J. Biol. Control, 21(1): 1-8, 2007

Kairomone formulations as reinforcing agents for increasing abundance of *Chrysoperla carnea* (Stephens) in cotton ecosystem

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ABSTRACT: **Adults and larvae of *Chrysoperla carnea* (Stephens) utilize different kairomones for oviposition, prey selection and acceptance. The larval kairomones containing scale extracts fortified with tricosane along with the eggs of *Corcyra cephalonica* (Stainton) as the supplementary diet, and the adult kairomone, acid hydrolyzed L-tryptophan were studied to increase the predatory activity of the *C. carnea* in cotton ecosystem at three locations during 2002 - 2004. Controls were maintained with the two releases of *C. carnea* and without any treatment. In all the locations, the number of eggs, larvae, pupae or adult *C. carnea* was more than the treated control and control. The number of aphids, jassids, and the incidence of bollworm were significantly less in kairomone treated plots, compared to other plots. However, no difference was observed in the activity of coccinellids predators. The scope for utilizing the kairomones as reinforcing agents for *C. carnea* is discussed.**

KEY WORDS: Abundance, *Chrysoperla carnea*, cotton, L-tryptophan, scale extract, tricosane

INTRODUCTION

Chemical communication in insects is a complex phenomenon, several of the entomophages utilize chemical cues from the host plants and host insects for host habitat location, host location and successful predation or parasitisation or oviposition. Chrysopids are important predators in cotton ecosystem, feeding on aphids, whiteflies, eggs and neonate larvae of bollworms (Singh *et al.*, 1994; Jalali *et al.*, 2003). Their role as efficient biocontrol agents has been well established on cotton (Bharpoda *et al.*, 2000; Balakrishnan *et al.*, 2004).

Adult chrysopids use the honeydew secretions from the homopteran as a chemical cue for their orientation and oviposition. L-tryptophan is an important component in honeydew for the attraction of adult chrysopids (Emden and Hagen, 1976). The role of acid hydrolyzed L-tryptophan as an attractant to chrysopids has been proved in the

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earlier studies (Hagen *et al.*, 1976; McEwen, 1993; Bakthavatsalam and Singh, 1996; Bakthavatsalam *et al.*, 2000) and the hydrochloride salt ($C_{11}H_{12}N_2O_2HCl$) of tryptophan formed during hydrolysis was found to be the basis for the attraction of *Chrysoperla carnea* (Stephens) (Harrison and McEwen, 1998). The spray of L-tryptophan in field conditions improved the abundance of chrysopids in potato (Ben-Saad and Bishop, 1976) and olive (McEwen *et al.*, 1994). The field trials on cotton, conducted during 2000 to 2002 revealed that the number of *C. carnea* eggs in the L-tryptophan treated plants was more compared to untreated control (Anonymous, 2001 and 2002). Similarly, the larval chrysopids utilize the chemicals from the scales of the prey insects for host location and feeding (Nordlund *et al.*, 1977; Lewis *et al.*, 1977; Bakthavatsalam and Singh, 1999; Hegde and Kulkarni, 2000; Singh and Paul, 2002). Attempts were earlier made to increase the predation potential of chrysopids through the use kairomones (Lewis *et al.*, 1977; Anonymous, 2000). However, till now a comprehensive study was not made to utilize combined adult and larval kairomone to increase the abundance of *C. carnea* in the field. In the present studies, kairomone formulations for adults and larvae were combined and used along with the supplementary diet on cotton to increase the abundance of *C. carnea* and the results are communicated in this article.

MATERIALS AND METHODS

Insect cultures

The test insect (*C. carnea*) Culture was maintained following the mass-culture protocol developed in PDBC (Singh *et al.*, 1994). Adults of *C. carnea* were reared on semi-synthetic diet and grubs were maintained using *Corcyra cephalonica* (Stainton) eggs.

Kairomonal formulations

The adult *C. carnea* attracting kairomone compound L-tryptophan was prepared as per the procedure described earlier by Bakthavatsalam and Singh, (1996). The Hydrochloric acid and L-tryptophan (specified dose) were sent separately to the test centers so as to enable them to mix the solutions in the specified quantity of water, keep for 3 days and to spray on a patch of 40 to 50 plants.

