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ASSESSMENT OF FORAGING POTENTIAL OF *Trichogramma brasiliensis* (ASHMEAD) AND *Trichogramma chilonis* ISHII TOWARDS COMBINATIONS OF OCTACOSANE, PENTACOSANE AND TRICOSANE BASED FORMULATIONS

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ABSTRACT

In an agroecosystem, the foraging response of natural enemies is governed by the non-nutritional volatile organic compounds that serve as olfactory cues. In this study, three straight chain saturated hydrocarbons in combinations i.e, Octacosane ($C_{28}H_{58}$), Pentacosane ($C_{25}H_{52}$) and Tricosane ($C_{23}H_{48}$) were formulated and their impact was studied to assess the foraging behavior of the two Trichogrammatids. Pentacosane ($C_{25}H_{52}$) and Tricosane ($C_{23}H_{48}$) combination was found to be a strong stimulator for *Trichogramma brasiliensis* (Ashmead). Overall significant stimulation in foraging efficiency for *Trichogramma chilonis* Ishii was noticed by Tricosane ($C_{23}H_{48}$) and Octacosane ($C_{28}H_{58}$) combination. Thus, these hydrocarbon combinations could be useful to provide effective biocontrol of Lepidopteran pests by stimulating the foraging behavior of *T. chilonis* and *T. brasiliensis*.

Keywords: Biological Control, Hydrocarbons, Semiochemicals, *Trichogramma*

INTRODUCTION

Humans need appropriate food which should be healthy, economical and of high nutritional value. Great loss occurs in crops due to insect pest infestation. Since centuries, humans have always needed better ways to protect crops in which Integrated Pest

Management (IPM) has proved to be a boon to produce high-quality products and has satisfied consumer's 'clean and green' demand as well. IPM system requires continuous adaptability with the integration of durable and toxicologically safe practices; preferably non-chemical ones **[1]** and also to act against the problem of insecticide resistance in pests **[2]**. This is possible by incorporating the recent trends of biological control. The scope and obligation to efficacious dynamic biological control were needed after the withdrawal of pesticides to ensure the stability of the environment and sustainability **[2, 3]**. Shifting from chemical control to the advanced strategy of IPM, several natural enemies for insect pest control are being used for protecting crops from pest infestation and to make pesticide free agri-produce and also provide protection from pesticidal resistance **[4]**. Hence; amplification of the biological control agents could be a remarkable strategy to increase crop yields leaving the conventional methods of farming behind for a greener future **[5-7]**. Among various natural enemies, genus *Trichogramma* has contributed a lot in the success of the biological control against Lepidopteran pests in varied crops **[8, 9]**. The Trichogrammatids have minute size, faster multiplication rate as well as a high degree of host specificity. The efficiency of Trichogrammatids may be enhanced by applying non-nutritional volatile cues i.e., Semiochemicals **[10-13]**. The presence of semiochemical cues particularly saturated straight chain hydrocarbons on host insects surfaces generates species specific responses in *Trichogramm* **[14-18]**. The information that volatile cues carry; causes an increase in the behavioural response from natural enemies **[19]**, thus making these infochemicals highly useful for research study **[20]**. The present study focuses on various combinations of Octacosane ($C_{28}H_{58}$), Pentacosane ($C_{25}H_{52}$) and Tricosane ($C_{23}H_{48}$) formulations on foraging behaviour of the egg parasitoids; of *Trichogramma brasiliensis* (Ashmead) and *Trichogramma chilonis* Ishii; which can be further used as an advancement in the biological control of the crops.

MATERIAL AND METHODS

Establishment of culture

The cultures of facilitated host *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) and isofemale lines of Trichogrammatids were established in Biological Control Laboratory, Amity University, Noida, Uttar Pradesh, India **[21, 22]**. Isofemale lines of Trichogrammatids {*Trichogramma brasiliensis* (Ashmead), N.

A. No. NBAII-MP-TRI-70*; Trichogramma chilonis* Ishii, N. A. No. NBAII-MP- TRI-13} were procured from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. Furthermore, the cultures of these Trichogrammatids were maintained in Bilogical Control Laboratory, Amity University, NOIDA (U. P.), India.

