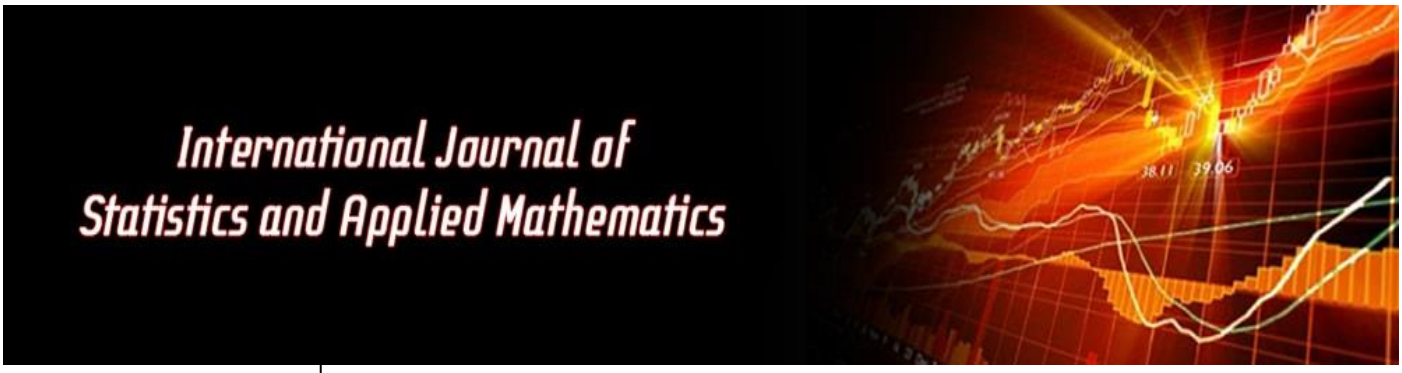

ISSN: 2456-1452
Maths 2024; SP-9(6): 180-184

www.mathsjournal.com
Received: 12-10-2024
Accepted: 13-11-2024

Allwin Loveson
Agricultural College and Research Institute, Killikulam, TNAU, Tamil Nadu, India

Srividhya S
Horticultural College and Research Institute, Paiyur, TNAU, Tamil Nadu, India

Thilagam P
Horticultural College and Research Institute, Paiyur, TNAU, Tamil Nadu, India

Jamuna E
ICAR-Krishi Vigyan Kendra, Tindivanam, TNAU, Tamil Nadu, India

Deivamani M
ICAR-Krishi Vigyan Kendra, Paparappatty, TNAU, Tamil Nadu, India

Corresponding Author:
Srividhya S
Horticultural College and Research Institute, Paiyur, TNAU, Tamil Nadu, India

Behavioural response of predatory coccinellid, *Cryptolaemus montrouzieri,* to different species of mealy bugs through olfactometer assay

Allwin Loveson, Srividhya S, Thilagam P, Jamuna E and Deivamani M

Abstract
The larvae and adults of coccinellids were known to detect their preys either by olfactory or visual stimuli. Through Y-tube olfactometer studies it was observed that *C. montrouzieri* showed a maximum orientation (74.0%) towards *P. citri* and the lowest response (57.8%) was towards *Phenococcus* sp. The olfactory response of *C. montrouzieri* was more (81.0%) towards prey with host leaves (guava) than prey alone (74.0%). Similarly, through four arm olfactometer studies, it was observed that 45.67 per cent of *C. montrouzieri* adults oriented towards *P. citri* while it was 21.0 and 14.33 per cent for *M. hirsutus* and *Phenococcus* sp respectively and also about 44.33 per cent of *C. montrouzieri* adults oriented towards *P. citri* + guava leaves while it was 22.33 and 16.67 percent for *M. hirsutus*+ mulberry and *Phenococcus*sp + cotton leaves, respectively. Through Pettersson olfactometer, it was observed that the time spent by *C. montrouzieri* adults with *P. citri* was more (4.10 min) when compared to *M. hirsutus* in the opposite arm (3.08).

