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Behavioural and electrophysiological responses of *Chrysoperla carnea* (Stephens) to kairomones - acid hydrolyzed / oxidized L-tryptophan and its breakdown products

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ABSTRACT: **Behavioural and electrophysiological responses of adult *Chrysoperla carnea* (Stephens) were studied in laboratory conditions (27±2°C and RH 70 %). The females of *C. carnea* in the ovipositional period were exposed to acid hydrolysed L- tryptophan using hydrochloric acid, oxidized L-tryptophan using hydrogen peroxide and quick amino acid oxidizers. The response of *C. carnea* females to both hydrolysed and oxidized L-tryptophan was greater at higher concentration (0.66%) than lower concentration (0.33%). The orientational response studies have shown that more number of adults was attracted to the acid hydrolyzed L-tryptophan than oxidized L-tryptophan. Ovipositional studies indicated that acid hydrolyzed L-tryptophan recorded higher oviposition, followed by oxidized L-tryptophan using quick oxidizers. The role of the quick oxidizers in fast breakdown of L-tryptophan and subsequent ovipositional attraction was studied by exposing *C. carnea* to 2 or 3-day-old acid hydrolysed/oxidized L-tryptophan. Higher oviposition was recorded in the 3-day-old formulation compared to 2-day-old acid hydrolyzed and oxidized L-tryptophan. The ultimate breakdown product of L-tryptophan, which was responsible for the attraction of L-tryptophan, was considered to be indole acetaldehyde. The attraction of *C. carnea* to indole acetaldehyde was also studied at 10, 20 mM. There was significantly more oviposition on the filter paper discs treated with 10mM indole acetaldehyde. The prospects of using these products as attractants are discussed.**

KEY WORDS: Acid hydrolyzed, *Chrysoperla carnea*, indole acetaldehyde, L-tryptophan, oxidized

INTRODUCTION

Chrysopids are considered as an important group of predators due to their desirable qualities, such as wide host range, high feeding potential, easy amenability to laboratory rearing, and resistance to pesticides and temperature. Chrysopids are widely used in the cotton ecosystem for the management of several pest species. It is known that chrysopids are attracted to, and feed on naturally occurring homopterous honeydew, which provides them nutrients necessary for oogenesis (Hagen, 1950; Elbadry and Fleschner, 1965; Hagen and Tassan, 1974). On chemical analysis it was observed that L-tryptophan is one of the components responsible for the attraction of chrysopids to the honeydew. Potato plants treated with L-tryptophan alone or combined with feed wheast attracted *C. carnea* and several other coccinellids (Ben Saad and Bishop, 1976). The addition of L-tryptophan either alone or with sucrose was found to attract more number of adults in lucerne fields (Liber and Niccoli, 1988). The number of adults of *C. carnea* was increased considerably on olive tree canopy after treatment with L-tryptophan (Mc Ewen *et al.*, 1994). L-trytophan, an active fraction in honeydew secretions, has been proved to be an effective attractant in cotton (Bakthavatsalam and Singh, 1996).

McEwen *et al.* (1993) described that the acid hydrolysis of L-tryptophan using 2M- hydrochloric acid increased the attraction to L-tryptophan after 3 days of

hydrolysis. However, Emden and Hagen (1976) observed that both acid hydrolyzed and hydrogen peroxide oxidized L- tryptophan attracted the chrysopids. It was also reported that indole acetaldehyde is the breakdown or decomposed product of L-tryptophan which was responsible for the attraction of chrysopids (Van Emden and Hagen, 1976). The present studies were initiated to find out; i. oxidation method that can be substituted to hydrolysis of L-tryptophan to avoid handling hazards of strong acids, ii. quick oxidation compounds to produce decomposition in less time so as to avoid the waiting period of 3 days, and iii. possibility of using indole acetaldehyde directly as an attractant for chrysopids.

