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**SYNOMONAL EFFECT OF COLE CROPS ON INDIVIDUAL AND ASSOCIATIVE  
LEARNING BEHAVIOUR OF *COTESIA PLUTELLAE***

**KUMAR A \*, ZAYEEM A AND KANAMENI S**

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector -125, Expressway,  
Noida Campus, U. P., India

**\*Corresponding Author: E mail: archnaaashi@yahoo.com; Tel No.: +91- 9711742253**

**ABSTRACT**

The effectiveness of pest management programs can be enhanced by integrating behavioural information of natural enemies in relation to their host. In present study individual and associative learning behavioural response of larval parasitoid *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) towards hexane leaf extract of three prominent cole crop varieties was investigated through Y tube olfactometer bioassays. Maximum individual and associative behavioural response of *C. plutellae* was observed towards hexane leaf extracts of knol khol variety White Vienna. The hexane leaf extracts of targeted cole crops were also subjected to gas liquid chromatography to detect their saturated hydrocarbon profile. Gas liquid chromatography of White Vienna revealed the presence of three favourable hydrocarbons viz., heneicosane (C<sub>21</sub>), docosane (C<sub>22</sub>) and nonacosane (C<sub>29</sub>). Rapid orientation of *C. plutellae* upon sensitization with favourable saturated hydrocarbons present in hexane extracts is indicative of its potential to utilize preferred chemical signals emanating from cole crops. This feature can be exploited worldwide to enhance the efficacy of *C. plutellae* in integrated pest management of cole crops.

**Keywords: Bioassays, Saturated Hydrocarbon, Semiochemicals, Gas Liquid  
Chromatography, Cole Crops**

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## INTRODUCTION

For ecofriendly management of pest population proper understanding of the behaviour of the natural enemies in relation to their host or prey and habitat (plants) is important. Natural enemies depend on plant pests for completion of their life cycle and hence survival. After oviposition, the female larval parasitoid must search for its host as soon as possible to propagate her progeny. These insect parasitoids are guided to their target pest by a wide array of volatile compounds referred to as semiochemicals secreted by several plant species [1, 2, 3, 4]. In particular, synomones play a vital role as attractants for natural enemies enhancing their host searching efficiency that ultimately leads to better pest elimination which is a beneficial strategy for integrated pest management (IPM) [5]. The cole crops represent an important group among vegetable crops. Diamondback moth *Plutella xylostella* (Linnaeus) (Lepidoptera: Plutellidae) is a major pest of the cole crops throughout the world due to its potential to develop resistance to synthetic pesticides as well as Bt formulations [6]. The solitary larval endoparasitoid, *Cotesia plutellae* Kurdjumov, (Hymenoptera: Braconidae) has been found to effectively minimize the crop loss under field

conditions [7]. Experience with odours encountered by parasitoids in their natural environment modifies their behaviour which increases or decreases their effectiveness [8, 9, 10, 11]. Therefore, the present investigation was aimed to demonstrate, how orientation response of *C. plutellae* females is enhanced due to repetitive sensitization with odours from different cole crops. Saturated Hydrocarbons present in different host plants were found to act as synomones for different parasitoids in diverse crop ecosystems [12, 13]. Thus, in the present study hexane leaf extracts were also analyzed through gas liquid chromatography to determine the saturated hydrocarbons present in them to understand the synomonal interaction.

## MATERIALS AND METHODS

### Host Insect Culture

The diamondback moth, *P. xylostella* was maintained on cabbage / cauliflower leaves, in active vegetative stage, in the culture room at  $26 \pm 2^\circ\text{C}$  and  $65\% \pm 5\%$  RH, 10L:14D photoperiod in open trays at the Biological Control Laboratory, Amity Institute of Biotechnology, Amity University Sector-125, Noida, Uttar Pradesh (INDIA) [14].