Keywords: *Biocontrol, C. montrouzieri,* Olfactometer assay, Kairomone, Mealybugs

Introduction
The members of coccidae are essentially of tropical and sub-tropical in nature. Coccids build up rapidly during hot weather period and are considered as “hard to kill” pests of fruit trees because of practical difficulties in managing these pests (Lower, 1968) [7]. The insecticides post-application stage hazards concerns the public, as they come from pesticide residues persisting on fruits (Fleischer and Waibel, 2003) [3]. Hence it necessitated the use of predator’s especially indigenous, locally adapted coccinellids for the management of *P. citri*. In general, most of the mealy bugs are controlled by parasitoids and predators in nature (Mani, 1986) [10]. Coccinellids are the very essential and important as many of them are predators of crop pests which includes aphids, mealybugs, scale insects, mites etc., and have been employed for their field utilization (Mani and Krishnamoorthy, 1989a) [8]. The value of coccinellids in biological control is more as both grubs and adults are feeding on the pest at a greater density.

Olfactometry is the measurement of the response of assessors to olfactory stimuli. An olfactometer is a device which dilutes a sample of odourous air with clean air. The larvae as well as adults of *C. montrouzieri* could sense the prey liberated chemicals and use them in targeting the source. The first instar larvae and adults were attracted to the odour emitted by *P. citri* more numbers than from other preys *viz*., *Pulvinariaregalis* Canard, *Mamestra brassicae* (Linnaeus), *Acyrthosiphonpisum* (Harris), *A. fabae* and *Myzuspersicae* (Sulzer). The presence of prey on the host plants enhanced the olfaction as indicated by the more number of predators attracted to them. Furthermore, the adults were lured to the odour of their own individuals in reasonable numbers but not the larvae (Kotikal and Sengonca, 1999) [6]. Earlier studies conducted by Sengonca *et al*., (1995) [11] on the extent of olfactory responses of *C. montrouzieri* and *Chrysoperlacarnea* to their respective preys showed both the predators, irrespective of their developmental stage, oriented more positively to their preys. Though *C. montrouzieri* is an important oligophagous predator, (Singh, 1994) [12] much is not known about the olfaction by different stages except the study made by Heidari and Copland (1992) [5].

The present study aims to know about the feeding preference and kairomonal effect of different species of mealy bugs through Y-Tube olfacto meter and four arm olfactometer assays.

Materials and Methods

Mass culturing of mealy bugs

Pink mealy bug, *Maconellicoccus hirsutus* (Green)

The pink mealy bug *M. hirsutus* was mass cultured on red pumpkin (*Cucurbita moschata* Duchesne) as suggested by Chacko *et al*. (1978) [2]. Fully matured unripe Ned pumpkin fruit (weighing about 2 to 3 kg) with well-defined ridges and grooves were selected. Fruit with stalk was selected for easy handling during culture work. The pumpkin was washed with water to remove unwanted dusts and was treated with 0.1% formalin to avoid mould attack before introduction of ovisacs or crawlers of *M. hirsutus* (Srinivasan, 1987) [13]. Then the pumpkin was treated with Dithane M-45 (1g/litre) and wounds on the fruit if any, were plugged with wax. The stalk of the fruit was smeared with molten wax to avoid contact with the mealybug while handling or transporting. To initiate culture, ovisacs of *M. hirsutus* were taken from mulberry and were placed over pumpkin and was kept in a wooden cage of 1 x 1 x 1' size and maintained under dark condition for uniform settlement of crawlers. After five to seven days, crawlers emerged from ovisacs settled on all sides of pumpkin and later on developed into fully matured mealybugs in 30-40 days. One fully infested pumpkin was sufficient to infest 3-4 fresh pumpkins and the crawlers were transferred with the help of tender Glyricidia leaves (*Glyricidia masculata* Steud.) whenever the infested pumpkin produced crawlers. Thus the cycle was repeated. The culture thus obtained was utilized for the various experimental studies.

