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FORMULATION OF CRUCIFER PLANT VOLATILES AND ASSESSMENT OF THEIR IMPACT ON PARASITIC EFFICIENCY OF *TRICHOGRAMMA CHILONIS*

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Abstract

In integrated pest management, utilization of chemical cues especially synomones emanating from plants can act as a highly valuable, self-sustaining, and eco-friendly alternative of chemical pesticides. Development of formulations of these chemical cues is an essential step to enhance their commercial utilization in field conditions. Present study focused on the development of formulations from synomonal cues present in leaf extracts using fuller's earth as a solid and inert base. Laboratory bioassays were carried out to assess the impact of storage duration and temperature on the quality of the targeted formulations. Saturated hydrocarbon profile of leaf extracts used in formulation preparation was also analyzed through Gas Chromatography. All the formulations elicited better response as compared to control. Formulation combination, FC1 - Girija (400000 mg/L) was found to register significantly higher mean percent parasitism for 15 ± 2ºC and 25 ± 2ºC storage temperatures which may be exploited commercially to enhance the efficacy of biological control agents.

Key words : Integrated Pest Management, Synomones, foraging efficiency, saturated hydrocarbons and Gas Chromatography.

Introduction

Biological Control, an essential component of Integrated Pest Management, is an environmentally sound and effective means of reducing or mitigating pests/pest effects. Release of natural enemies, for instance, reduces pest population dramatically, and thereby, minimizes crop losses (Uefune *et al.* 2011 and Jalali *et al.* 2016). Natural enemies are guided by a complex of non-nutritional, volatile cues commonly referred as semiochemicals (Bakthavatsalam *et al*. 2007; Flint & Doane 2009). The message that plant volatiles especially synomonal cues convey, results in enhanced behavioral response from natural enemies making them valuable tools for integrated pest management (Udai *et al.* 2018). Studies on the control of crop pests through the use of volatile cues has increased because of their various advantages, including the perceptions of these compounds as environmentally desirable as well as the real need for new pesticides with eco-friendly, novel modes of action (Yadav & Paul 2009). Development of plant leaf extract based formulations with clay as solid, inert base could work as an effectual alternate for pest management by enhancing the efficiency of natural enemies (Akol *et al*. 2003; Parmashivan & Paul 2005; Bakthavatsalam *et al*. 2007). Furthermore, formulation of plant leaf extracts makes them easy for handling, transportation and storage.

Among the natural enemies, the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) has received most attention because of its polyphagous nature against many lepidopterous pests of economically high valued crops (Fatouros *et al*. 2008). Over 100 insect pests have been reported worldwide which damage important crucifer crops like cabbage and cauliflower.

Hence the present study aimed at development of formulations of synomonal cues present in plant leaf extract by using fuller's earth clay as solid, inert base. Impact of storage duration and temperature on response eliciting capability of various formulation combinations on foraging behaviour of *Trichogramma chilonis* Ishii, a prevalent egg parasitoid in cruciferous crop was also evaluated. In plant extracts, hydrocarbons have been found to act as synomonal cues in different crop ecosystems (Paul *et al*. 2008). Therefore, the hexane leaf extracts were also subjected to gas chromatography

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to determine their saturated hydrocarbon profile.

Materials and Methods

Insect rearing protocols

The host insect, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), and isofemale lines of egg parasitoid species, *Trichogramma chilonis* Ishii, were obtained from Biological Control Laboratory, Amity Institute of Biotechnology, Amity University, Sec-125, Noida, U.P. Cultures of *C. cephalonica* adults were established in oviposition cages as described by Sreekumar and Paul (2000). Their eggs were collected, cleaned and sterilized using UV; glued on paper egg cards in a single layer using Arabic gum and placed in glass vials (diameter = 2.5 cm, length = 10 cm). The 0-24-h-old *C. cephalonica* eggs were offered to female *Trichogramma* for oviposition and the vials were kept at 26±2 °C and 65±5% RH for emergence (Asfiya Zayeem and Archna Kumar 2012).

Preparation of synomonal extract

From two crucifer crop varieties Girija and Madhuri Atkai, 30g leaf sample was collected during flowering phase of the crop from the farmer's fields of the state of Uttar Pradesh, India, for preparation of plant extracts for bioassays and gas- liquid chromatography studies. Leaf samples of each variety (having three replications) were washed in distilled water, air-dried and soaked overnight in HPLC grade hexane. The filtrates were kept in sodium sulfate for 1h, passed though silica gel column and distilled at 60-70ºC in a water bath. The extracts were rinsed in hexane, poured in a small tube and evaporated completely in a water bath. The concentration 400000 mg/L was prepared by adding distilled HPLC grade hexane to the extracts (Archna *et al*. 2009).

