ISSN 2250-3153

Effect of Various Concentration of Octacosane, Pentacosane and Tricosane on Foraging Behavior of Trichogrammatids

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Abstract- Semiochemicals especially their alkane constituents play vital role in governing the foraging behavior of egg parasitoids. Thus in the present study various concentrations of the three straight chain alkanes were prepared to determine the specific concentration(s) optimizing the foraging behavior of Trichogramma brasiliensis Ashmead and Trichogramma chilonis Ishii. Laboratory bioassays were carried out to assess the impact of various concentrations of octacosane, pentacosane and tricosane on the parasitic efficiency of targeted trichogrammatids. T. chilonis was found to exhibit enhanced parasitism for concentrations ranging from 7 000mg/L to 15 000mg/L with maximum at 11 000mg/L for Pentacosane (17.23±0.56) whereas T. brasiliensis exhibited favorable response towards almost all the concentrations of tricosane and pentacosane showing more efficiency at 11 000mg/l (19.44±2.00) and 14 000mg/l(18.89±0.70) respectively. These studies indicate that alkanes can act as semiochemical source and can be exploited to enhance foraging efficiency of egg parasitoids in integrated pest management programs.

Index Terms- Alkanes, Integrated Pest Management, Semiochemicals, Trichogramma.

I. Introduction

In nature, tritrophic interactions of host plant, host insects and their natural enemies are mediated by a complex array of stimuli, of which the role of semiochemicals is commendable (Loughrin *et al*., 1994, Paul *et al*., 2008). The central role played by semiochemicals emanating from plants and host insects, during different phases of the natural enemies' searching behavior is well established (Hilker *et al.*, 2002; Borges *et al.*, 2003; Lou *et al.*, 2005; Manrique *et al.*, 2005; Yong *et al.*, 2007; Tabone *et al*., 2010). Stimulus in form of infochemicals guide parasitoids to their host leading eventually to oviposition in or on the host (Moraes *et al.*, 2005; Fatouros *et al.*, 2008). Parasitoids exhibit varied foraging behavior in response to sensitization by semiochemical stimulus. The ability of infochemicals to enhance the orientation efficiency of parasitoids, culminating in control of the pest infestation forms the basis of IPM strategies (Powell, 1991; Dicke and Vet, 1999; Romeis *et al*., 2005). Due to heavy reliance of natural enemies on infochemicals for optimal performance as biological control agents in IPM strategies identification of the chemical nature of these cues is vital. Various studies have reported these cues to be mainly hydrocarbons in nature (Yadav *et al*., 2002; Paul *et al*., 2008). Several follow-up studies have found saturated, straight chain hydrocarbons like tricosane, pentacosane, hexacosane, pentadecane, hexatriacontane, docosane, to act as biologically active cues and enhance the foraging efficiency of Trichogrammatids. Polyphagous nature of Trichogrammatids against many lepidopterous pests of economically high valued crops like rice, cotton tomato, and cole crops makes them one of the most promising biological control agents during field releases (Stinner, 1977; Bobendreier *et al*., 2003; Fatouros *et al*., 2008). Hence in present study various concentrations of straight chain saturated hydrocarbons viz., octacosane, pentacosane and tricosane were prepared to determine specific concentration(s) optimizing the foraging behavior of two Trichogrammatids *viz.*, *Trichogramma brasiliensis* Ashmead and *Trichogramma chilonis* Ishii.

II. MATERIALS AND METHODS

Insect rearing protocols

The host, rice meal moth *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), and isofemale lines of Trichogrammatids, *T. brasiliensis* and *T. chilonis* were obtained from a stock culture maintained at the Biological Control Laboratory, Amity Institute of Biotechnology, Amity University, Sec-125, Noida, U.P. Culture of *C. cephalonica* was established in rearing cages as described by Sreekumar and Paul (2000). Eggs of *C. cephalonica* 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 *T. brasiliensis* for oviposition and the vials kept at 26±2 °C and 65±5% RH for emergence (Bharti *et al*., 1994, Archna *et al*., 2009).

Preparation of saturated hydrocarbon concentrations

Three straight chain saturated hydrocarbons *viz*., tricosane, pentacosane, and octacosane- obtained from sigma-Aldrich were used for the preparation of test samples. Five different concentrations *viz.*, 7 000 mg/L; 11 000 mg/L; 14 000 mg/L; 15 000 mg/L and 25 000 mg/L, were prepared for each targeted hydrocarbon by adding appropriate quantity of distilled HPLC grade hexane. All concentrations of targeted hydrocarbons were tested for their activity with *T. brasiliensis* and *T. chilonis*.