Citrus mealy bug, *Planococcuscitri* Risso

Citrus mealybug was mass cultured separately on red pumpkin as described for *M. hirsutus.*

Mass culturing of coccinellids

Mass culturing of *Cryptolaemus montrouzieri* Mulsant

As *C. montrouzieri*is the dominant coccidophagous species, it was multiplied on pumpkin infested with *M. hirsutus* and *P. citri*, separately. One fully infested pumpkin for each species of mealybug was taken separately in a wooden cage of 1 x 1 x 1' size and 50 adults of *C. montrouzieri* (15 day old) comprising both sexes (1:1) were introduced on the infested pumpkin. As supplementary food for the adults vials containing 50 per cent honey solution was kept inside the cage. The cages were kept under dark for mating and oviposition of beetles (Mani and Krishnamoorthy, 1997) [9]. During this period, beetles deposited their eggs either singly or in groups in the ovisacs of female mealybugs. The grubs were visible in such cages within a week after the introduction of the beetles. Grubs of *C. montrouzieri* pupated on the pumpkin or anywhere inside the breeding cage after a period of 20 days. To facilitate easy removal of pupa, dried guava leaves were kept at the base of the pumpkin in the cages. Emerged adults were collected and introduced into pumpkin with 15 days old mealybugs for further multiplication. Thus the cycle was repeated. Various life stages of *C. montrouzieri* were taken from this laboratory cultures as and when they were required for conducting laboratory and field experiments.

The cultures of two mealybug species, *M. hirsutus* and *P. citri* and the predator, *C. montrouzieri* were maintained in the laboratory with 25.5±2.1° C and 69.9±5.5% RH at the department of Agricultural Entomology, TamilNadu Agricultural University, and Coimbatore.

Olfactometer studies

Y-Tube olfactometer bioassay

All the experiments were conducted in a glass Y-tube olfactometer. The Y-tube olfactometer bioassay method (Bertschy *et al*., 1997) was used to assess the insect's performance for one out of two odour sources. The length of the Y stem and arm were

20 cm and 10 cm, respectively and the diameter was 2 cm. Air was drawn into the olfactometer through tephlon tube with the help of a vacuum pump with a speed of

40 kpa. Purified air though charcoal filter enters each arm of the olfactometer and flows over the respective odour source in an inserted glass chamber. The air leaves the olfactometer through the central tube. There, one insect was introduced at a time and has to move upwind as in nature. At the fork, it has to take a choice between the odour sources. Each arm led from a sample container that incorporated the source of test material. The total number of insects released per experiment was 30 and the total time allowed per insect was 10 minutes. For every batch of five insects the olfactometer was washed with a solvent in the following order; tap water, detergent, tap water, analytical grade methanol and distilled water and then kept in hot air oven for ten minutes. In the first set of experiment, one arm was placed with source materials of *P. citri, M. hirsutus* and *Phenococcus*sp individually for a single experiment and the other arm was left devoid of source material as control. The response of *C. montrouzieri* was observed for each experiment and was replicated thrice. In another set of experiments, one arm was placed with mealybugs along with its host *viz.*, infested guava leaves of *P. citri*, mulberry leaves of *M. hirsutus* and cotton leaves of *Phenococcus* sp and the another arm was kept devoid of source as control. The experiment was replicated thrice and the response of *C. montrouzieri* was observed and recorded.

Four-Arms Bioassay

The four arm olfactometer was made of odourless acrylic introduced by Pettersson during 1970 and modified by Vet *et al.* (1983), and has been employed by a great number of scientists primarily for testing the responses of insects. It consists of an enclosed Perspex arena (15cm dia.) with a central chamber and four side arms with a length of 7cm and 3cm diameter. Air was drawn from the centre of the olfactometer using a vacuum pump, establishing discrete air currents in the side arms. The three arms were placed with source materials of *P. citri*, *M. hirsutus* and *Phenococcus*sp leaving one arm as control. One set of experiment was conducted with mealybugs alone and another along with host materials as followed in Y-tube bioassay. A total of 30 insects were released in the central arena and observations were recorded for every 15 minutes until an hour ends. All the experiments were replicated thrice. At the end of each experiment, all olfactometers were cleaned by gentle washing with soapy water, followed by rinsing and wiping with analytical grade methanol. The olfactometer should be set in a suitable position avoiding strong direst light from a particular direction. However, to counteract bias caused by lighting, each olfactometer was turned by an angle of 90° after each replicate.