Preparation of Synomonal extract based Formulation

Formulations were prepared by mixing 5 ml of 400 000 mg/L concentration of hexane leaf extracts with 1g of fuller's earth clay as an inert base (Choudhary *et al*. 2006). Six such formulation combinations *viz*., FC1 - Girija (400000 mg/L), FC2 - Madhuri Atkai (400000mg/L), FC3 - 25% FC2+75% FC1, FC4 - 50% FC2+50% FC1, FC5 - 75% FC2+25% FC1 and FC6- Hexane washed fuller's earth clay, were prepared. Each formulation was prepared in two sets and stored in opaque vials; one set was stored in fridge at 15 ± 2ºC and the other set in BOD incubator at 25 ± 2ºC and 65 ± 5% RH.

Bioassay Protocol:

Two bioassays were conducted simultaneously, one with formulations combinations stored at 15 ± 2ºC and other with formulation stored at 25 ± 2ºC. Each bioassay was carried out by placing egg cards (30 eggs per card) sprayed with the respective formulations (@10mg/egg card) in 150 × 15 mm Petri dishes. In each Petri dish, 10, 0-24h old Trichogrammatids females were released in the center. The Petri dishes were replicated six times for each treatment. The parasitoids were observed at five minutes interval for 45 minutes and the total number of parasitoids that visited the egg card referred as 'parasitoid activity index (PAI)' was counted. Thereafter, the parasitoids were removed carefully from each egg card. The cards were kept individually in homeopathic vials (1 dram size) and the number of blackened eggs was counted to estimate the parasitism (Archna *et al*. 2009). Laboratory conditions for the experiment were 26 ± 2°C, 65 ± 5% RH and light intensity 160 LUX according to Asfiya Zayeem and Archna Kumar (2012). Such bioassays were repeated after every week for two months.

Gas chromatography analysis

The hexane leaf extracts were analyzed for the presence of saturated hydrocarbons by gas liquid chromatography fitted with a flame ionization detector (FID) and capillary column (VF-1mS, 15m) packed on Varian 430-GC. The operating conditions used were column temperature 100ºC to 320ºC @ 10ºC/minutes and injector and detector temperature was 320ºC. Nitrogen was used as a carrier gas with a flow rate of 20 ml/ minutes. Injection volume was 1µl. The hydrocarbons were identified, by comparing with the standards obtained from Sigma Aldrich USA. The concentration of unknown saturated hydrocarbons was calculated by using the formula according to Archna *et al*. 2009 as follows:

Concentration of Area of unknown saturated hydrocarbon
Concentration of

unknown saturated =
X known saturated

Hydrocarbon Area of known saturated
hydrocarbon Hydrocarbon

Statistical analysis

For analyzing interaction of *T. chilonis* with five different formulations stored at two different temperature conditions, parasitoid activity index (PAI) and parasitism data was tabulated. The parasitism values were transformed into and percent parasitismby using the following formula:

Percent parasitism = (Number of blackened eggs per card / Total number of eggs per card) × 100. Data obtained for percent parasitism and parasitoid activity index (PAI) was analyzed by 3-way ANOVA, using applied Indostat Software version 8.5 developed by Windostat Hyderabad, India. The difference between the means of various treatments was compared by LSD test at 5% significance level.

Results

Effect of formulation combinations stored at 25 ± 2ºC on *T. chilonis*

All the formulation combinations stored at 25 ± 2ºC were found to elicit significantly higher response as compared to control ($P<0.05$). Hexane leaf extract formulation FC3 stored at 25°C elicited maximum parasitoid activity index (10.50±0.22) for *T. chilonis* in week 1 followed by FC2 in week 2 (10.00±1.27). Mean foraging response was observed to be highest in week 1 (8.30±0.26).

Impact of different formulation combinations on fecundity of *T. chilonis* observed up to eight weeks indicated that FC2 registered highest percent parasitism in week 4, 6 and 8 (42.78±4.98, 21.11±2.38 and 21.67±8.64) whereas FC3 elicited maximum parasitisation in week 1 and 7 (33.89 ±.3.98 and 26.11±2.00). Highest parasitisation rate was observed for FC1, FC4 and FC5 during week 5, 2 and 3 respectively (37.78±4.28, 30.00±3.34 and 25.00±4.77). FC1 registered the highest mean percent parasitism (25.28±2.35) among all the combinations tested at 5% significant level. Impact of all formulation combinations on mean parasitism was higher as compared to control (Table 1 and 2).

