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L-tryptophan as an ovipositional attractant for *Chrysoperla carnea* (Stephens) (Neuroptera : Chrysopidae)

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ABSTRACT : Laboratory and field cage experiments were conducted to select the best kairomone as an ovipositional attractant for *Chrysoperla carnea* (Stephens). Acid hydrolysed L-tryptophan (15 days after storage) was found to be highly attractive to the males and females of *C. carnea* in no choice method, using wind tunnel method and in multiple choice method using the kairomone treated filter paper. Pure commercial honey, 25 per cent and 50 per cent honey solutions were attractive in no choice method but not in the multiple choice method. Highest number of eggs were laid on the filter paper sprayed with 15 days old L-tryptophan in laboratory study. However, in the field cage studies, only 3 day old L-tryptophan recorded highest oviposition on cotton plants treated with the kairomones, probably the breakdown product of L-tryptophan is not effective in long range if stored beyond 7 and 15 days. Possibility of using L-tryptophan in the field is discussed.

KEY WORDS : *Chrysoperla carnea*, honey, L-tryptophan, ovipositional attractant

Chrysopids have been recorded as key natural enemy of various crop pests (Canard *et al.*, 1984; Singh and Narasimham,1992). Release of chrysopids has been recommended against the pests of cotton (Dhandapani *et al.*, 1992; Singh *et al.*, 1994; Mishra and Mandal,1995). Several methods have been suggested for increasing their field efficiency by manipulating their behaviour using different attractants (Ridgway and Murphy, 1984). Honey dew and L-tryptophan invoke behavioural response in *C.carnea* (Mc Ewen *et al.*, 1993) and have been used in the field as attractants (Hagen *et al.*, 1970; Hagen *et al.*, 1976). Laboratory

and cage experiments were carried out to select the best kairomone for use in the field as an efficient attractant and the results are presented in this paper.

MATERIALS AND METHODS

Insect culture

The cultures of *C. carnea* used for the experiments were drawn from the laboratory cultures. The healthy cocoons weighing more than 6 mg were selected and emerging adults were used between 7 and 15 days after emergence (peak oviposition period) unless and otherwise specified.

Kairomone

Different concentrations of honey solution (by volume) were prepared by mixing the commercial grade of honey with distilled water. Solution of L-tryptophan was prepared by acid hydrolysis (McEwen *et al.*, 1993). Storing of acid hydrolysed solution of L-tryptophan has high significance with respect to their use in the field, hence the experiments were conducted by keeping L-tryptophan solutions at 7°C in a refrigerator for 2 , 3, 4, 5, 7 and 15 days. Distilled water was used as a control.

Olfactometer studies

The experiments were conducted during 1995 and 1996. Laboratory experiments were conducted at 24 ± 2°C and 60 -70 per cent relative humidity. No choice and multiple choice methods were followed to find the orientation and oviposition behaviour of the adults of *C. carnea* in response to the kairomones.

No choice method

Two identical acrylic boxes (non absorbent, odourless and transparent) of 15 x 15 x 15 cm were connected through a cylindrical acrylic sheet of 30 cm length and 3 cm diameter. Kairomonal extracts prepared were taken in a cotton swab and hung on one of the chambers (termed bait chamber). The test adults were starved for 24 h as suggested by McEwen *et al.* (1993) and 15 of them were released on the other acrylic sheet box (termed test chamber). A wind flow of 1 m/sec was maintained from bait chamber to test chamber with the help of a mini fan fitted with an electronic regulator kept along the side of the bait chamber. The number of adults entering the bait chamber within 60 minutes was noted. Each treatment was replicated 10 times. Distilled water was used as control.

Multiple choice method

In the second experiment the kairomonal substances were sprayed on brown paper discs (6 cm dia) and dried in shade for half an hour. All the paper discs were glued to the lid of an acrylic box (30 x 30 x 30 cm) and seventy adults (50% females) which were starved for 24 h were released. The number of adults visiting each filter paper was recorded for half an hour at an interval of 5 minutes. The

maximum number of adults recorded on each paper was considered for statistical analysis. The experiment was repeated 10 times.

Oviposition behaviour

The adults used for the multiple choice method were left overnight on the acrylic sheet box and provided with the adult feed. The number of eggs deposited on each of the filter paper was recorded on the next day morning. The experiment was repeated 10 times .

Field cage experiment

Gravid *C. carnea* females were used for the experiment. Twenty cotton plants grown on pots at the boll stage were selected and kept in a field cage (3 x 3 x 3 m). The pots were spaced 60 cm between rows and 40 cm between the plants.

The honey and L-tryptophan solutions were sprayed at 1630 hours on the cotton plant and allowed to dry. After 30 minutes of spraying, twenty females and 10 males of *C. carnea* were released into the cage. The number of eggs laid on the treated plants was recorded on the next day morning. Control was maintained with water spray. Each treatment was replicated ten times.The data were subjected to analysis of variance.

RESULTS AND DISCUSSION

The adults showed characteristic antennal palpation and upwind movement in the tunnel whenever they were responsive to the kairomones. The speed of the adults was more and their movement was in straight direction compared to slow and staggered movement to the non kairomones (control). The adult took 30 seconds to 25 minutes to reach the bait chamber. Some of the adults directly landed on the cotton swab in the bait chamber.