Bioassay Protocol

Petridish bioassays were carried out by placing egg cards (30 eggs per card) sprayed with the respective hydrocarbon concentrations (@50 µl /egg card) in 150 x 15 mm petri dishes. In each petri dish, 10, 0-24h old Trichogrammatid 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 number of blackened eggs was counted to estimate the parasitism (Singh *et al*., 1998). Laboratory conditions for the experiment were 26 ± 2°C, 65 ± 5% RH and light intensity 160 LUX (Padamavathi and Paul, 1998, Kumar *et al*., 2012).

Statistical analysis

The data was tabulated and percent parasitism was calculated using the following formula:

Percent parasitism = (Number of blackened eggs per card / Total number of eggs per card) X100.

Data obtained for percent parasitism and PAI was analyzed by 2 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. Comparison of PAI and parasitism for different concentrations of crude hydrocarbon extracts with pure hexane (control) were carried out using paired T test.

III. RESULT

Effect on foraging efficiency of *T. chilonis*

Highest parasitoid activity index was registered for 15000mg/L concentration of tricosane for egg parasitoid *T. chilonis*, which was significantly different from control at 5% level of significance (14.00±1.16) (P=0.0001; t=10.6279). Parasitoid activity index was found to be significantly high for 25000mg/L concentration of octacosane (3.50± 0.22) (P=0.0422; t=2.71) and 11000 mg/L and 14000 mg/L concentrations of Pentacosane (7.50± 0.22, 6.17± 0.83) (P=0.0001; t=12.47 and P=0.0422; t=2.71) respectively (Table 1).

For egg parasitoid *T. chilonis*, 11000 mg/L concentration of pentacosane (17.23± 0.56) (P=0.0009; t=7.06) was found to register the highest mean percent parasitism. 7000mg/L and 11000mg/L concentrations of octacosane (12.78± 1.03; 9.45± 0.56) (P=0.0009; t=7.06 and P=0.002; t=5.97) respectively recorded significantly higher parasitism as compared to control at 5% level of significance. Parasitization was recorded to be significantly high for 11000mg/L, 14000mg/L and 15000 mg/L concentrations of Pentacosane. 15000mg/L concentration of Tricosane registered significantly higher parasitism as compared to control (15.00± 3.19) (P=0.008; t=4.23) (Table 2).

Effect on foraging efficiency of *T. brasiliensis*

Highest parasitoid activity index was registered for 25000mg/L concentration of tricosane (10.00±0.68) (P=0.01; t=4.10).. For pentacosane, 14000mg/L concentration reported significant level of response (8.00±0.37) (P=0.0001; t=19.03) whereas for octacosane 25000mg/L concentration shows significant level of parasitoid activity (4.17±0.17) (P=0.0001; t=13.00) (Table 3).

11000mg/L concentration of tricosane elicited the highest mean percent parasitism (19.44+2.00) (P=0.0006, t=7.68) for *T. brasiliensis*, followed by 14000mg/L concentration of pentacosane (18.89+0.70) (P=0.0002, t=10.27) which were significantly different from control at 5% level of significance. All concentrations of pentacosane, except 15000 mg/L, were found to register a significantly high percent parasitism. Parasitism was recorded to be significant for all concentrations of Tricosane (Table 4).

IV. DISCUSSION

Foraging behavior of egg parasitoids is guided by a wide array of semiochemicals (Hilker and Meiners, 2006). Hendry *et al*., (1976) analyzed five host plants of *Heliothis zea* Boddie to determine the presence of hydrocarbons acting as synomones for *Trichogramma evanescens* Westwood. They identified series of hydrocarbons present in host plants ranging from C_{21} to C_{25} in varying quantities**.** Dutton et al. (2000) Yonggen *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.* Similarly in hexane extracts of ten different varieties of tomato *(Lycopersicon esculentum* Mill) obtained in the vegetative and flowering phase of growth, the synomonal response of the prominent egg parasitoid .*T. chilonis* was observed (Paul *et al*., 2008) which seemed to be associated mainly with tricosane (C_{23}), heneicosane (C_{21}), pentacosane (C_{25}) and hexacosane (C_{26}) during the vegetative period and heneicosane (C_{21}), hexacosane (C_{26}) during the flowering period. In present study, saturated hydrocarbons, tricosane, pentacosane and octacosane were found to elicit significant parasitism for *T. chilonis*. Among the eleven saturated hydrocarbons tested by Padmavathi and Paul (1998), tricosane, octacosane and docosane have been reported to enhance the activity and parasitic efficiency of *T. chilonis*. In another study, *Trichogramma exiguum* Pinto and Platner was found to respond positively to pentacosane, hexacosane, pentadecane, hexatriacontane, tricosane and docosane (Paul *et al*., 2002). This is in accordance with the current findings where tricosane and pentacosane were found to register a significant parasitization for *T. brasiliensis* whereas no significant parasitism was reported for octacosane. *T. chilonis* was found to exhibit enhanced parasitization at concentrations ranging from 7000mg/L to 15000mg/L whereas *T. brasiliensis* exhibited favorable response towards almost all the concentrations of tricosane and pentacosane. Less response towards high concentrations of octacosane could be due to bulkiness of octacosane that led to deposition of flakes of this hydrocarbon on egg cards hindering the parasitization of eggs by the parasitoids.