Hydrocarbon formulation preparation

Three straight chain saturated hydrocarbons *viz;* Octacosane ($C_{28}H_{58}$), Pentacosane ($C_{25}H_{52}$) and Tricosane ($C_{23}H_{48}$) obtained from Sigma-Aldrich were used for the preparation of samples. Their selection was based on previous literature. Initially, 10,000 mg/L concentration of Octacosane (A), Pentacosane (B) and Tricosane (C) were prepared by adding an appropriate quantity of HPLC grade hexane. Furthermore it was assumed as 100%. After this 50%+50%, 75%+25%, and 25%+75%, dilutions were prepared for:

Octacosane+Pentacosane,

Pentacosane+Tricosane

Tricosane+Octacosane combinations.

All concentrations were utilized for stable formulation preparation by impregnating them in inert Fuller's earth clay **(Table 1)**.

Table 1: Detail of formulation combinations and concentrations

Concentration Combination	C1	C2	C3	C4	C5	C6 (Control)
A+B	100%A	100%B	50% A +50% B	75% A +25% B	25%A +75%B	Hexane washed Fuller's earth clay
B+C	100%B	100%C	50% B +50% C	75% B +25%C	25%B +75% C	Hexane washed Fuller's earth clay
C+A	100%C	100%A	50% C +50% A	75% C +25%A	25%C +75% A	Hexane washed Fuller's earth clay

Petridish bioassays

The *C. cephalonica* eggs were collected and washed with hexane to remove active cues from the surface of host eggs. Tricho cards were processed by pasting 30 eggs on 2 x 2 cm^2 paper. The experimental arena consisted of six petri dishes (150 mm × 15 mm), each containing six tricho cards arranged randomly and equidistantly towards the periphery of petri dishes. In each petri dish, five tricho cards were treated with 1 mg of each targeted formulation and the sixth card (control) was treated with 1mg Fuller's earth clay washed with 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 performed at 26±2°C, 65%±5 RH and 160 lux light intensity. Orientation response of egg

parasitoids was recorded every 5 min. for 45 min. After bioassays, tricho cards were incubated at 26±2°C and 65%±5 RH. On the 5th day, parasitization (PARA) was observed by counting the number of blackened eggs on each tricho card **[23]**.

Statistical analysis

Orientation responses from petri dish bioassays with two Trichogrammatids was tabulated and parasitoid activity index (PAI) was calculated. PARA data was converted into percent parasitization (% PARA). Heterogeneous variances in raw data were subjected to normalization through transformation (Square-Root and ArcSine) 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 Graph Pad (www. graphpad. com) was done to obtain a two-tailed *p*-value and *t*-value of responses with a significant difference of individual concentrations with that of control **[24, 25]**. In each interaction for mean PAI and mean % PARA % stimulation (+) / inhibition (-) over control were also calculated (PAI, $\% X_{PAI}$; %PARA, $\% X_{\%PARA}$) **[26]**.

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

$\% X_{PAI / \%PARA}$ = % 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

RESULT AND DISCUSSION

Assessment impact of different concentrations of Octacosane, Pentacosane and Tricosane combinations on mean PAI and mean % PARA showed that all three combinations showed significantly varied responses towards *T. brasiliensis* and *T. chilonis*. Hydrocarbon combinations interaction studies showed that the overall significant stimulation in parasitoid activity for *T. brasiliensis* was noticed by Octacosane and Pentacosane concentrations (202.25%, p=0.0005, t=8.0131) and highest parasitism stimulation was shown by Pentacosane and Tricosane (177.82%, p=0.0244, t=3.1843). Different hydrocarbons and their combinations also appeared to induce varied stimulations and inhibition in foraging behavior of *T. brasiliensis*. Octacosane and Pentacosane combination which showed an overall highest parasitoid activity also indicated that all four tested combinations of these hydrocarbons were able to generate positive stimulation for targeted parasitoid. In this group, it was observed that equal

concentrations of both the hydrocarbons and 100% of Octacosane induced significant stimulation in *T. brasiliensis* **(**46.67±12.97, p=0.0366 t=2.8313 and 13.89±3.15, p= 0.0380, t=2.8004). In case of Pentacosane and Tricosane, 10000 mg/L concentration of Tricosane and 25% Pentacosane + 75% Tricosane combination showed significant stimulation response, respectively (27.22±7.62, p=0.0244, t=3.1843 and 26.67±5.77, p=0.0240, t=3.2005) and the individual pure form of Tricosane showed little higher parasitism. In case of Tricosane and Octacosane combination, no significant parasitizing stimulation was observed for targeted parasitoid. Hence, it is evident that Pentacosane act as a strong stimulator for *T. brasiliensis* **(Table 2 and 3)**.