Effect of formulation combinations stored at 15± 2ºC C on *T. chilonis*

Hexane leaf extract formulation FC3 stored in 15± 2ºC demonstrated maximum parasitoid activity index (9.67±0.67) and percent parasitism (45.55±3.91) for *T. chilonis* in week 1. Response among various formulation combinations indicated that FC1 registered highest percent parasitism during week 3, 4, 6, 7 and 8 (26.67±7.10, 28.33±9.46, 20.56±3.69, 21.67±2.24 and 17.22±9.33) whereas FC2 showed a comparatively high response in week 2 and 5 (29.44±5.99 and 31.11± 5.63). Among all the combinations tested, FC1 registered the highest mean PAI and mean percent parasitism (4.67±0.50, 22.22±2.21). Maximum mean percent parasitism was found to be highest in week 1 (31.56±3.25).

Impact of all formulation combinations on mean parasitism was higher as compared to control up to 7th week of storage. However, there was no significant impact of temperature of storage on the

Table 1:. Evaluation of Mean Parasitoid Activity Index of *Trichogramma chilonis* up to eight weeks by applying Synomonal formulation combinations.

	FC1		FC2		FC3		FC4		FC5		FC6		Mean	
	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C
Week1	9.00±0.37	8.33±0.33	7.33±0.21	5.17±0.17	10.50±0.22	9.67±0.67	7.50±0.22	6.67±0.33	7.17±0.31	6.50±1.15	3.50±0.22	2.67±0.42	8.30±0.26	7.27±0.39
Week2	7.33±1.52	1.50±0.76	10.00±1.27	2.50±0.34	4.67±0.42	3.00±0.68	5.00±0.86	1.83±0.31	8.33±0.92	3.67±0.96	3.33±0.33	3.17±0.65	7.07±0.58	2.50±0.31
Week3	6.50±0.50	5.00±0.26	3.67±0.33	3.67±0.33	2.67±0.33	4.33±0.33	6.50±0.50	3.33±0.67	3.00±0.47	3.33±0.42	4.17±0.47	2.33±0.33	4.47±0.36	3.93±0.21
Week4	4.67±0.62	9.33±0.96	4.33±0.56	3.33±0.67	3.67±0.56	3.67±1.09	5.67±0.62	3.00±0.37	4.00±0.52	4.67±0.42	5.00±0.58	3.33±0.62	4.47±0.27	4.80±0.54
Week5	0.67±0.21	5.50±0.96	0.67±0.21	4.17±0.54	3.33±0.21	2.33±0.62	2.00±0.63	5.00±1.03	2.67±0.21	2.67±0.67	2.00±0.58	4.67±1.02	1.87±0.24	3.93±0.40
Week6	2.00±0.93	3.50±1.31	4.33±1.33	2.83±0.70	4.33±0.72	3.33±0.96	4.00±0.52	1.67±0.62	3.67±0.56	2.00±0.73	2.17±0.31	1.33±0.49	3.67±0.40	2.67±0.40
Week7	6.67±1.41	3.050±1.03	4.00±0.86	1.17±1.60	2.33±0.33	3.67±1.50	4.00±0.97	2.50±0.62	3.17±1.10	3.17±0.98	3.67±0.8	5.50±0.56	4.03±0.49	2.80±0.45
Week8	5.17±1.25	0.67±0.21	8.67±0.80	0.50±0.22	7.83±1.28	0.67±0.33	4.17±0.75	0.00±0.62	8.67±1.17	0.50±0.22	7.17±2.82	1.00±0.45	6.90±0.57	0.47±0.10
Mean	5.25±0.49	4.67±0.50	5.37±0.49	2.95±0.26	4.92±0.43	3.83±0.45	4.85±0.32	3.00±0.34	5.08±0.42	3.31±0.35	3.88±0.42	3.00±0.29		

* FC1 - Girija (400000 mg/L), FC2 - Madhuri Atkai (400000mg/L), FC3 - 50% FC2+50% FC1, FC4 - 25% FC2+75% FC1, FC5 - 75% FC2+25% and FC6- Hexane washed fuller's earth clay.