## Parasitoids

The nucleus culture of *C. plutellae* was obtained from the Project Directorate of Biological Control (PDBC), Bangalore. Two/three pairs of parasitoid wasps were kept overnight for mating in a Perspex glass cage (30 x 30 x 30 cm size) and offered with second and third instar larvae of diamondback moth for parasitization for about 24–48 hours as described by [15], with slight modifications. The adult parasitoids were fed with 10% glucose / honey solution, pollen and opened raisins. Parasitized larvae were transferred to new trays after 48 hrs and reared following normal rearing procedure till the formation of parasitoid pupae in silken cocoons on the leaf surface. The pupae thus formed were collected and stored in a refrigerator at 10°C till further use. Upon emergence, the adults were examined under a binocular microscope for the segregation of the females and males for further multiplication and use in experiments.

## Synomonal Extracts Preparation

From each targeted crop variety 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 collected, washed with water, air dried and soaked overnight in hexane. The filtrate was kept in sodium sulphate for 1 h, and passed through silica gel column. The extract was then distilled at 60 - 70 °C in a water bath. It was then rinsed with hexane and taken out in a small tube and evaporated completely by keeping the tube in a water bath. This was then used both for gas liquid chromatography and bioassay studies by adding appropriate quantity of distilled HPLC grade hexane. The concentrations used in bioassay were 4 00 000 mg/L, 2 00 000 mg/L and 1 00 000 mg/L.

## Gas Liquid Chromatography Analysis

These 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 100 °C to 320 °C @ 5 °C / min and injector and detector temperature was 320°C. Nitrogen was used, as a carrier gas with a flow rate of 20 mL/ min. Injection volume was 1 µL. 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 [16] as follows.

$$\text{Concentration of Unknown Saturated Hydrocarbon} = \frac{\text{Area of Unknown Saturated Hydrocarbon}}{\text{Area of Known Saturated Hydrocarbon}} * \text{Concentration of Known Saturated Hydrocarbon}$$

### Bioassay Protocol

The individual and associative learning behaviour response of *C. plutellae* towards various concentrations of different cole crop extracts were investigated using a Borosil glass Y-tube olfactometer (length of each arm = 200mm, internal diameter = 15 mm). The upper arms of the Y-tube were considered as centres for source (extract) and control (hexane). The lower arm of the Y tube served as inlet point for release of the parasitoid(s). Air flow @ 7.15 mL / min was maintained using a blower fitted to the upper arms of the olfactometer. The cole crop extracts were applied @ 50µl onto a Whatman No.1 filter paper strip (30 mm x 10 mm). Source and control strips were placed individually inside the upper arm of Y tube. Four such replication tubes were arranged. Single / 5 mated female parasitoids were released at the stem of the Y tube olfactometer and their response was recorded by counting the number of parasitoids opting for the host plant odour against control. Each assay was run for

20 minutes at  $26 \pm 2^\circ\text{C}$  and  $65\% \pm 5\%$  RH and 150 Lux light density. The position of each parasitoid was recorded after every two minutes.

### Statistical Analysis

The response of parasitoid wasps to various concentrations of target host plant leaf extracts was tabulated and analyzed by 2-way ANOVA, using applied indostat software version 8.0 developed by Windostat Hyderabad, India. The difference between the means of two treatments within a group was compared by LSD test at 5% significance level. Subsequently, significant difference in the response elicited by the source as compared to control was determined using paired t-test.

## RESULTS

### Individual Synomonal Interaction

Response of individual female *C. plutellae* was found to be significantly higher towards

hexane extract of all varieties as compare to control at 5% significance level ( $P < 0.05$ ). knol khol variety White Vienna registered highest foraging response *C. plutellae* female ( $2.58 \pm 0.22$ ) followed by cauliflower variety PSBK1 ( $2.54 \pm 0.22$ ). Among all the concentrations, 4 00 000 mg/L concentration of White Vienna leaf extracts elicited most significant synomonal response for single female parasitoid ( $2.88 \pm 0.20$ ;  $P < 0.001$ ;  $t = 6.26$ ) (Table 1).