Overall significant stimulation in foraging efficiency for *T. chilonis* was noticed by Tricosane and Octacosane combination, respectively (1258.20%, p<0.0001, t=21.9107; 116.36%, p=0.0057, t=4.6317). This combination also reflected that 100% Octacosane and 25% Tricosane +, 75% of Octacosane were able to produce significant stimulation response. (46.67±4.39, p=0.0057, t=4.6317 and 32.22±3.18, p=0.0111, t=3.9293). Although the various concentration of two other combinations i.e., Octacosane + Pentacosane and Pentacosane + Tricosane combinations were able to generate positive stimulation in *T. chilonis* but none of them were to be found significant at 5% significance level **(Table 4 and 5)**. The effect of three hydrocarbon based formulations on overall parasitizing stimulation on two trichogrammatids revealed that Pentacosane and Tricosane showed higher overall parasitization stimulation activity of *T. brasiliensis* (177.82%) followed by Octacosane and Pentacosane combination (142.8%), whereas Tricosane and Octacosane combination showed 116.36% overall parasitizating stimulation of *T. chilonis*. Although, inhibition (-5%) was also shown by Octacosane and Pentacosane combination towards parasitizing response of *T. chilonis* **(Graph 1)**.

Various saturated hydrocarbons were reported for influencing parasitization of *Trichogramma spp*. **[27]**. Elevation in foraging responses by *T. brasiliensis* and *T. japonicum* were detected towards Tricosane (C_{23}) during a study conducted on five Trichogrammatids **[28]**. During a study on the impact of eleven saturated hydrocarbons on Trichogrammatids it was observed that egg cards treated with Tricosane showed the highest level of parasitism by *T. chilonis* which was at par with Octacosane **[29]**.

Variation in the quantity and concentration of saturated hydrocarbons influenced the parasitization efficiency of Trichogrammatids **[30]**. *T. brasiliensis* and *T. japonicum* with kairomonal formulation (Tricosane @ 0.0001 g/10 mL) and kailonite clay were claimed to be the best combination for any IPM program **[28]**.

Present study suggested that difference in orientation response of targeted egg parasitoids *viz*., *T. chilonis* and *T. brasiliensis* towards three saturated hydrocarbon combination based formulations appears due to the difference in compatibility of each hydrocarbon with its base and its own impact. Other authors also quoted similar pattern in their studies which showed accordance with present research work.

Table 2: Effect of different concentration combinations of Octacosane, Pentacosane and Tricosane on Parasitoid Activity Index of *Trichogramma brasiliensis*

	C1	C2	C3	C4	C5	C6 (control) Y	MEAN X	Difference (X-Y)
A+B	8.83±0.60	7.17±0.60	13.50±1.17	1.50±0.22	9.33±0.67	2.67±0.49	8.07±0.78	5.40
Significancy at 5% level	P < 0.0001 t= 12.9210	P< 0.0001 t= 13.1747	P=0.0005 t = 8.0131	NS	P = 0.0008 t = 7.2548			
% Stimulation	230.71	168.54	405.62	-43.82	249.44			
B+C	4.33±0.76	6.83±0.60	5.50±0.76	8.17±0.95	10.00±0.58	0.00±0.00	6.97±0.48	6.97
Significancy at 5%	P = 0.0023 t = 5.7009	P< 0.0001 t = 11.3714	P = 0.0008 t = 7.2012	P = 0.0003 t = 8.6351	P< 0.0001 t= 17.3205			
% Stimulation	-	-	-	-	-			
C+A	5.33±1.15	13.17±0.95	16.67±0.76	6.17±0.60	10.67±0.76	9.67±0.88	10.40±0.87	0.73
Significancy at 5%	NS	P = 0.0074 t = 4.3412	P = 0.0003 t = 9.0370	NS	NS			
% Stimulation	-44.88	36.19	72.39	-36.19	10.34			

Values of each interaction is mean of six replicates; Values after ± indicate Standard Error; NS=Non significant at 5% level

Table 3: Effect of different concentration combinations of Octacosane, Pentacosane and Tricosane on Mean Percent Parasitism of *Trichogramma brasiliensis*