The results indicated that highest number of adults were attracted to 15 days old L-tryptophan. L-tryptophan solutions stored for 2 to 7 days, pure honey and honey solutions were comparatively less attractive but superior than the control at P=0.05 (Table 1). Dean and Satasook (1983) observed that both L and D isomers of tryptophan were attractive to *C. carnea* but their finding is in variance with the present investigation where DL-tryptophan, (mixture of D and L isomers of tryptophan) was found unattractive to *C. carnea*. In general females were more responsive than the males. McEwen *et al.* (1993) observed that 3 day old L-tryptophan is more attractive to *C. carnea*, contrary to the present observations, probably attributed to the different methodologies used for testing.

Similar results were obtained in the multiple choice experiment where the 15

Table 1. Orientation behaviour of adults of *C. carnea* in no choice method

Treatment	No. of adults entering the bait chamber
Honey solution (25%)	3.2 (1.89)
Honey solution (50 %)	4.9 (2.30)
Pure honey	5.2 (2.28)
DL-tryptophan	1.8 (1.41)
L-tryptophan (2 day old)	3.7 (2.02)
L-tryptophan (3 day old)	3.8 (1.98)
L-tryptophan (4 day old)	4.8 (2.27)
L-tryptophan (5 day old)	4.8 (2.24)
L-tryptophan (6 day old)	4.8 (2.17)
L-tryptophan (7 day old)	5.2 (2.35)
L-tryptophan (15 day old)	7.3 (2.78)
Water Control	1.2 (1.21)
CD (P=0.05)	(0.40)

Figures in parenthesis are $\sqrt{X + 0.5}$ transformed values

days old L-tryptophan recorded highest number of adults visiting the filter papers, while honey solution recorded lowest number of adults visiting the filter papers (Table 2), probably due to the stickiness of the honey solutions.

Table 2. Orientation behaviour of adults of *C. carnea* in mulitple choice method

Treatment	Mated adults	Unmated males	Unmated females
L-tryptophan 15 days old	12.8 (3.59)	12.6 (3.45)	18.9 (4.15)
L-tryptophan 7 days old	5.8 (2.47)	6.3 (2.52)	12.1 (3.45)
Honey 50% solution	3.9 (2.03)	7.4 (2.75)	11.1 (3.35)
Pure honey	3.0 (1.81)	2.9 (1.72)	8.5 (2.95)
Water	4.4 (2.15)	3.3 (1.78)	7.4 (2.72)
No spray	2.4 (1.65)	2.4 (1.68)	6.5 (2.65)
CD (P=0.05)	(0.46)	(0.81)	(0.55)

Figures in parenthesis are $\sqrt{X + 0.5}$ transformed values

Table 3. Egg laying pattern of *C. carnea* on the kairomone treated papers in multiple choice method

Treatment	No. of eggs laid
L-tryptophan (15 days old)	47.7 (6.59)
L-tryptophan (7 days old)	25.7 (4.70)
Honey (50%)	1.3 (1.18)
Pure honey	2.9 (1.67)
Water	20.4 (4.29)
No spray	21.8 (4.36)
CD (P=0.05)	(1.39)

Figures in parenthesis are $\sqrt{X + 0.5}$ transformed values

The oviposition behaviour was also found to be influenced by the kairomones. Highest number of eggs were laid on the filter papers treated with 15 day old L-tryptophan followed by 7 day old L-tryptophan. Papers treated with the honey solution did not record any egg laying, even lesser than the water spray and untreated control (Table 3), probably the adults were deterred by the stickiness of the honey solutions.

The results obtained in the field cage experiments deviate from the laboratory experiments. All the plants treated with L-tryptophan recorded higher number of eggs compared to control. Three day old L-tryptophan treated plants recorded highest number of eggs (Table 4) contrary to the laboratory observations where 15 days old L-tryptophan recorded higher eggs and adult orientation. This may be due to pronounced breakdown of the

Table 4. Oviposition pattern on the kairomone treated cotton plants under caged conditions

Kairomone substance	No. of eggs / 20 plants
Honey solution (25%)	2.80 (1.65)
Honey solution (50 %)	2.80 (1.65)
Honey (Pure)	1.60 (1.25)
L-tryptophan (3 days old)	28.90 (4.89)
L-tryptophan (7 days old)	13.80 (3.63)
L-tryptophan (15 days old)	6.90 (2.43)
Water spray (Control)	0.30 (0.85)
CD (P=0.05)	(1.12)

Figures in parenthesis are $\sqrt{X + 0.5}$ transformed values

L-tryptophan during storage resulting in the volatiles being not perceived at the longer range. This also suggests that the storage of hydrolysed L-tryptophan is not possible. Honey solutions, though found to be attractive in the olfactometer tests, recorded the least number of eggs in the field cage study also.

The results indicated that L-tryptophan which is one of the components of the artificial honey dew (Hagen *et al.*, 1976) was highly attractive to *C. carnea*, corroborating earlier studies (Van Emden and Hagen, 1976). The tryptophan a non-volatile compound becomes volatile when hydrolysed, due to the breakdown product, indole acetaldehyde(Van Emden and Hagen, 1976).

Attraction of natural enemies to alfalfa and potato fields by spray of L-tryptophan has been documented in earlier studies (Hagen *et al.*, 1976; Ben Saad and Bishop, 1976 a,b). Dean and Satasook (1983) opined that *C.carnea* were unlikely to be attracted to the tryptophan under field conditions. However, Liber and Niccoli (1988) and McEwen *et al.* (1994) established that spraying of L-tryptophan increased the effectiveness of chrysopids in olive plants as confirmed in the present investigations. Further studies are required to find out slow hydrolysing agents or oxidising agents which can augment slow release of the breakdown product over a period of time without much deterioration in the quality of the volatile. Field studies with proper dosage and economic analysis of the efficacy of the L-tryptophan are necessary for proper recommendation of the product.

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