	SEm	CD at 5%
Temperature × Temperature	0.16	0.31
Week x. Week	0.31	0.62
Concentration × concentration	0.27	0.53
Temperature × concentration	0.38	0.75
Week × concentration	0.77	1.51

Table 2: Evaluation of Mean Percent parasitism of *Trichogramma chilonis* up to eight weeks by applying Synomonal formulation combinations.

	FC1		FC2		FC3		FC4		FC5		FC6		Mean	
	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C	25°C	15°C
Week 1	28.34± 10.36	18.33± 7.29	21.11± 6.42	25.00± 7.14	33.89± 3.98	45.55± 3.91	31.11± 8.93	33.89± 7.17	22.78± 4.50	35.00± 7.03	18.89± 4.99	21.11± 2.84	27.44± 3.15	31.56± 3.25
Week 2	26.66± 5.37	20.56± 3.38	26.67± 1.22	29.44± 5.99	22.78± 1.81	21.11± 4.69	30.00± 3.34	17.78± 4.10	23.89± 1.34	27.22± 5.67	16.66± 1.49	13.89± 3.79	26.00± ±1.35	23.22± 2.18
Week 3	18.89± 5.49	26.67± 7.10	14.44± 2.05	15.56± 5.14	22.78± 3.38	16.11± 7.27	20.00± 7.50	23.33± 6.94	25.00± 4.77	21.67± 9.38	13.34± 5.77	10.00± 5.44	20.22± 2.18	20.67± 3.13
Week 4	34.45± 8.46	28.33± 9.46	42.78± 4.98	17.22± 7.01	21.11± 6.70	7.22± 20.56	27.78± 7.13	21.67± 8.11	17.78± 8.85	18.89± 8.85	15.56± 7.03	16.67± 6.77	28.78± 3.49	21.33± 3.47
Week 5	37.78± 4.28	24.44± 5.88	25.56± 7.03	31.11± 5.63	25.56± 6.76	18.89± 2.05	23.89± 4.60	16.11± 2.50	15.56± 2.81	18.33± 0.75	13.33± 4.30	16.11± 3.98	25.67± 2.58	27.78± 1.92
Week 6	18.33± 5.36	20.56± 3.69	21.11± 2.38	19.44± 6.05	20.00± 2.43	15.00± 3.19	11.67± 3.73	15.00± 5.07	15.56± 4.53	3.89± 2.50	14.44± 3.72	11.67± 3.92	17.33± 1.72	14.78± 2.09
Week 7	24.44± 3.06	21.67± 2.24	15.00± 4.77	7.22± 2.34	26.11± 2.00	20.00± 3.85	13.33± 2.11	12.22± 2.05	13.33± 5.09	10.56± 1.81	12.78± 3.59	13.89± 1.59	18.44± 1.84	14.33± 1.48
Week 8	13.33± 4.87	17.22± 9.33	21.67± 8.64	10.56± 4.59	15.00± 7.14	5.00± 2.40	7.78± 4.01	17.22± 7.32	10.56± 6.11	11.67± 6.37	11.67± 3.63	13.33± 6.27	13.67± 2.78	12.33± 2.81
Mean	25.28± 2.35	22.22± 2.21	23.65± 2.11	19.45± 2.19	23.40± 1.73	20.28± 2.19	20.69± 2.20	19.65± 2.11	18.06± 1.84	18.40± 2.40	14.58± 1.53	14.58± 1.64		

* FC1 - Girija (400000 mg/L), FC2 - Madhuri Atkai (400000mg/L), FC3 - 50% FC2+50% FC1, FC4 - 25% FC2+75% FC1, FC5 - 75% FC2+25% and FC6- Hexane washed fuller's earth clay.

	SEm	CD at 5%
Temperature × Temperature	1.12	2.20
Week x. Week	2.24	4.39
Concentration × concentration	1.94	3.81
Temperature × concentration	2.74	5.38
Week × concentration	5.48	10.76

Table 3: Hydrocarbon Profile of Crucifer Crop Varieties Girija and Madhuri Atkai In flowering phase of growth.

Hydrocarbon Profile	Name of hydrocarbon	Madhuri Atkai(mg/L)	Girija (mg/L)
Favourable	Heneicosane (C_{21})	747.96	908.8
	Docosane (C_{22})	383.38	7465.3
	Tricosane (C_{23})	293.74	5525.5
	Pentacosane (C_{25})	Not Detected	6406.0
	Nonacosane (C_{29})	568.30	3963.9
Others	Tetracosane (C_{24})	126.96	Not Detected
	Heptacosane (C_{27})	Not Detected	6465.2
	Triacontane (C_{30})	814.13	6899.4
	Hentriacontane (C_{31})	1626.47	Not Detected
	Dotricontane (C_{32})	9703.98	Not Detected

parasitization efficiency of these formulations (table 1 and 2).