	C1	C2	C3	C4	C5	C6 (control) Y	MEAN X	Difference (X-Y)
A+B	13.89±3.15	12.78±7.12	46.67±12.97	0.00±0.00	21.11±6.36	7.78±3.06	18.89±4.19	11.11
Significancy at 5% level	P=0.038 t=2.8004	NS	P = 0.0366 t = 2.8313	NS	NS			
% Stimulation	78.53	64.27	499.87	-100.00	171.34			
B+C	25.00±6.31	27.22±7.62	18.89±10.94	25.56±7.59	26.67±5.77	8.88±4.61	24.67±3.26	15.79
Significancy at 5%	NS	P=0.0244 t = 3.1843	NS	NS	P=0.0240 t = 3.2005			
% Stimulation	-181.53	206.53	112.73	187.84	200.34			
C+A	7.78±3.19	17.78±10.81	18.89±8.97	11.67±4.69	19.44±7.71	8.89±3.06	15.11±3.30	6.22
Significancy at 5%	NS	NS	NS	NS	NS			
% Stimulation	12.49	100.00	112.49	31.27	118.67			

Values of each interaction is mean of six replicates; Values after ± indicate Standard Error; NS=Non significant at 5% level

Table 4: Effect of different concentration combinations of Octacosane, Pentacosane and Tricosane on Parasitoid Activity Index of *Trichogramma chilonis*

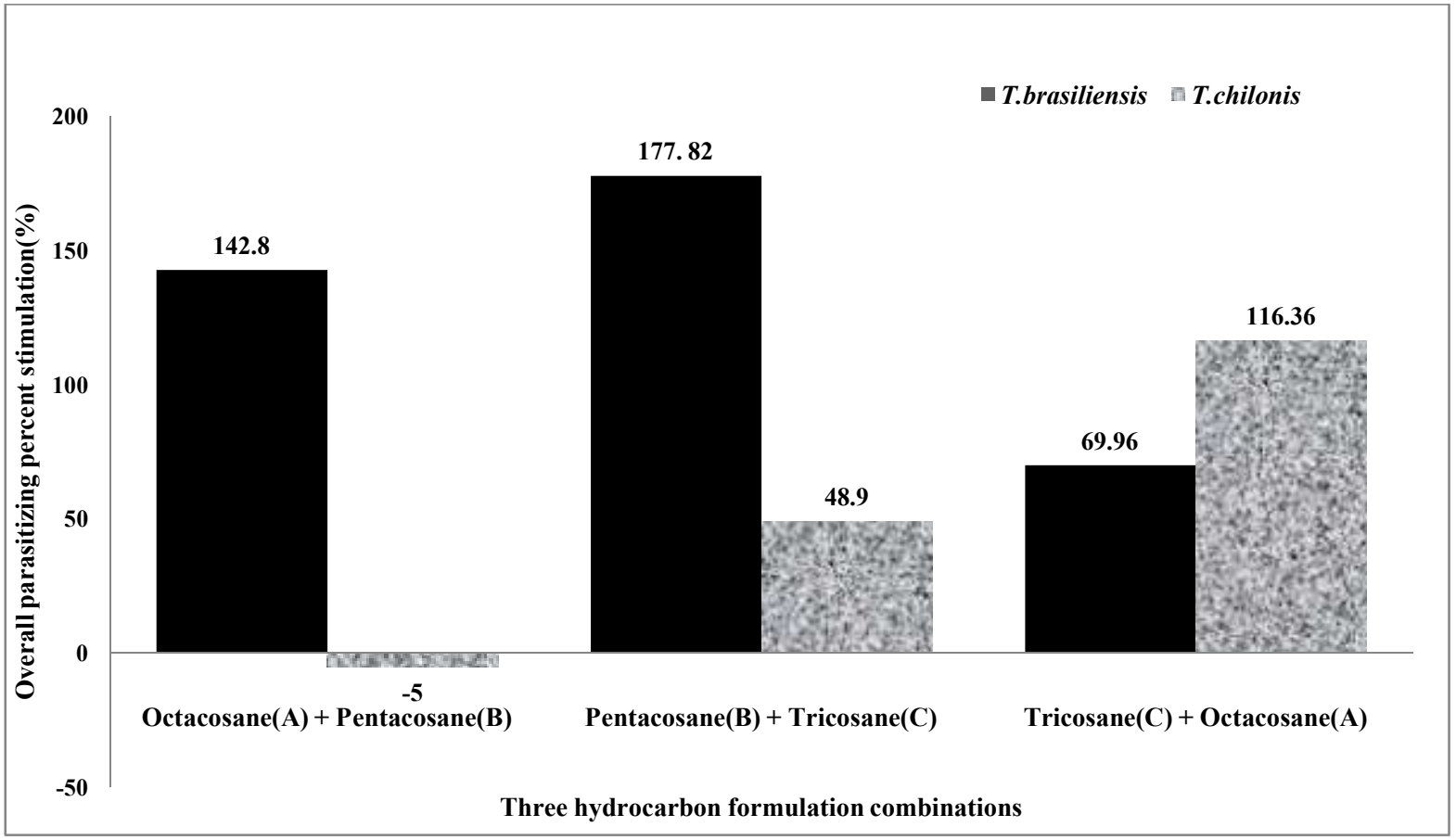
	C1	C2	C3	C4	C5	C6 (control) Y	MEAN X	Difference (X-Y)
A+B	6.67±0.67	6.33±0.67	9.17±0.31	6.33±0.42	5.17±0.48	4.33±0.42	6.73±0.29	2.4
Significancy at 5% level	P =0.0026 t = 5.5340	P =0.0327 t = 2.9277	P =0.0007 t = 7.3899	P =0.0327 t = 2.9277	P =0.0041 t = 5.0000			
% Stimulation	54.04	46.19	111.78	46.19	19.40			
B+C	8.50±0.76	5.50±0.76	3.83±0.60	5.83±0.60	2.50±0.56	5.33±0.99	5.23±0.47	-0.1
Significancy at 5%	NS	NS	NS	NS	NS			
% Stimulation	59.47	3.19	-28.14	9.38	-53.10			
C+A	2.83±0.60	13.83±0.60	9.83±0.40	9.00±1.06	10.00±0.82	0.67±0.21	9.10±0.73	8.43
Significancy at 5%	P= 0.0155 t = 3.6056	P< 0.0001 t= 21.9107	P < 0.0001 t = 19.2068	P= 0.0004 t= 8.4275	P =0.0002 t= 9.7780			
% Stimulation	322.39	1964.18	1367.16	1243.28	1392.54			

