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BRINJAL SYNOMONES AS STIMULANTS FOR THREE TRICHOGRAMMATIDS

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ABSTRACT

Infestation of *Leucinodes orbonalis* Guenee (brinjal fruit and shoot borer) on *Solanum melongena* L. (brinjal/eggplant) is a huge distress to the farmers in India. Despite using latest hybrid plant varieties and advanced farming tools, there is a considerable yield loss to brinjal due to *L. orbonalis*. Chemical pesticides provide short term relief but their long term atrocities can't be neglected. Globally, bio-intensive pest management (BIPM) programs utilizing *Trichogramma spp.* have advocated increased vegetable crop production with better fruit quality and higher commercial value. Considering the stimulation in behavioural responses of insect natural enemies by synomones derived from plant sources, present study was conceptualized. Synomonal extracts in hexane were prepared from leaf samples collected from vegetative and flowering stages of brinjal variety Pusa Uttam (DBR-31) grown in pots in laboratory and field conditions. The impact of four synomonal extracts were observed on *Trichogramma chilonis* Ishii, *Trichogramma japonicum* Ashmead and *Trichogramma pretiosum* Riley through petri-dish bioassays and their chemical composition was analysed through gas liquid chromatography (GLC). *T. pretiosum* exhibited highest foraging response among the three Trichogrammatids under the influence of synomones derived from vegetative stage field condition and this behavioural change may be attributed to the presence of docosane (C_{22}), tetracosane (C_{24}), pentacosane (C_{25}) and tritriacontane (C_{33}). Augmentative release of *T. pretiosum* along with formulation of the preferred alkanes may be utilized as a potential biological control agent in brinjal crop fields for eco-friendly and self-sustainable *L. orbonalis* management.

Keywords: Alkanes, insect pest, *Leucinodes orbonalis*, natural enemy, parasitization, parasitoid, *Solanum melongena*

INTRODUCTION

Solanum melongena L., commonly called brinjal or eggplant, is the 4th commercially economical vegetable crop of India. Brinjal accounted for 6.81% of the total vegetable production in India **[1]**. It is noticeable that due to the cultivation of high yielding varieties and adopting recent agricultural practices, productivity of the brinjal has increased. However, susceptibility of this crop to insect pests is still a major concern.

National Bureau of Agricultural Insect Resources (NBAIR), Bangalore has reported 41 insect pests of brinjal crop in India that may belong to the group of specialist or generalist insect pests. *Leucinodes orbonalis* Guenee (brinjal fruit and shoot borer) contributes to the major yield loss (60-80%) in different states **[2, 3]**. Chemical pesticides have always supervened upon classical biocontrol pest management methods. Nevertheless, agriculturists cannot ignore the fact that harmful inorganic compounds have a desolating effect on beneficial insect populations as well as other agro-dependent organisms, including humans **[4]**. Due to the injudicious use of pesticides and their catastrophic impact on the environment, biological control is now being accepted as a better prototype for trimming down the use of hazardous chemicals in the agro-ecosystem. It encompasses the exploitation of insect natural enemies to control the pest population below threshold level **[5]**. This will provide an eco-friendly pest control measure to the agriculturists. Various insect natural enemies have been reported as parasitoids of *L. orbonalis* in India **[6]**. Among these, *Trichogramma spp.* (Hymenoptera: Trichogrammatidae) have emerged as the most preferred insect natural enemy due to their polyphagous nature, short life cycle and high performance rate in integrated pest management (IPM) programs **[7, 8]**. The foraging response of Trichogrammatids is highly governed by the olfactory cues elicited from host plants **[9, 10, 11]**. Such infochemicals are categorized as synomones **[12]**.

In light of the above facts, authors have attempted to analyse the parasitization potential of three isofemale lines of Trichogrammatids (*Trichogramma chilonis* Ishii, *Trichogramma japonicum* Ashmead and *Trichogramma pretiosum* Riley) under the influence of synomonal extracts of brinjal variety Pusa Uttam (DBR-31).

MATERIALS AND METHODS

Brinjal variety maintenance

All the laboratory bioassays were carried out during the year 2018-19 under controlled conditions in Pest Control Laboratory, AIB, AUUP, NOIDA (U.P.), India. The brinjal variety Pusa Uttam (DBR-31) was grown in laboratory and field conditions by following standard agronomical practices. Healthy plants were identified and 30 gm leaf samples from the targeted stages *i.e.*, vegetative stage and later from flowering stage were collected. Leaf samples were utilized for synomone extract preparation in HPLC grade hexane **[13]**.