The host derived larval kairomone solution was prepared by extracting the *C. cephalonica* scales in hexane (AR grade) @ 1 g in 100 ml using magnetic stirrer set at 60° C for 30 minutes and another 30 minutes at room temperature 27±1°C. The extract was filtered and then fortified with 100 mg of tricosane (Sigma Aldrich) and the solution was made up to 100 ml to get 1000ppm stock solution. The kairomone extract was sprayed on the Whatmann No.1 filter paper bits and used in the experiments. *C. cephalonica* egg cards were used to provide supplementary diet. Kairomone solutions were prepared freshly to ensure their use within 24 hours.

Experimental Field Layout

The field experiments were conducted during 2002- 2004 at three locations. Two trials were conducted during 2002 & 2003 at White field (near Bangalore) on the cotton grown by Sathya Sai cotton mills. The other two trials were conducted during 2002 & 2004 - one at University of Agricultural Sciences (UAS), Dharwad and another at UAS Regional Agricultural College, Raichur in collaboration with the scientists working at respective places.

The experimental fields in all the locations were divided into three patches each with 1000 cotton plants and separated by at least 25 meters so as to accommodate the following three treatments -

i. Kairomone Treated Plants

In the kairomone treated block the early second instar larvae of *C. carnea* (3-4 days old) were released at the rate of two larvae per plant. The next day after the release, the kairomone treated Whatmann No.1 filter papers and UV irradiated *C. cephalonica* egg card bits were placed randomly in 16 spots (@ 3 cards, each card comprising approximately 50,000 eggs was cut into 16 bits), and stapled on the cotton plants as supplementary food. After 20-25 days of 1st release, L-tryptophan solution was sprayed on the cotton plants, coinciding with the likely emergence of adult *C. carnea*. On the 29-30th day of first release again the kairomones treated filter papers were stapled along with the *C. cephalonica* egg card bits (without release of chrysopid larvae). On the 50th day of first release, L- tryptophan was sprayed again.

ii. Treated control plot

In the treated control, the larvae of *C. carnea* were released @ 2 larvae per plant for two times at 25 days interval after each release. However no kairomonal treatments were given.

iii. Untreated control plot

In the untreated control, neither chrysopid larvae were released nor kairomone treatments given.

The performance of the treatment was assessed by making observations on number of egg, larval, pupal and adult *C. carnea* in the 50 tagged cotton plants at pre-treatment, post-treatment and at the time of harvest. The incidence of sucking pests (aphids and leafhoppers), bollworm, *Helicoverpa armigera* (Hübner) and boll damage were also recorded on the tagged plants. The abundance of other predators like coccinellids was also recorded.

For statistical analysis, the number of insects from the 50 plants was grouped into seven replications each containing seven plants and student's t test was conducted between the pre-treatment with the respective post-treatments.

RESULTS AND DISCUSSION

During the year 2002, in the pre-treatment observations the population of chrysopids was more, mainly because of the egg masses of another indigenous chrysopid, *Apertochrysa* sp. However, in the final observation the number of *C. carnea*, especially the number of eggs was more. During 2003, the number of eggs was more in the kairomone treated plots than the untreated and treated controls (Table 1).

At Dharwad, the number of chrysopids increased gradually in each observation in the treated plots. The number of eggs and larvae predominating the treated plots indicated the fresh incidence of the chrysopids (Table 2). The number of chrysopids was low in the treated control and least in the untreated control (Table 2). However there was no indication of increase in the population of other predators like coccinellids (Table 3). The population of aphids and jassids was significantly controlled in the kairomone treated plots compared to other treatments, an indication of increased activity of chrysopids (Table 4). Similarly, the number of *H. armigera* eggs and larvae were low in kairomone treated plots contributing for a higher yield in the kairomone treated plots (Table 5).