Values of each interaction is mean of six replicates; Values after ± indicate Standard Error; NS=Non significant at 5% level

Table 5: Effect of different concentration combinations of Octacosane, Pentacosane and Tricosane on Mean Percent Parasitism of *Trichogramma chilonis*

	C1	C2	C3	C4	C5	C6 (control) Y	MEAN X	Difference (X-Y)
A+B	43.89±9.72	38.89±6.70	36.67±10.44	29.44±12.43	30.56±12.78	37.78±8.10	35.89±4.53	-1.29
Significancy at 5% level	NS	NS	NS	NS	NS			
% Stimulation	16.17	2.94	-2.94	-22.08	-19.11			
B+C	31.11±11.54	11.67±7.24	1.11±1.11	30.00±9.35	0.56±0.56	10.00±5.31	14.89±3.96	4.89
Significancy at 5%	NS	NS	NS	NS	NS			
% Stimulation	211.10	16.70	-88.90	200.00	-94.40			
C+A	15.56±3.72	46.67±4.39	23.33±6.09	14.44±3.51	32.22±3.18	12.22±3.72	26.44±2.85	14.22
Significancy at 5%	NS	P =0.0057 t =4.6317	NS	NS	P =0.0111 t =3.9293			
% Stimulation	27.33	281.91	90.92	18.17	163.67			

Values of each interaction is mean of six replicates; Values after ± indicate Standard Error; NS=Non significant at 5% level



Graph 1: The overall percent stimulation on parasitization as showed by three hydrocarbon formulations

CONCLUSION

Assessment impact of different concentrations of Octacosane ($C_{28}H_{58}$), Pentacosane ($C_{25}H_{52}$) and Tricosane ($C_{23}H_{48}$) combinations served as favourable that influenced the higher foraging behaviour in *T. brasiliensis* and *T. chilonis* There is need to assess the individual concentrations of Octacosane (C_{28}), Pentacosane (C_{25}) and Tricosane (C_{23}) and their combinations to prepare stable formulations as stimulants for *T. brasiliensis* and *T. chilonis*, to provide an eco-friendly and self-sustainable insect pest control.

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