Gas liquid chromatography (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) **[14]**.

Petri-dish bioassay

Synomonal extracts were utilized for bioassay studies. The stock extract (4,00,000 mg/L) was serially diluted (2,00,000 mg/L, 1,00,000 mg/L, 50,000 mg/L and 25,000 mg/L). 30 µL each of the above mentioned five synomonal concentrations and the base solvent hexane, which act as control, were applied randomly on Tricho-cards for Petri-dish bioassay. Bioassays were carried out with the three Trichogrammatids and their parasitoid activity index (PAI) was tabulated after every 5 min. for a total period of 45 min. Tricho-cards were then incubated for five days to estimate the parasitization (PARA) by counting blackened eggs. Data obtained for PARA was converted to percent parasitization (% PARA) **[15]**.

Statistical analysis

The tabulated raw data were subjected to transformation (PAI: Square root transformation; %PARA: Arc Sin Transformation) for analysis through Three Way-Analysis of Variance (ANOVA) using Windostat software version 8.5 **[14]**.

RESULTS

Parasitoid activity index of Trichogrammatids

At individual concentrations, *T. chilonis* exhibited a highly significant PAI towards Brinjal variety Pusa Uttam synomones derived from vegetative stage lab condition at the concentration of 50,000 mg/L (2.33) followed by 1,00,000 mg/L concentration (2.02). *T. pretiosum* also tend to show a significant response at concentrations 4,00,000 mg/L (1.34) and 25,000 mg/L (1.32) towards cues obtained from vegetative stage field condition. The overall mean PAI of five treatments (T1 to T5) with respect to control was found to be highest and significant for *T. chilonis* (3.03) towards synomones from vegetative stage lab condition **(Table 1)**.

However, on evaluating the overall PAI of the three Trichogrammatids, it was observed that *T. pretiosum* had a higher preference for extracts derived from field grown plants whereas *T. chilonis* preferred extract from lab grown plants. *T. japonicum* demonstrated preference for control **(Figure 1)**.

Parasitization rate by Trichogrammatids

At individual concentrations, *T. chilonis* displayed a highly significant %PARA at the concentration of 50,000 mg/L (29.36) followed by 1,00,000 mg/L concentration (23.18) under the influence of Brinjal variety Pusa Uttam synomones derived from vegetative stage lab condition. Response was also found to be significant at 2,00,000 mg/L concentration (18.69) followed by 4,00,000 mg/L concentration (17.27) of the same extract. The overall mean % PARA of five treatments (T1 to T5) with respect to control was found to be highest and significant for *T. chilonis* (45.92) towards synomones from vegetative stage lab condition **(Table 2)**.

On evaluating the overall % PARA response of three Trichogrammatids, *T. pretiosum* was found to be exhibit highest parasitism followed by *T. japonicum* under the influence of extracts derived from field grown plants. *T. chilonis* exhibited better response under the influence of extracts derived from lab grown plants **(Figure 1)**.

Efficacy of synomonal extracts

Synomones prevalent in the extract from vegetative stage field condition evoked higher foraging response as compared to control. % PARA responses by Trichogrammatids was also observed to be high for cues from vegetative stage lab condition **(Figure 2)**. Lower concentrations of synomonal extracts were preferred choice for foraging by the Trichogrammatids (T4>T5>T3>T2>T1) **(Table 1; Table 2)**.

Chemical composition of brinjal synomones

GLC profile confirmed that non-polar saturated hydrocarbons ranging from C_{22} to C_{35} were present in variable number and concentrations in the four extracts derived from Brinjal variety Pusa Uttam. Extract from vegetative stage field condition had a blend of all the foresaid hydrocarbons with a very high concentration of C_{25} (83.31 mg/L) and C_{33} (13.08 mg/L). C_{22} and C_{24} were found in very low concentration but were exclusively present in vegetative stage field synomone. Extracts from flowering stage lab/field condition had a mixture of different hydrocarbons but were present in very low concentrations, except for C_{33} (13.61 mg/L) and C_{35} (15.46 mg/L) in flowering stage lab condition. Extract from vegetative stage lab condition contained only three hydrocarbons *viz.*, C_{31} (0.45 mg/L), C_{33} (5.46 mg/L) and C_{35} (2.67 mg/L) **(Table 3)**.