MATERIAL AND METHODS

i. Host culture

Cultures of *C. carnea* were maintained at ambient laboratory conditions (27 ± 2°C and RH 70 %). The larvae were reared on UV- irradiated eggs of *Corcyra cephalonica* (Stainton) while the adults were reared on the semi-synthetic diet containing protinex, honey, yeast and fructose as described earlier (Anonymous, 1994; Jalali *et al.*, 2003).

ii. Preparation of kairomonal substances

Two concentrations of L-tryptophan, *i.e.*, 0.33 and 0.66 per cent were prepared by acid hydrolysis as described by Mc Ewen *et al.* (1993). Honey was diluted with water to make 50 per cent solution. For oxidation of L-tryptophan, commercial pharmacological grade hydrogen peroxide (40%) was used. Two ml of hydrogen peroxide was added to 98 ml of solution containing 0.33 and 0.66g of L-tryptophan and after two or three days the kairomone was tested. Quick oxidizer like amino acid oxidizer (Sigma Aldrich*), derived from the venom of snakes, was also added to L-tryptophan and the formulations were tested after two or three days.

Indole acetaldehyde at concentrations of 10 and 20 mM was prepared by oxidizing indole acetic acid (SD Fine Chemicals*) with sodium borohydride. Four mg of sodium borohydride dissolved in one ml of water was added to 9 ml of indole acetic acid. To dissolve indole acetic acid completely, a pinch of sodium bicarbonate was added little by little. This solution was kept overnight at 4°C before use. Further, if the bubbles persisted after 24 hours a drop of formaldehyde was added or heated at 50°C for 20 minutes.

iii. Electroantennogram studies

The electroantennogram response of the adults of *C. carnea* was studied in the laboratory to generate basic information on the response of adults to the kairomones. The antennae were excised along with their base and mounted in the electrodes filled with electrolyte in the manipulator and the electrical responses of the antenna were recorded using the electroantennogram (SYNTECH®, The Netherlands). The stimulus was given for one second and the response was measured for 5 seconds. The stimulus flow was maintained at the rate of 0.5m/sec. Fifty per cent honey solution was used as reference. The experiment was repeated five times and the electrical response in terms of mV was measured. Absolute net EAG_x responses (-mv) to the test cues were calculated by subtracting the mean absolute EAG response of control stimulation immediately preceding ($Control_x$) and following ($Control_{x+1}$) the presentation of the absolute EAG response of the test cue. The data were transformed to $\log_{10}$ values and analysed through ANOVA.

iv. Orientational response

Orientational response of *C. carnea* to the adults was studied in a wind tunnel. The wind tunnel was made of transparent, odorless, acrylic sheet of 3 mm thickness with two chambers referred to as bait-chamber and test-chamber, respectively which were connected through one metre wind tunnel as described by Bakthavatsalam and Singh (1996). Fifteen adults of *C. carnea* were released in the bait-chamber and the number of adults reaching the bait-chamber in 60 minutes was recorded. Each treatment was replicated 10 times and the data were analysed through completely randomized block design.

v. Ovipositional response

Seventy adults of *C. carnea* were released in an acrylic sheet box (30 X 30 X 30 cm) made of odourless, transparent, non-adsorbent acrylic sheet of 3mm thickness. The known quantity of kairomones was sprayed on brown paper discs and dried in shade for half an hour. The paper discs were stuck to the top of the box for oviposition. Fifteen mated adult females were left overnight and the number of eggs laid on the paper discs was counted on the next morning. Five replications were maintained for each treatment.

RESULTS AND DISCUSSION

i. Electroantennogram responses

Highest absolute net response was recorded to honey solution (50%), followed by acid hydrolyzed L-tryptophan (0.66% and 0.33%) and oxidised L- tryptophan using quick oxidizers (0.66%) and all these treatments were statistically on par with each other (Table 1 & Fig. 1).

Least response by *C. carnea* antenna was shown to oxidized L-tryptophan using H_2O_2 followed by higher concentration (0.66%) of the same product. When the response was normalized using honey as reference, the response to acid hydrolyzed L-tryptophan (0.66%) and oxidized L-tryptophan (0.66%) was higher than all other cues.

ii. Orientational and ovipositional respnse of *C. carnea* to hydrolyzed and oxidized L- tryptophan

Among the different compounds tested, highest number of adults visited the filter papers treated with acid hydrolyzed L - tryptophan (0.66% and 0.33%), followed by oxidized L-tryptophan using quick oxidizers (Table 2). The females of *C. carnea,* following contact with L-tryptophan, showed reduced mean walking speed (inverse orthokinesis), increased turning frequency (positive klinokinesis), and increased mean turning angles (Mc Ewen *et al.,* 1993). The oxidized L-tryptophan using hydrogen peroxide did not evoke any response, contrary to the observations of Van Emden and Hagen (1976).