An another experiment was conducted with four arm Pettersson olfactometer by taking the best preferred hosts of

C. montrouzieri by connecting directly with the computer using OLFA computer program for the collection and analysis of behavioral data with the four arm olfactometer. The program was developed by Francesco Nazzi, Udine, Italy, 1995.

Results and Discussion

Y-tube olfactometer bioassay

Response of *C. montrouzieri* to different species of mealybugs (No choice test)

The result of Y-tube olfactometry studies with reference to the orientation of *C. montrouzieri* towards different mealybug species is presented in table 1. There was significant difference on the orientation of *C. montrouzieri* to different mealybug species. About 74.0 per cent of *C. montrouzieri* adults oriented towards *P. citri* while it was 64.4 and 57.8 per cent for *M. hirsutus* and *Phenococcus* sp respectively. This clearly showed that *P. citri* was the most preferred host for *C. montrouzieri* than other mealybug species tested.

Response of *C. montrouzieri* to different species of mealybugs along with its host (No choice test)

The result of Y-tube olfactometry studies with reference to the orientation of

C. montrouzieri towards different mealybug species with their hosts is presented in table 2. Here also significant variation was observed on the orientation of *C. montrouzieri* to different mealybug species with their hosts. About 81.0 per cent of *C. montrouzieri* adults oriented towards *P. citri* + guava leaves, while it was 66.3 and 66.7 per cent for

M. hirsutus + mulberry and *Phenococcus*sp + cotton, respectively. Hence it is inferred that *P. citri* along with guava leaves attracted more *C. montrouzieri* beetles than other species with their hosts tested. There was about 1.9 to 7.0 per cent increase in the attraction of *C. montrouzieri* over mealybug species alone.

Under no choice condition the response of *C. montrouzieri* to different species of mealybugs along with its host revealed that the olfactory response to *P. citri* was more (81.0%) when it was along with host leaves and was less (74.0%) when there was mealybug alone. The increase in orientation of *C. montrouzieri* towards its prey and host might be due to synergistic effect of the odours of prey and host leaves. Similar kind of results were obtained in multi choice test also.

Four-arm bioassay

Response of *C. montrouzieri* to different species of mealybugs (Multi choice test)

The orientation response of *C. montrouzieri* showed distinct deviation between different mealybug species and were statistically significant (Table 3). However, there was no difference observed at 15 minutes between the control and the mealy bug arms. At 30 minutes and later a significantly higher number of adults were oriented in the mealy bug arms than the control arm of the four arm olfactometer. At 45 minutes interval, about 45.67 per cent of *C. montrouzieri* adults oriented towards *P. citri* while it was 21.0 and 14.33 per cent for *M. hirsutus* and *Phenococcus* sp respectively. About 34.16 per cent of *C. montrouzieri* adults were attracted to *P. citri* in an hour whereas *M. hirsutus* and *Phenococcus*sp were 18.58 and 14.16 per cent. Only 4.19 per cent were oriented towards control arm and the remaining 28.91 per cent of *C. montrouzieri* adults were hovering in the central arena of the olfactometer. This clearly showed that

P. citri was the most preferred host for *C. montrouzieri* than other species tested (Figure 1).

Among the mealybug species, the two most preferred species were selected and the olfactory response of *C. montrouzieri* were studied using four arm olfactometer by OLFA Package using computer revealed that the time spent in the arm with *P. citri* was more (4.10 min) when compared to *M. hirsutus* in the opposite arm (3.08 min)