#### Associative Synomonal Interaction

Associative learning behavioural response of the larval parasitoid *C. plutellae* was maximum for knol khol variety White Vienna ( $7.83 \pm 0.73$ ) followed by cauliflower variety PSBK1 ( $7.58 \pm 0.35$ ). 4 00 000 mg/L concentration for both White Vienna and Golden acre extracts elicited highest foraging response for five mated females ( $8.75 \pm 0.89$ ;  $8.75 \pm 0.54$ ). However, 100,000 mg/l concentration for White Vienna also registered equally high response for *C.*

*plutellae* females ( $8.75 \pm 1.01$ ) (Table 2). Response observed was significant for almost all concentrations as indicated by paired t test.

#### Synomonal Hydrocarbons from Host Plants

Gas liquid chromatograph of targeted extracts in flowering phase of growth indicated the presence of favourable as well as unfavourable saturated hydrocarbons ranging from  $C_{21}$  to  $C_{32}$  varying in number and concentration eliciting varied degree of orientation response from *C. plutellae*. Gas liquid chromatography of White Vienna extracts, which elicited highest individual as well as associative response, had three favourable hydrocarbons heneicosane ( $C_{21}$ ) (261.51 mg/L), docosane ( $C_{22}$ ) (301.67 mg/L), and nonacosane ( $C_{29}$ ) (189.73 mg/L). Variety PSBK -1 indicated the presence of four favourable hydrocarbons. Concentration of docosane ( $C_{22}$ ) was highest in all Cole crop leaf extracts (Table 3).

**Table 1: Synomonal Response of Individual *C. plutellae* Towards Hexane Leaf Extracts of Three Cole Crop Varieties**

Cole Crop Varieties	Concentrations of Hexane Extracts (mg/L)	Orientation Towards Source (s)	Orientation Towards Control (c)	Difference in Orientation (s-c)	P Value	t Value
GOLDEN ACRE	400 000	2.75 ± 0.28	0.88±0.24	1.87±0.04	0.0022	3.6965
	200 000	2.13 ± 0.24	1.00±0.18	1.13±0.08	0.0060	3.1953
	100 000	2.63 ± 0.22	1.00±0.13	1.63±0.09	0.0002	4.7789
	Total	2.50 ± 0.25	0.96±0.18	1.54±0.07		
PSBK-1	400 000	2.63 ± 0.22	0.88±0.15	1.75±0.07	0.0002	4.8693
	200 000	2.25 ± 0.36	1.00±0.13	1.25±0.23	0.0128	2.8247
	100 000	2.75 ± 0.26	0.63±0.18	2.12±0.08	0.0001	6.4875
	Total	2.54 ± 0.22	0.83±0.15	1.71±0.07		
White Vienna	400 000	2.88 ± 0.20	0.63±0.18	2.25±0.02	0.0001	6.2605
	200 000	2.38 ± 0.18	1.00±0.13	1.38±0.05	0.0002	4.7932
	100 000	2.50 ± 0.29	0.88±0.24	1.62±0.05	0.0053	3.2568
	Total	2.58 ± 0.22	0.83±0.18	1.75±0.04		

Values are Mean of Sixteen Observations ± Standard Error

	SEm	CD at 5%
<b>For source:</b>		
Variety × Variety	0.13	0.26
Concentration × Concentration	0.19	0.38
Variety × Concentration	0.30	0.60
<b>For control:</b>		
Variety × Variety	0.12	0.24
Concentration × Concentration	0.15	0.30
Variety × Concentration	0.26	0.51

**Table 2: Synomonal Responses of Five Mated Females of *C. plutellae* Towards Hexane Leaf Extracts of Three Cole Crop Varieties**