In ovipositional response studies, highest number of eggs was laid on the paper discs treated with acid hydrolyzed L-tryptophan, followed by L-tryptophan oxidized with quick oxidizers. Statistically, both treatments were on par (Table 2). Least number of eggs laid was by *C. carnea* females on paper discs treated with L-tryptophan (0.33%) oxidized using amino acid oxidizer.

iii. Ovipositional response of *C. carnea* to hydrolysed / oxidized L-tryptophan after 2 or 3 days of waiting period

Three day old formulations recorded highest oviposition by *C. carnea* compared to 2-day-old formulations. The acid hydrolyzed L- tryptophan (0.33%) and oxidized L- tryptophan (0.66%) using amino acid quick oxidizers recorded highest oviposition. Least oviposition was observed in the untreated control (Table 3). Earlier study made by Bakthavatsalam and Singh (1996) also made similar observations regarding *C. carnea* females laying highest number of eggs on 3-day-old acid hydrolyzed L-tryptophan.

iv. Response of *C. carnea* to Indole Acetaldehyde

In the multiple choice tests, highest number of adults visited the filter papers treated with 20 m M and 10 mM indole acetaldehyde. Similarly, more number of eggs was recorded on paper discs treated with 10 mM indole acetaldehyde, compared to acid hydrolyzed L-tryptophan. Previously it had been believed that acid hydrolysis of L-tryptophan breaks the amino acid doen into various compounds which are attractive to lacewing adults (Van Emden and Hagen, 1976). Later, it was

Table. 1. Electroantennogram response of females of *C. carnea* to hydrolyzed and oxidized L-tryptophan

Treatment (Concentration)	Mean absolute net response (- mV)
1. Acid hydrolysed L-tryptophan (0.33%)	0.6018 (-0.2814)
2. Oxidised L-tryptophan using H_20_2 (0.33%)	0.2178 (-0.7232)
3. Oxidized L-tryptophan using quick oxidizers (0.33%)	0.4612 (-0.4581)
4. Acid hydrolyzed L-tryptophan (0.66%)	0.6550 (-0.3057)
5. Oxidized L-tryptophan using H_20_2 (0.66%)	0.2952 (-0.6091)
6. Oxidized L-tryptophan using quick oxidizers (0.66%)	0.5082 (-0.3777)
7. Reference (honey 50%)	0.7872 (-0.1615)
CD (P = 0.05)	-0.2699

Figures in parentheses are $\log_{10}$ transformed values.