(Table 4). Whereas in the central arena of four arm olfactometer the total time spent was 0.96 min. *C. montrouzieri* made first choice to *P. citri* than *M. hirsutus.*

Response of *C. montrouzieri* to different species of mealybugs along with its host

The response of *C. montrouzieri* adults to different mealybug species along with their hosts showed distinct difference and were statistically significant. However, there was no difference observed at 15 minutes between the control and the mealybug with host arms. At 30 minutes and later a significantly higher number of adults were attracted in the mealybugs with host arms than the control arm of the four arm olfactometer (Table 5). At 60 minutes interval, about 44.33 per cent of *C. montrouzieri* adults oriented towards *P. citri* + guava leaves while it was 22.33 and 16.67 percent for *M. hirsutus* + mulberry and *Phenococcus*sp + cotton leaves, respectively. About 41.67 per cent of *C. montrouzieri* adults were attracted to *P. citri* + guava leaves in an hour whereas *M. hirsutus* + mulberry and *Phenococcus*sp + cotton leaves were 18.33 and 16.99 per cent. Only 8.08 per cent were oriented towards control arm and the remaining 14.93 per cent of *C. montrouzieri* adults were hovering in the central arena of the olfactometer. This clearly showed that *P. citri* along with guava leaves oriented more adults than other species with their hosts tested, which may be due to the kairomones produced by the leaves. In four arm olfactometery studies, after 15 minutes of *C. montrouzieri* release there was no significant difference in the orientation between the control arm and the mealy bug arms. However, at 30 minutes after the *C. montrouzieri* release there was significant difference in the orientation of *C. montrouzieri* to different treatment arms (Table 3). At 45 minutes interval, about 45.67 per cent of *C. montrouzieri* adults oriented towards *P. citri* while it was 21.0 and 14.33 per cent for *M. hirsutus* and *Phenococcus*sp respectively (Table 4). Similarly, *P. citri* with guava leaves had attracted more *C. montrouzieri* than the control and also the other two mealy bugs with their host. Similar findings were reported by Garcia and Ribeiro (1983) [4] indicating a significant attraction of *C. septempunctata* to the host plant with aphids than to the host plant alone. During the field survey it was evident that more number of *C. montrouzieri* were found in the trees infested with *P. citri* than other mealybugs and it was confirmed by olfactometry assays also. The results obtained from four arms olfactometer assays also in accordance with the results of Y-tube olfactometer.

Table 1: Olfactory response of *C. montrouzieri* to different mealybug species under no choice condition (Y-Tube olfactometer assay)

Mealybug species	Olfactory response (%) of *C. montrouzieri*		
	Minimum	Maximum	Mean ± SD
P. citri	68.2	80.8	74.0±6.3
P. hirsutus	60.0	69.6	64.4±4.8
*Phenococcus*sp	52.4	60.9	57.8±4.7

F value (P=0.05): 7.043

Table 2: Olfactory response of *C. montrouzieri* to different mealybug species with their hosts in Y-tube olfactometer assay No choice condition

Mealybug species	Olfactory response (%) of *C. montrouzieri*			
	Minimum	Maximum	Mean ± SD	% increase over mealybug species alone
P. citri + Guava	76.9	85.2	81.0±7.9	7.0
M. hirustus + Mulberry	57.1	70.8	66.3±0.0	1.9
*Phenococcus*sp + Cotton	66.7	66.7	66.7±4.1	5.8

F-Value (P=0.05): 7.118

Table 3: Olfactory response of *C. montrouzieri* to different species of mealybugs under multi choice condition