Hydrocarbon Profile

Gas chromatograph of hexane extracts of Girija and Madhuri Atkai in flowering phase revealed the presence of favourable as well as unfavorable saturated hydrocarbons ranging from C_{21} to C_{32} varying in number and concentration eliciting varied level of synomonal response from *T. chilonis*. Gas chromatograph of hexane leaf extract of Girja, whose formulation was preferred the most by the targeted egg parasitoid indicated the presence of seven hydrocarbons; Heneicosane (C_{21}), Docosane (C_{22}), Tricosane (C_{23}), Pentacosane (C_{25}), Heptacosane (C_{27}), Nonacosane (C_{29}) and Triacontane (C_{30}). Among these quantities of the favorable hydrocarbon Docosane (C_{22}), was the highest (7465.3). Cole crop variety Madhuri Atkai showed the presence of four favourable hydrocarbons, Heneicosane (C_{21}), Docosane (C_{22}), Tricosane (C_{23}) and Nonacosane (C_{29}) (table 3). Among these quantities of the favorable hydrocarbon Dotriacontane (C_{32}) was the highest (9703.98).

Discussion:

Synomonal cues benefit the plant by attracting natural enemies of the herbivores and profit parasitoids by guiding them to potential hosts or prey on the plant (Uefune *et al.* 2011; Asfiya Zayeem and Archna Kumar 2012). Plant volatiles cues especially synomones emanating from various plant sources play central role in enhancing the parasitic efficiency of prominent egg parasitoid genus Trichogrammatids (Tandon & Bakthavatsalam 2007; Rani *et al.* 2008). Seenivasagan *et al.* (2009) observed that the foraging activity of larval parasitoid, *Cotesia plutellae* Kurdjumov is varied and highly influenced by the volatiles emanating from seven different cruciferous plants. Development of formulation is a crucial link between extraction of synomonal cues and their application in field, which in turn dictates economy. In this study several fuller's earth clay based synomonal formulation combinations to provide a stable base for plant produced volatiles were prepared. In a study conducted by Akol *et al.* (2003) parasitoids were found to be significantly more attracted to volatiles from cabbage and host-infested cabbage sprayed with the powder formulation than to clean air. Paramasivan & Paul (2005) also prepared eight different semiochemical dust formulations using kaolinite clay as carrier with leaf extracts of flowering phase of maize. The semiochemical dust formulation prepared using equal proportions of flowering phase leaf extract of Mahekanchan, TCSH-1 and egg of *Chilo partellus* Swinhoe registered the highest parasitism in the field, followed by the dust formulation prepared using the flowering phase leaf extract of TCSH-1. Results of present study are in accordance with the above findings and indicate that all the formulation combinations elicited a significantly higher parasitisation from *T. chilonis* as compared to control. However varied response was observed for different formulation combinations. Studies conducted by Dutton *et al.* (2000), Yonggen *et al.* (2006), Chaudhary *et al.* (2006), and Rani *et al.* (2008) revealed that variation in the quantity and concentration of saturated hydrocarbons influenced the parasitization efficiency of Trichogrammatids. Yadav *et al.* (2001) also reported the presence of Pentacosane in potato *(Solanum tuberosum)* and soybean *(Glycine max)* and classified Pentacosane as favourable saturated hydrocarbon for *T. exiguum.* Paul *et al.* (2008) studied the impact of saturated hydrocarbons present in vegetative and flowering phase of ten different varieties of tomato (*Lycopersicone sculentum* Mill) on *T. chilonis.* They detected the presence of saturated hydrocarbons ranging from C_{14} to C_{29} in varying numbers and concentrations and found synomonal activity to be associated mainly with Heneicosane (C_{21}), Tricosane (C_{23}), Pentacosane (C_{25}) and Hexacosane (C_{26}) during the vegetative period and with Heneicosane (C_{21}) and Hexacosane (C_{26}) during the flowering period. Present findings are in accordance with these studies and suggest that higher parasitization registered for FC1 for both storage temperatures could be attributed to varied number and higher concentration of saturated hydrocarbons in Girija as compared to Madhuri Atkai.

Conclusion

It is suggested that semiochemical formulation of FC1 - Girija (400000 mg/L) in crude form could be used for the efficient pest management by *T. chilonis* in the field through enhancing their parasitization rate.

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