V. CONCLUSION

Present study results indicate that bulkiness and hence mass of hydrocarbons can influence their semiochemical activity and lesser the concentration of heavier hydrocarbons, more is their semiochemical activity. Utilization of optimal concentrations of favorable hydrocarbons can be exploited to enhance the foraging efficiency of Trichogrammatids in integrated pest management programs.

ACKNOWLEDGMENT

We are extremely grateful to Department of Science and Technology for granting financial support for this study. The authors are also thankful to the Director General, AIB, Amity University for providing the required facilities.

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Table 1: Effect of three saturated hydrocarbons on mean parasitoid activity index of *T. chilonis*:

	C1 (7000 mg/L)	C2 (11000 mg/L)	C3 (14000 mg/L)	C4 (15000 mg/L)	C5 (25000 mg/L)	C6 Hexane (control)	Mean
Octacosane	1.83±0.31	0.67±0.21	1.50±0.22	2.50±0.22	3.50±0.22	1.50±0.21	2.00±0.20[c]
Pentacosane	5.50±0.81	7.50±0.22	6.17±0.83	3.50±1.15	1.67±0.33	3.67±0.21	4.87±0.49[b]
Tricosane	5.00±0.82	5.33±0.42	2.83±0.66	14.00±1.16	4.00±0.45	1.50±0.67	6.23±0.80[a]

*Value within column marked with different letters are significantly ($P < 0.05$) different.

	SE	Cd
hydrocarbon x hydrocarbon	0.35	0.69
Concentration x concentration	0.42	0.84
hydrocarbon x concentration	0.85	1.68

Table 2: Effect of three saturated hydrocarbons on mean percent parasitism of *T. chilonis*:

	C1 (7000 mg/L)	C2 (11000 mg/L)	C3 (14000 mg/L)	C4 (15000 mg/L)	C5 (25000 mg/L)	C6 Hexane (control)	Mean
Octacosane	12.78±1.03	9.45±0.56	2.78±0.56	2.22±0.70	2.78±0.55	3.33±0.86	6.00±0.85[b]
Pentacosane	8.33±2.95	17.23±0.56	16.11±2.00	16.11±1.59	8.88±1.11	7.78±1.41	13.33±1.19[a]
Tricosane	7.22±3.38	7.22±1.81	5.00±2.40	15.00±3.19	6.67±2.11	4.45±1.41	8.22±1.28[b]

*Value within column marked with different letters are significantly ($P < 0.05$) different.

	SE	Cd
Concentration x concentration	1.09	2.15
hydrocarbon x hydrocarbon	1.33	2.64
hydrocarbon x concentration	2.66	5.27

Table 3: Effect of three saturated hydrocarbons on mean parasitoid activity index of *T. brasiliensis*:

	C1 (7000 mg/L)	C2 (11000 mg/L)	C3 (14000 mg/L)	C4 (15000 mg/L)	C5 (25000 mg/L)	C6 Hexane (control)	Mean
Octacosane	2.50±0.22	2.67±0.21	1.50±0.22	4.00±0.26	4.17±0.17	2.00±0.00	2.97±0.21[c]
Pentacosane	2.00±0.45	4.33±0.42	8.00±0.37	0.83±0.17	5.67±0.21	1.50±0.22	4.17±0.50[b]
Tricosane	6.00±0.45	6.67±0.42	9.67±0.92	9.83±1.60	10.00±0.68	3.33±1.17	8.43±0.81[a]

*Value within column marked with different letters are significantly ($P < 0.05$) different.

	SE	Cd
hydrocarbon x hydrocarbon	0.25	0.50
Concentration x concentration	0.31	0.61
hydrocarbon x concentration	0.62	1.22

Table 4: Effect of three saturated hydrocarbons on mean percent parasitism of *T. brasiliensis*:

	C1 (7000 mg/L)	C2 (11000 mg/L)	C3 (14000 mg/L)	C4 (15000 mg/L)	C5 (25000 mg/L)	C6 Hexane (control)	Mean
Octacosane	7.22±0.56	0.56±0.56	3.33±0.00	6.12±0.56	1.67±0.75	6.67±0.86	3.78±0.45[b]
Pentacosane	12.22±0.70	12.22±0.70	18.89±0.70	4.44±0.70	11.67±0.75	4.44±0.70	11.89±0.90[a]
Tricosane	10.00±1.49	19.44±2.00	17.22±3.38	11.11±0.70	16.11±1.59	5.56±0.70	14.78±1.83[a]

*Value within column marked with different letters are significantly ($P < 0.05$) different.

	SE	Cd
hydrocarbon x hydrocarbon	0.51	1.01
Concentration x concentration	0.63	1.24
hydrocarbon x concentration	1.25	2.48