Table 1. Abundance of *C. carnea* in kairomone treated cotton plants during 2002-03 at Whitefield

Treatment	*C. carnea* eggs (No. / 7 plants)			
	2002		2003	
	Pre-treatment	Final	Pre-treatment	Final
Kairomone plot	3.86	1.00	0.43	5.57**
Treated Control plot	0.71	0.57	0.14	2.00
Untreated Control plot	2.43	0	1.00	1.00

** Significantly different from pre-treatment at 1% level under students' t test

Table 2. Impact of kairomones on the abundance of chrysopid during 2002 at Dharwad

Treatment	*C. carnea* (No. / 7 plants)											
	Before treatment			After first spray			After second spray			Pre harvest count		
	Egg	Grub	Adult	Egg	Grub	Adult	Egg	Grub	Adult	Egg	Grub	Adult
Kairomone plot	1.28	1.14	0.71	2.00	2.71	1.71	4.14*	4.57*	3.71*	5.29*	5.85*	3.43*
Treated control plot	1.57	1.00	0.57	2.14	3.29	2.14	3.42*	3.86*	3.29*	3.29*	4.71*	3.00*
Untreated Control plot	1.42	1.28	0.57	0.85	1.28	0.86	0.57	0.71	0.71	1.00	1.58	1.14

* Significantly different from pretreatment under Students' t test

Table 3. Impact of kairomones on abundance of coccinellids during 2002 at Dharwad

Treatment	Mean number of Coccinellids /7 plants							
	Before treatment		After first spray		After second spray		After third spray	
	Grub	Adult	Grub	Adult	Grub	Adult	Grub	Adult
Kairomone plot	3.43	1.85	3.71	2.14	4.28	1.71	6.00	10.14**
Treated control plot	3.85	1.43	3.57	1.42	3.86	1.29	6.43	8.71
Untreated Control	3.71	1.57	3.57	1.71	3.85	1.43	6.14	7.71

** Significantly different at 1% level under Students' t test

Table 4. Incidence of sucking pests in kairomone treatment during 2002 at Dharwad

Treatment	Aphids (No. / 7 plants)				Leafhoppers (No. / 7 plants)			
	Pre-treatment	After first spray	After second spray	Pre harvest count	Pre-treatment	After first spray	After second spray	Pre harvest count
Kairomone plot	67.57	58.14	27.29*	11.0*	11.14	9.00	5.57	3.85
Treated control plot	68.71	45.29	42.43	28.14*	9.85	7.43	6.71	5.85
Untreated Control plot	67.00	72.86	81.86	130.29	10.43	10.85	11.57	12.43

* Significantly different from pre-treatment under Students' t test

In the experiments conducted at Raichur, the same trend was observed with more abundance of the *C. carnea* population in the kairomone treated plots, followed by treated control (Table 6). The damage to the bolls was comparatively lower in kairomonal treated plot than the treated control and untreated control (Table 7).

Use of L-Tryptophan was reported to increase the abundance of chrysopids in olive and potato (Ben Saad and Hagen, 1976; McEwen *et al.*,1994). On cotton the spraying of acid hydrolysed tryptophan was found to attract oviposition in the sprayed plants (Anonymous, 2001; 2002). However, Dean and Satasook (1983) expressed that

Table 5. Incidence of bollworm *H. armigera* in the kairomone treatment during 2002 at Dharwad

Treatment	Eggs (No. / 7 plants)				Larvae (No. / 7 plants)				Cotton Yield q/ ha
	Pre-treatment	After first spray	After second spray	Pre harvest count	Pre-treatment	After first spray	After second spray	Pre harvest count	
Kairomone plot	8.14	7.00	4.43*	3.71**	6.00	5.57	3.86*	2.43*	11.25
Treated control plot	9.00	6.00	4.70	4.43*	6.29	4.86	4.43	3.00	11.15
Untreated Control	8.00	8.70	6.71	5.71	6.71	5.57	3.85	3.29	10.25