Mealybug species	Time Interval (min)	Range	Mean ±SD	% response over total no of adults	Mean
P. citri	15	5-6	5.3±0.6	17.67	34.16
	30	7-11	9.0±2.0	30.00	
	45	13-15	13.7±1.2	45.67	
	60	12-14	13.0±1.0	43.33	
M. hirsutus	15	3-5	4.0±1.0	13.33	18.58
	30	5	5.0±0.0	16.67	
	45	6-7	6.3±0.6	21.00	
	60	6-8	7.0±1.0	23.33	
*Phenococcus*sp	15	3-4	3.7±0.6	12.33	14.16
	30	4-5	4.3±0.6	14.33	
	45	4-5	4.3±0.6	14.33	
	60	4-5	4.7±0.6	15.66	
Control	15	3-7	5.0±2.0	16.66	4.19
	30	2	2.0±0.0	0.06	
	45	0-1	0.7±0.6	0.02	
	60	0	0.0±0.0	0.0	

Table 4: Assessment of olfactory response of *C. montrouzieri* to *M. hirsutus* and *P. citri* using Pettersson olfactometer time spent

Beetle	Treatment arm (*P. citri*)	Control arm I	Treatment arm (*M. hirsutus*)	Control ARM II	Centre
1	5.1*	1.3	2.5	0.5	1.5
2	4.3	0.6	3.5	0.6*	1.0
3	3.2*	2.0	2.7	1.0	1.1
4	4.0*	1.2	2.6	0.8	1.4
5	5.6*	0.3	2.6	0.5	1.0
6	4.6	1.3	3.0*	0.3	0.8
7	4.1	1.7	3.2*	0.6	0.4
8	3.3	1.3*	4.0	1.2	0.2
9	3.9*	0.8	2.8	0.9	1.6
10	3.3	1.6	3.9*	0.6	0.6
Mean	4.1	1.21	3.08	0.7	0.96

T-Value (P= 0.05): 2.82, * First Choice

Table 5: Olfactory response of *C. montrouzieri* to different species of mealybugs along with its host under multi choice condition

Mealybug species	Time Interval (min)	Range	Mean ±SD	% response over total no of adults	Mean
P. citri + Guava Leaves	15	9-13	11.3±2.1	37.67	41.67
	30	11-15	12.7±2.1	42.33	
	45	12-14	12.7±1.2	42.33	
	60	11-16	13.3±2.5	44.33	
M. hirsutus + Mulberry leaves	15	3-5	4.0±1.0	13.33	18.33
	30	4-7	5.3±1.5	17.67	
	45	5-7	6.0±1.0	20.00	
	60	4-8	6.7±2.3	22.33	
*Phenococcus*sp + Cotton leaves	15	3-4	4.7±1.2	15.66	16.99
	30	4-6	5.0±1.7	16.66	
	45	5-7	5.7±1.2	19.00	
	60	4-6	5.0±1.0	16.67	
Control	15	3-6	4.7±1.5	15.66	8.08
	30	2-3	2.3±0.6	7.67	
	45	0-2	1.0±1.5	3.33	
	60	0-3	1.7±1.0	5.67	