Table 1: Parasitoid activity index (PAI) responses of Trichogrammatids towards synomones derived from Brinjal variety Pusa Uttam growing in laboratory/field condition at vegetative/flowering growth stages

Pusa Uttam Growth Stages	Trichogrammatids	Treatments #						Overall Mean †
		T1	T2	T3	T4	T5	Control (Hexane)	
Vegetative stage (Lab)	*T. chilonis*	2.51	2.53	3.52	3.83	2.75	1.50	3.03
	T. japonicum	3.33	3.71	3.19	3.55	3.69	5.40	3.49
	T. pretiosum	1.19	0.79	0.88	0.71	1.19	0.71	0.95
Vegetative stage (Field)	*T. chilonis*	0.88	2.53	2.02	2.81	2.49	2.46	2.15
	T. japonicum	4.29	5.08	4.77	4.90	4.43	4.13	4.69
	T. pretiosum	2.42	2.01	1.95	1.69	2.40	1.08	2.09
Flowering stage (Lab)	*T. chilonis*	2.96	3.19	3.87	3.75	4.16	4.59	3.59
	T. japonicum	2.63	2.07	1.50	4.45	3.83	4.48	2.90
	T. pretiosum	1.05	0.94	1.41	0.71	1.25	0.88	1.07
Flowering stage (Field)	*T. chilonis*	3.71	3.59	3.63	3.10	2.80	3.31	3.36
	T. japonicum	1.43	1.49	2.09	1.49	1.32	2.33	1.56
	T. pretiosum	1.44	0.94	0.71	0.88	0.79	0.85	0.95
Mean		2.32	2.41	2.46	2.65	2.59	2.64	2.49
$F_{30, 360} = 1.64$, $P < 0.05$		$SE_{(m)}$=0.47		$SE_{(d)}$=0.66		$CD_{.05}$=1.29		$CD_{.01}$=1.70

Legends: T1, mean PAI at 4,00,000 mg/L; T2, mean PAI at 2,00,000 mg/L; T3, mean PAI at 1,00,000 mg/L; T4, mean PAI at 50,000 mg/L; T5, mean PAI at 25,000 mg/L; #, Mean of six replicates; †, Mean of treatments from T1 to T5; Lab, potted plants grown in laboratory condition; Field, potted plants grown in field condition

Table 2: Percent parasitization (%PARA) responses of Trichogrammatids towards synomones derived from Brinjal variety Pusa Uttam growing in laboratory/field condition at vegetative/flowering growth stages

Pusa Uttam Growth Stages	Trichogrammatids	Treatments $						Overall Mean ‡
		T1	T2	T3	T4	T5	Control (Hexane)	
Vegetative stage (Lab)	*T. chilonis*	43.07	44.49	48.99	55.17	37.89	25.80	45.92
	T. japonicum	36.13	33.49	40.91	41.06	44.48	52.30	39.21
	T. pretiosum	10.24	4.06	4.06	4.06	8.91	4.06	6.26
Vegetative stage (Field)	*T. chilonis*	5.13	13.02	19.30	24.81	26.41	23.25	17.73
	T. japonicum	32.43	45.75	45.96	41.16	43.52	30.59	41.76
	T. pretiosum	21.39	19.06	14.13	12.22	22.85	8.56	17.99
Flowering stage (Lab)	*T. chilonis*	32.64	37.44	42.96	44.42	50.55	57.70	41.60
	T. japonicum	23.83	20.99	11.96	38.49	32.70	34.92	25.59
	T. pretiosum	5.13	4.06	9.99	4.06	4.06	4.06	5.46
Flowering stage (Field)	*T. chilonis*	25.12	27.97	29.10	24.73	24.21	27.77	26.22
	T. japonicum	9.92	15.65	11.09	11.46	5.13	13.99	10.65
	T. pretiosum	10.38	7.81	4.06	4.06	4.06	6.95	6.07
Mean		21.28	22.81	23.57	25.47	25.40	24.16	23.71
$F_{30, 360} = 1.34$, $P > 0.05$		$SE_{(m)}$=5.70		$SE_{(d)}$=8.04		$CD_{.05}$=15.82		$CD_{.01}$=20.72