** Significant at 1% level

* Significant at 5% level

Table 6. Impact of *C. carnea* kairomones on abundance during 2004 at Raichur

Treatment	*C. carnea* adult (No. / 7 plants)		
	Pretreatment	1 day after 1st spray	1 day after 2 nd spray
Kairomone Plot	1.71	8.40**	14.28**
Treated control plot	1.71	5.14**	6.57**
Untreated control	1.71	2.71	3.28

** Significantly different at 1% level under Students' t test

Table 7. Incidence of bollworms and yield in the experimental plots during 2004 at Raichur

Treatment	Fruiting bodies damaged (%)	Good open bolls / plant	Bad open bolls / plant	Yield Q/ha
Kairomone plot	21.34	22.12	8.20	22.44
Treated control	26.84	18.12	12.22	20.12
Untreated control	48.44	11.44	26.48	13.15
CD (P=0.05)	2.44	2.55	3.65	4.34

though the Tryptophan attracted females of *C. carnea*, the numbers will not be sufficient enough to control infesting aphids. In the present studies, control of *H. armigera* and other bollworms may be attributed to the combined use of larval and adult kairomones along with food supplements.

The performance of several parasitoids and predators was reported to have enhanced with the use of kairomone at field conditions. Parasitism by *Meteorus rubens* (Nees) on *Agrotis ipsilon* Hufnagel increased when sprayed with molasses (3%) and kairomone (Zaki *et al.*, 1997). Kairomones from *A. ipslilon* and *Spodoptera littoralis* Boisduval increased parasitization by *Cotesia rufricrus* Haliday and *Microplitis demolitor* Wilkinson (Zaki, 1996). Gross *et al.* (1984) reported increase in parasitization by *Trichogramma pretiosum* Riley when the kairomones were sprayed along with tricosane @ 395 mg/ ha on soybean that were artificially infested with the eggs of *Helicoverpa zea* Boddie. Role of moth scales and tricosane as a kairomone has been established earlier (Nordlund *et al.*, 1977; Lewis *et al.*, 1977; Bakthavatsalam and Singh, 1999). In the present studies, scale extracts fortified with Tricosane was found to supplement the deficiencies.

Application of moth scales of *Choristoneura fumiferana* (Clemens) on the spruce fir was considered to interfere in the host searching behaviour of *Trichogramma* spp. (Jennings and Jones, 1986). However, in the present studies we have observed that the abundance of chrysopids was considerably increased when kairomone formulations were used along with the supplementary diet. Earlier studies have proved that the supplementary diets were very essential without which the natural enemies spent their time and energy unnecessarily leading to the death of the natural enemies.

At present, kairomones for the larval chrysopids, do not have shelf life and has to be used immediately, therefore studies are necessary to improve the shelf life of the larval kairomones. The present system of using acids like Hydrochloric acid (HCl) for hydrolysis of the L-tryptophan has some disadvantage like the waiting period of 3 days and use of strong acids, which may pose handling hazards. Unfortunately, no substitute has been identified so far in spite of efforts made to substitute HCl with weak acids like citric acid, acetic acid or hydrogen peroxide and quick amino acid oxidizers (Anonymous, 2000; Anonymous, 2002, 2003).

By the use of the kairomone the number of releases and doses of chrysopids may be reduced since the kairomones facilitate concentration of the adults at the treated sites. Although there is further scope to increase the concentration and quantity of L-tryptophan, due to high cost, these could not be ventured. This method has also the potential to be used for the other entomophages like Trichogrammatids, which utilize same kairomonal clues (Lewis *et al.*, 1975). There is also great scope for increasing the efficiency of the entomophages with the modification of the components. The success on the cotton crop may also be utilized in other crops with the fine-tuning of the technology.

ACKNOWLEDGEMENTS

The authors are grateful to the Director, Project Directorate of Biological Control, Bangalore; Director of Research, University of Agricultural Sciences, Dharwad; Professor and Head, Department of Entomology, University of Agricultural Sciences, Dharwad and the Head, UAS, Raichur for the facilities provided. Technical assistance provided by Mr. C. Bharati Dasan and Ms. K. V. Usha is gratefully acknowledged.

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(Received: 17.08.2006; Revised: 12.04.2007; Accepted: 18.04.2007)