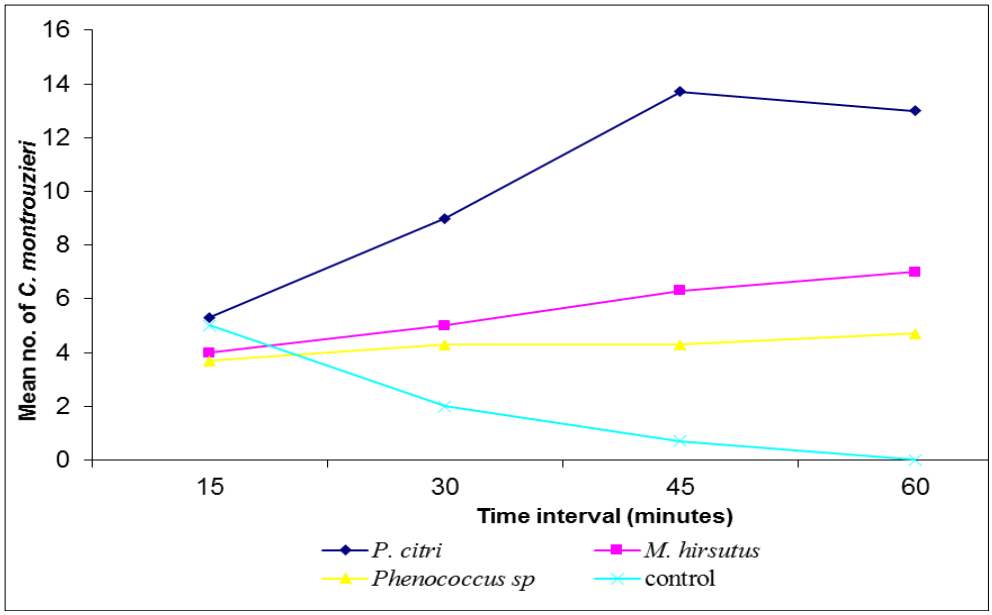

Fig 1: Olfactory response of *C. montrouzieri* to different species of mealybugs using four arm olfactometer

Conclusion

Thus, it is evident that adults of *C. montrouzieri* sense the prey liberated chemicals and use the same to reach the source. Such of those volatiles need to be identified for further exploitation in the biological control of mealybugs in different crop ecosystems.

References

1. Bertschy C, Turlings TCJ, Bellotti A, Dorn S. Chemically mediated attraction of three parasitoid species to mealybug infested cassava leaves. Fla Entomol. 1997;80:383-95.
2. Chacko MJ, Bhat PK, Ananda Rao LV, Deepale Singh MB, Ramanaryana EP, Sreedharan K. The use of the ladybird beetle, *Cryptolaemus montrouzieri* for the control of coffee mealybug. J Coffee Res. 1978;8:14-19.
3. Fleischer G, Waibel H. Pesticide policy and integrated pest management. In: Maredia KM, Dakouo D, Mota-Sanchez D, editors. Integrated pest management in the global arena. Cambridge, USA: CABI Publishing, 2003, p. 49-63.
4. Garcia V, Ribeiro JA. Olfactory as a selection method for *aphidophagous coccinellids. Arquipelago Ciencias* da Natureza. 1983;4:31-41.
5. Heidari M, Copland MJW. Host finding by *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae), a predator of mealybugs (Homoptera: Pseudococcidae). Entomophaga. 1992;37:621-625.
6. Kotikal YK, Sengonca C. Olfactory responses of mealybug predator, *Cryptolaemus montrouzieri Mulsant* (Coleoptera: Coccinellidae) to the kairomones of prey arthropods, their host plants and the predator itself. J Biol Control. 1999;13:1-6.
7. Lower HF. Hard to kill pests of fruit trees. J Agri South Austral. 1968;72:75-7.
8. Mani M, Krishnamoorthy A. Occurrence of mealybugs and their natural enemies on custard apple around Bangalore, S. India. J Biol Control. 1989;3:77.
9. Mani M, Krishnamoorthy A. Australian ladybird beetle, *Cryptolaemus montrouzieri*. Madras Agric J. 1997;84:237-48.
10. Mani M. Distribution, bioecology and management of the grape mealybug, *Maconelli coccushirsutus* (Green) with special reference to its natural enemies. Unpubl Ph.D. Thesis, Univ Agric Sci, Bangalore; 1986. 201 p.
11. Sengonca C, Kotikal YK, Schade M. Olfactory reactions of *Cryptolaemus montrouzieri Mulsant* (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) in relation to period of starvation. J Pest Sci. 1995;68(1):9-12.
12. Singh SP. Fifteen years of AICRP on Biological control. Technical Bulletin No 8. Project Directorate of Biological Control, Bangalore, India, 1994.
13. Srinivasan TR. Studies on biology and control of grape mealybug, *Maconelli coccushirsutus* Green (Homoptera: Pseudococcidae). Unpubl M.Sc. (Ag.) Thesis, Tamil Nadu Agric Univ, Coimbatore, India, 1987, p. 56.
14. Stubbs M. Another look at prey detection by coccinellids. Ecol Entomol. 1980;5:179-182.