Legends: T1, mean PAI at 4,00,000 mg/L; T2, mean PAI at 2,00,000 mg/L; T3, mean PAI at 1,00,000 mg/L; T4, mean PAI at 50,000 mg/L; T5, mean PAI at 25,000 mg/L; $, Mean of six replicates; ‡, Mean of treatments from T1 to T5; Lab, potted plants grown in laboratory condition; Field, potted plants grown in field condition

Table 3: Gas liquid chromatography (GLC) profile of synomonal extracts from Brinjal variety Pusa Uttam

Components of alkane standard		Retention time	Concentration (mg/L) of unknown alkanes in Brinjal variety Pusa Uttam leaf samples			
			VSL	VSF	FSL	FSF
C_{22}	Docosane	14.34	ND	0.60	ND	ND
C_{23}	Tricosane	15.20	ND	0.32	1.07	ND
C_{24}	Tetracosane	16.03	ND	0.75	ND	ND
C_{25}	Pentacosane	16.83	ND	83.31	0.19	0.11
C_{26}	Hexacosane	17.60	ND	1.11	0.13	ND
C_{27}	Heptacosane	18.35	ND	2.02	0.44	0.15
C_{28}	Octacosane	19.18	ND	1.75	0.25	ND
C_{29}	Nonacosane	20.11	ND	1.37	0.45	0.13
C_{30}	Triacontane	21.21	ND	0.73	0.14	ND
C_{31}	Hentriacontane	22.51	0.45	2.00	1.36	0.58
C_{32}	Dotriacontane	24.08	ND	1.07	0.72	0.19
C_{33}	Tritriacontane	26.00	5.46	13.08	13.61	1.85
C_{34}	Tetratriacontane	28.35	ND	1.37	1.34	ND
C_{35}	Pentatriacontane	31.24	2.67	8.09	15.46	1.09

Legends: C, carbon; VSL, Vegetative stage lab condition; VSF, Vegetative stage field condition; FSL, Flowering stage lab condition; FSF, Flowering stage field condition; ND, not detected

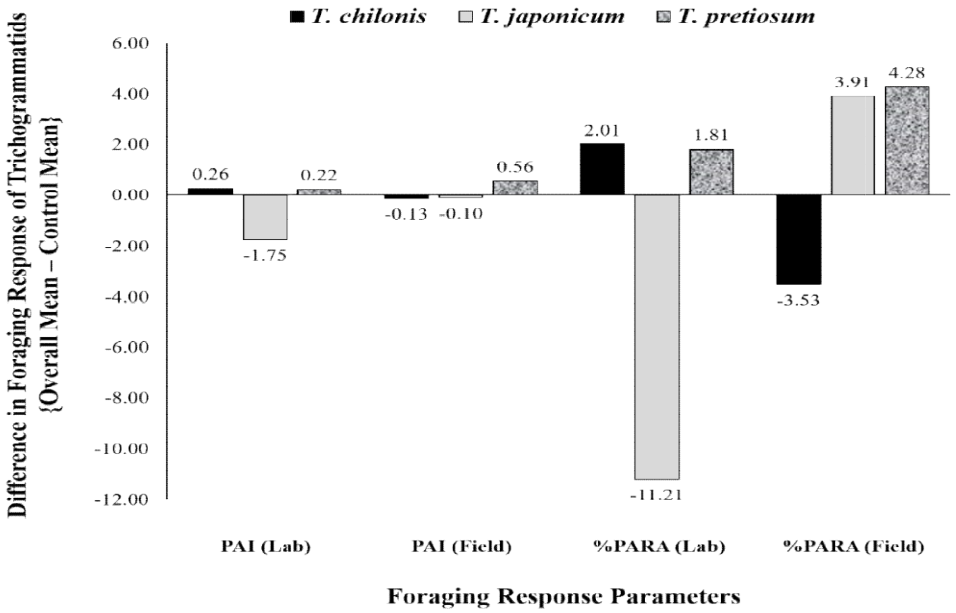

Figure 1: Realisation of the most potential *Trichogramma spp.* through their foraging responses towards synomones derived from Brinjal variety Pusa Uttam growing in laboratory/field condition

