. Attractiveness and parasitisation rate of *T. brasiliensis* and *T. chilonis* towards Bt cotton hybrids Bioseed 6488-I and Mrc 6025 was highly noticeable.

Lower concentrations of K-21 plant extracts were found to generate higher foraging response. Paul *et al.* (2008) worked on the hexane extracts of ten different varieties of tomato (*Lycopersicon esculentum* Mill) grown in vegetative and flowering stages and studied their effect on *T. chilonis*. Varietal difference in eliciting allelochemical responses from the egg parasitoid was noticed and lower concentration was reported to be the preferred concentration for effective orientation response and parasitism.

GLC profile of targeted extracts also revealed the presence of thirteen alkanes (C_{22}–C_{34}) in different numbers and concentrations. Present study indicates that presence of higher range hydrocarbons like octacosane (C_{28}), nonacosane (C_{29}), triacontane (C_{30}), hentriacontane (C_{31}), dotriacontane (C_{32}) and tritriacontane (C_{33}) may be responsible for enhanced foraging response of targeted Trichogrammatids. Madhu *et al.* (2000) studied the impact of eight plant extracts including tomato on *T. japonicum* and *T. brasiliensis* and observed that C_{28} was present in higher concentration in the selected tomato cultivar and was expected to elicit higher parasitism by the Trichogrammatids. Bauer *et al.* (2004) and Paul *et al.* (2008) also observed the emission of various alkanes (C_{21}–C_{35}) from aerial parts of tomato cultivars and found to be responsible for generating higher rate of parasitism by Trichogrammatids.

Overall study on orientation response (PAI)/percent parasitisation rate (%PARA) of three Trichogrammatids towards plant extracts in hexane (from four growth stages) came out to be significant {PAI ($F_{30, 360}$ = 1.62, P = 0.0227); %PARA ($F_{30, 360}$ = 1.61, P = 0.0245)} at 5% level of significance. VSF extract of K-21 can directly be used in lower concentrations to enhance the efficiency of Trichogrammatids as evidenced by response of various lower concentrations. Alternatively, the formulation of octacosane (C_{28}), nonacosane (C_{29}), triacontane (C_{30}), hentriacontane

(C_{31}), dotriacontane (C_{32}) and tritriacontane (C_{33}) in appropriate concentration can be applied to Trichogrammatids to elevate their foraging behaviour.

Conclusion

Study revealed that regular augmentation of Trichogrammatids in tomato crop ecosystem amalgamated with allelochemical cues released by tomato variety K-21 can prove to be an effective biocontrol procedure against economically important insect pests of tomato. It will be an eco-friendly and self-sustainable strategy to control the insect pest population in a crop ecosystem to threshold levels without application of hazardous chemical pesticides. Utilization of stimulative volatile cues and frequent releases of Trichogrammatids can bring a huge relief to agriculturists, mankind and environment.

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