Cole Crop Varieties	Concentrations of Hexane Extracts (mg/L)	Orientation Towards Source (s)	Orientation Towards Control (c)	Difference in Orientation (s-c)	P Value	t Value
GOLDEN ACRE	400 000	8.75±0.54	5.00±0.85	3.75±-0.31	0.0034	3.72
	200 000	5.00±0.48	4.00±0.21	1.00±0.27	0.0069	3.3166
	100 000	7.50±0.66	4.25±1.14	3.25±-0.48	0.0038	3.6571
	Total	7.08±0.56	4.42±0.73	2.66±-0.17		
PSBK-1	400 000	7.50±0.26	6.00±0.67	1.50±-0.41	0.0433	2.2827
	200 000	7.00±0.93	5.00±0.77	2.00±0.16	0.0874	1.8762
	100 000	8.25±0.13	5.25±1.19	3.00±-1.06	0.0344	2.4132
	Total	7.58±0.35	5.42±0.88	2.16±-0.53		
White Vienna	400 000	8.75±0.89	2.25±0.39	6.50±0.50	0.0001	9.4087
	200 000	6.00±0.30	6.50±0.15	-0.5±0.15	0.1661	1.4832
	100 000	8.75±1.01	5.25±0.69	3.50±0.32	0.0001	7.000
	Total	7.83±0.73	4.67±0.41	3.16±0.32		

Values are mean of twelve observations ± standard error

	SEm	CD at 5%
<b>For source:</b>		
Variety × Variety	0.64	1.34
Concentration × Concentration	0.55	1.11
Variety × Concentration	0.96	1.92
<b>For control:</b>		
Variety × Variety	0.49	1.02
Concentration × Concentration	0.62	1.23
Variety × Concentration	1.00	2.02

Table 3: Hydrocarbon Profiles of Three Cole Crop Varieties in Flowering Phase of Their Growth

Hydrocarbons	Concentrations in mg/L		
	Golden Acre	PSBK1	White Vienna
<b>Favourable</b>			
Heneicosane (C <sub>21</sub> )	1 061.64	1260.23	261.51
Docosane (C <sub>22</sub> )	2 563.67	1626.79	301.67
Tricosane (C <sub>23</sub> )	1 469.46	712.27	ND
Pentacosane (C <sub>25</sub> )	889.16	ND	ND
Nonacosane (C <sub>29</sub> )	616.27	105.53	189.73
<b>Unfavourable</b>			
Tetracosane (C <sub>24</sub> )	1 099.45	413.39	ND
<b>Others</b>			
Heptacosane (C <sub>27</sub> )	372.77	222.92	ND
Triacosane (C <sub>30</sub> )	10 076.68	22131.33	34 550.63
Hentriacontane (C <sub>31</sub> )	1 236.03	5198.30	4 570.21
Dotriacontane (C <sub>32</sub> )	88.74	ND	ND

ND = Not detected

## DISCUSSION

Induction of plant defence in response to herbivore includes the emission of volatile compounds called synomones that act as attractants for natural enemies of herbivores. Synomones attract predators and parasitoids which elucidate the tritrophic interaction in a crop ecosystem. The Y-tube olfactometer tests conducted to observe the response of *C. plutellae* females to synomonal volatile from

Blue Lake cabbage and Early Phenomenal cauliflower indicated that odours of both the brassica varieties were preferred to the control odour (water) [17]. *C. plutellae* predominantly uses plant derived volatiles in its host searching behaviour [15]. Studies on the interaction of two herbivore species that simultaneously attack crucifer plants and their respective parasitic wasps observed the specific response of two specialist parasitic

wasps, *C. plutellae* and *Cotesia glomerata* (Linnaeus) to info-chemicals originating from cabbage plants (*Brassica oleracea* var. *capitata*) infested by each of their host larvae (*P. xylostella* and *Pieris rapae* Linnaeus). The results indicated presence of infochemically mediated tritrophic interactions [18]. Among the green leaf volatiles of cabbage Z3-6: Ac, Z2-6: Ald and Z3-6: OH elicited significant response in *C. plutellae* [19]. Y tube olfactometer studies on differential attractiveness to plant odours of *Cotesia marginiventris* (Cresson) found that the wasp preferred cowpea over maize odour [20]. Based on behavioural assays, they showed that not only the quantity of volatile emission but also the quality composition of the volatile blends was important for the attraction of *C. marginiventris*. Jasmonic acid induced cruciferous plants to emit volatiles thus enhancing the overall response of *C. plutellae* to the treated crucifers [21]. In present study Y tube Bioassays were carried out to elucidate the individual and cumulative synomonal effect of selected leaf extracts on *C. plutellae* female under laboratory conditions. The Bioassays demonstrated that individual and associative behavioural response was high for Knol Khol variety White Vienna. Gas liquid chromatography studies of White Vienna in flowering phase