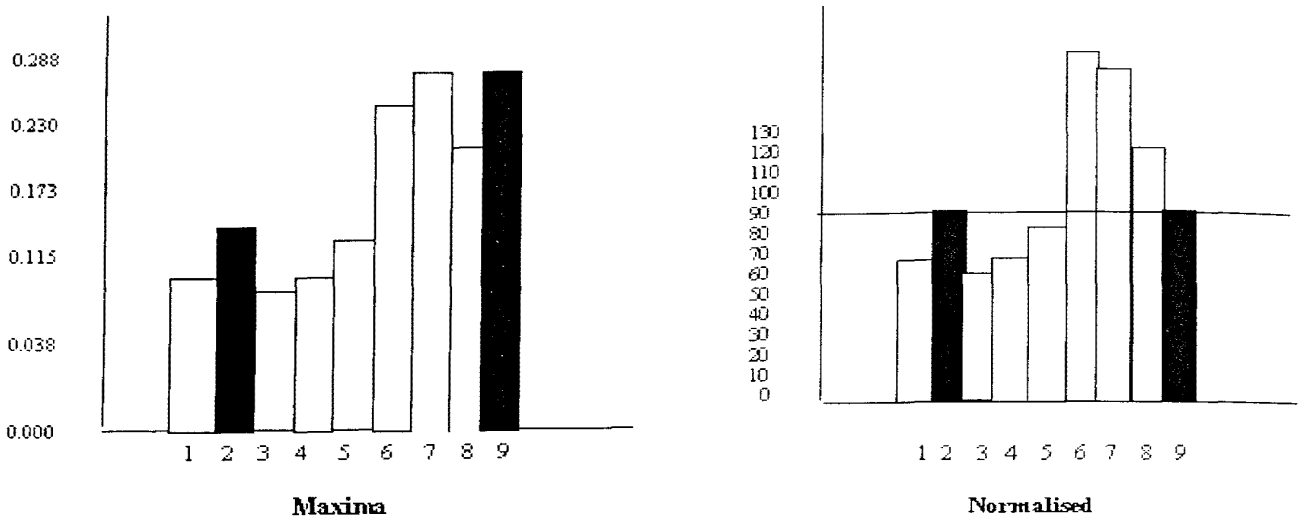

Fig.1. A typified EAG response of gravid female of *C. carnea* to kairomones [1. Air, 2. honey (reference), 3. Acid hydrolysed L-tryptophan (AHLT) (0.33%), 4. Hydrogen peroxide oxidized L- tryptophan (H_2O_2LT) (0.33%), 5. Amino acid oxidiser oxidized L-tryptophan (AAOLT) (0.33%), 6. AHLT (0.66%), 7. H_2O_2LT (0.66%), 8. AAOLT (0.66%) and 9. Honey - (reference)]

Table 2. Orientational and ovipositional response of *Chrysoperla carnea* to the acid hydrolysed and oxidized L-tryptophan

Treatment (Concentration)	No of adults responsive	No of eggs laid
1. Acid hydrolysed L-tryptophan (0.33%)	7.1	38.0
2. Oxidized L-tryptophan using H_2O_2 (0.33%)	2.8	26.2
3. Oxidized L-tryptophan using quick oxidizers (0.33%)	4.8	12.6
4. Acid hydrolyzed L-tryptophan (0.66%)	7.7	28.1
5. Oxidized L-tryptophan using H202 (0.66%)	2.8	21.1
6. Oxidized L-tryptophan using quick oxidizers (0.66%)	4.6	37.2
7. Control	1.8	2.0
CD (P=0.05)	0.9	4.2

observed that the solubility of L-tryptophan was significantly greater in 2M hydrochloric acid than in water. In acidic solution, L-tryptophan formed the hydrochloride salt ($C_{11}H_{12}N_2O_2HCl$) with no evidence of further reaction of decomposition occurring. The hydrochloride salt was seem to be readily soluble in water. Solutions containing L-tryptophan in hydrochloric acid have been shown to be attractive to adults of *C. carnea* (Harrison and McEwen, 1998).

Contrarily, in our studies indole acetaldehyde recorded ovipos on, which was also supported by Van Emden and Hagen (1976) who observed ovipositional attraction in *C. carnea* to indole acetaldehyde produced by heating 1H-indole 3- aldehyde with ninhydrin. Though in our studies some attraction could be observed for indole acetaldehyde, the usage of this compound will be uneconomical due to its prohibitive cost and technicality involved in the conversion of indole acetic acid to indole acetaldehyde. Nevertheless, further studies may be initiated to use indole acetaldehyde in an economical concentration for attracting of chrysopids.

Most of these studies were conducted at laboratory

Table 3. Ovipositional response of *C. carnea* to hydrolyzed / oxidized L-tryptophan after 2 and 3 days of waiting period

Treatment (concentration)	No of eggs laid	
	2 days after hydrolysis/ oxidization	3 days after hydrolysis/ oxidation
1. Acid hydrolyzed L-tryptophan (0.33%)	29.0	39.2
2. Oxidized L-tryptophan using hydrogen peroxide (0.33%)	24.3	29.0
3. Oxidized L-tryptophan using quick oxidizers (0.33%)	16.1	14.1
4. Acid hydrolyzed L-tryptophan (0.66%)	23.3	28.9
5. Oxidized L-tryptophan using peroxide (0.66%)	14.9	22.1
6. Oxidized L-tryptophan using quick oxidizers (0.66%)	17.2	39.1
7. Control	14.0	2.0
CD (P = 0.05)	8.0	5.8

Table 4. Orientation and ovipositional response of *C. carnea* to Indole acetaldehyde

Treatment	No. of adults responsive	No. of eggs laid
1. Indole acetaldehyde 10 m M	3.20	42.00
2. Indole acetaldehyde	3.60	16.20
3. Acid hydrolysed L-tryptophan	1.30	0.80
CD (P = 0.05)	0.60	9.60

and net house conditions and further studies are necessary to test the compounds such as indole acetaldehyde in the field for substituting acid hydrolyzed L-tryptophan, which will ultimately reduce using the strong acids as well as avoid the waiting period.

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