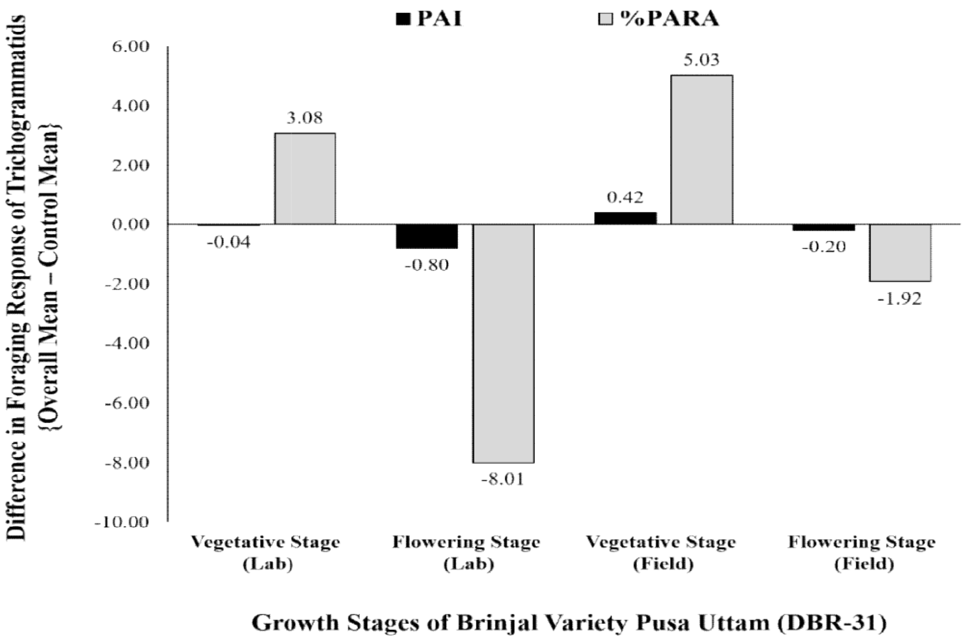

Figure 2: Impact of growth stage and environment condition on Brinjal variety Pusa Uttam and thereafter its effect on foraging responses of three Trichogrammatids

DISCUSSION

The release of saturated hydrocarbons from leaf surfaces of damaged/undamaged host plants due to insect pests and their role in inter-kingdom communication has been well documented. Present study also observed the role of alkanes in stimulating higher foraging responses in Trichogrammatids. Synomones from vegetative stage lab condition were preferred as stimulants by *T. chilonis* for high parasitism, which might be attributed to the presence of only three hydrocarbons *viz.*, C_{31}, C_{33} and C_{35}. Stimulation in behaviour at such lower concentration may be attributed to the fact that there was no interference by other hydrocarbons (not detected). *T. pretiosum* exhibited highest foraging responses, followed by a high parasitism response by *T. japonicum*, towards synomones from vegetative stage field condition. C_{25} and C_{33} along with exclusive presence of C_{22} and C_{24} might be the synomonal components responsible for eliciting highest foraging responses by *T. pretiosum*. The inference derived from present study very well corroborates with available literature. Halinski *et al.* have advocated the presence of saturated hydrocarbons in abundance in leaf cuticular waxes of brinjal **[16]**. Szafranek *et al.* mentioned C_{20}-C_{36} as the most often encountered hydrocarbons in plant cuticular waxes **[17]**. According to Lara *et al.*, n-alkanes (C_{23}-C_{36}) prevailed in the surface waxes of brinjal fruit with dominance of C_{31} and C_{33} **[18]**. Yadav *et al.* relegated C_{25} as favourable saturated hydrocarbon for *T. exiguum* **[19]**. Paul *et al.* demonstrated that *T. brasiliensis* and *T. exiguum* exhibited very high PAI and %PARA responses under the influence of C_{25} and C_{26} **[20]**. Higher concentration of C_{22}, C_{24}, C_{25} and C_{26} were recorded in damaged plant leaf extracts of brinjal and their role in elevated PAI and % PARA behaviour of *T. chilonis* was discussed **[21]**. Field studies in the past have encouraged the utilization of Trichogrammatids as egg parasitoid in brinjal crop ecosystem and advocated their effectiveness against *L. orbonalis* **[22, 23]**.

CONCLUSION

Findings from the current research will pave way for the application of *T. pretiosum* along with favourable synomonal formulations in optimal concentration in brinjal crop ecosystems so as to limit *L. orbonalis* population to economic threshold level. This eco-friendly and self-sustainable strategy can form an integral part of the bio-intensive pest management (BIPM) programs and provide hope for the agriculturists to condemn the use of chemical pesticides.

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