revealed the presence of three favourable hydrocarbons, heneicosane (C<sub>21</sub>), docosane (C<sub>22</sub>) and nonacosane (C<sub>29</sub>) in lower concentration as compare to other two cole crop varieties. Present study also indicated that orientation behaviour of parasitoid varied with composition and concentrations of different saturated hydrocarbons in synomonal source. The source of chemical cues in a system were elucidated where the host was concealed and the parasitoid had no direct contact with the host larvae or its frass [22]. Behavioural bioassays with *Pholetesor bicolor* (Nees), a larval parasitoid of the apple leafminer *Phyllonorycter pomonella* (Zeller) showed that the herbivore-damaged leaf epidermis (mine) elicited ovipositional probing of parasitoid females. Hexane extracts of mines elicited the same ovipositional probing behaviour. In addition, gas chromatographic analyses showed qualitatively and quantitatively different profiles of the components of the host-plant complex. The highest quantities and also the highest number of compounds were recovered from mine extracts. Compounds identified in the mine included six alkanes, Heptacosane to tritriacontane (n-C<sub>27</sub> to n-C<sub>33</sub>) and squalene (C<sub>30</sub>H<sub>50</sub>). Thus, it was concluded that this leafminer parasitoid uses plant-derived semiochemicals for host location and

ovipositional probing behaviour. In a related study [23] volatiles of JA-treated plants of six rice varieties compared for attractiveness to *Anagrus nilaparavate* Pand et Wang, an egg parasitoid of rice plant hoppers were also found to differ among varieties, both in total quantity and quality of the blends emitted. 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 *Trichogramma chilonis* Ishii was observed [16] which seemed to be associated mainly with tricosane (C<sub>23</sub>), heneicosane (C<sub>21</sub>), pentacosane (C<sub>25</sub>) and hexacosane (C<sub>26</sub>) during the vegetative period and heneicosane (C<sub>21</sub>), hexacosane (C<sub>26</sub>) during the flowering period. The non-hydrocarbon fraction and hydrocarbon fraction together elicited a positive effect on oviposition behaviour in *C. plutellae* females [24]. The effect of induction by exogenous application of jasmonic acid (JA) on the responses of Brussels sprouts plants and on host-location behaviour of associated parasitoid wasps was studied [25]. Feeding by the biting-chewing herbivores *P. rapae* and *P. xylostella* resulted in significantly increased endogenous levels of JA, a central component in the octadecanoid signaling pathway that mediates induced plant defense. Three species

of parasitoid wasps, *C. glomerata*, *Cotesia rubecula* Marshall, and *Diadegma semiclausum* Hellen, differing in host range and host specificity, were tested for their behavioural responses to volatiles from herbivore-induced, JA-induced, and non-induced plants. All three species were attracted to volatiles from JA-induced plants compared to control plants; however, they preferred volatiles from herbivore-induced plants over volatiles from JA-induced plants. Attraction of *C. glomerata* depended on both timing and dose of JA application. JA-induced plants produced larger quantities of volatiles than herbivore-induced and control plants, indicating that not only quantity, but also quality of the volatile blend is important in the host-location behaviour of the wasps.

## CONCLUSION

All these studies corroborate the present study that the favoured response by *C. plutellae* treated with the hexane leaf extract of White Vienna and PSBK1 may be due to the presence of these favourable hydrocarbons, thus confirming the role of actively released plant volatiles for enhancing the host seeking behaviour of parasitoids.

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