Chemically-mediated behaviour of the predator *Rhynocoris marginatus* (FAB.) (Heteroptera : Reduviidae) searching insect prey

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Summary

The reduviid predator, *Rhynocoris marginatus* (Fab.) (Heteroptera : Reduviidae) is a potential biocontrol agent for the Asian armyworm, *Helicoverpa armigera* (Hubner), tobacco caterpillar, *Spodoptera litura* (Fab.) (Lepidoptera : Noctuidae) and *Mylabris pustulata* (Thunberg) (Coleoptera : Meloidae). The influence of info-chemicals from these three species of groundnut pests on the prey-searching and probing behaviour of the reduviid predator, was studied. The water fraction of the hexane extract of *H. armigera* elicited maximum response in *R. marginatus* when compared to that of *M. pustulata* and *S. litura*. Fourth (17.81min) and fifth (30.18min) instar nymphs exhibited maximum handling time with *H. armigera* extract as compared to other instars. Minimum response was observed in the case of *S. litura* extract.

Keywords : Biocontrol agent, *Helicoverpa armigera, Spodoptera litura, Mylabris pustulata*, behaviour.

Introduction

Rhynocoris marginatus, a polyphagous predator present in India, is an effective biological control agent of cotton and groundnut crop pests (AMBROSE & CLAVER, 1999; SAHAYARAJ, 1999, 2002a). Prey chemicals have been found to elicit the host searching behaviour of this heteropteran predator (SAHAYARAJ & PAULRAJ, 2001). The chemicals, which govern the prey and predator interaction, are generally called info-chemicals. It includes kairomones and allomones, which influence the prey-predator interaction (ANANTHAKRISHNAN, 2002). The chemical cues emanating from the pests act as arrestants or stimulants to the predators and increase their search for the prey (SINGH *et al.*, 2002). According to DUFFEY (1980), kairomones and allomones are especially present in the nutrient storage sites like gut, haemolymph and fat body. Several studies have been carried out to find out the role of kairomones from lepidopteran pests in eliciting a host searching behaviour of many generalist predators (YASUDA & WAKAMURA, 1996; YASUDA, 1997; BAKTHAVATSALAM *et al.*, 1999, 2000; SINGH *et al.*, 2002; DUNKELBLUM *et al.*, 2000). In reduviids, the chemical-based prey-predator interaction was studied by MARAN (1999) and SAHAYARAJ & PAULRAJ (2001). Though most of the above studies were carried out with organic solvent extracts of the pests, no work was done on the water extract of any pest. Hence the present study aims to investigate the behaviour of *R. marginatus* towards the water fraction of the hexane extracts of *Mylabris pustulata, Spodoptera litura* and *Helicoverpa armigera* so that the info-chemicals present in the water fraction, that elicit the host-seeking response in *R. marginatus* can be identified.

Materials and Methods

Insect rearing

The predator, *R. marginatus* was collected from groundnut ecosystem, Palayamkottai, Tamil Nadu, India and maintained on *Corcyra cephalonica* STAINTON larvae under laboratory conditions (28 ± 2ºC, L13:D11 photoperiod and relative humidity 73 ± 4%) by the method developed by SAHAYARAJ (2002b). Different life stages of *S. litura* and *H. armigera* and adult *M. pustulata* were collected from the same locality reared on groundnut leaves and flowers, respectively, under the same laboratory conditions. Laboratory emerged fifth instar larvae of *S. litura* and *H. armigera* and adults of *M. pustulata* (both male and female) were used for this study.

Infochemical extracts of the pests

The infochemicals emanating from *M. pustulata, S. litura* and *H. armigera* were extracted using hexane (SINGH *et al.*, 2002). Hundred live *M. pustulata* adults were weighed and placed in a stoppered bottle to which 75 ml of hexane was added. The bottle was shaken at room temperature (28ºC) for 2 hours and kept at 50ºC in water bath for 20 min. The extract was filtered through Whatmann No.1 filter paper. The extract was then fractionated into hexane-soluble and water-soluble fractions and the latter fraction was used for the experiment. The same procedure was followed for *S. litura* and *H. armigera.*

Behavioural Bioassay

A two-armed glass olfactometer designed by SAHAYARAJ & PAULRAJ (2001) was used to study the feeding behaviour of *R. marginatus*. A small piece of filter paper (1×1 cm) impregnated with the water-soluble fraction of *H. armigera* extracts was used as test and filter paper impregnated with distilled water was used as control. Ninety-six hours starved second instar nymphs of *R. marginatus* were released singly into the central chamber and their behaviour (approaching and handling) was observed for one hour continuously.Before introducing each nymph into the chamber, it was cleaned with 0.1% sodium hypo chloride. Similar procedure was carried out for III, IV, and V instar *R. marginatus* with both *M. pustulata* and *S. litura* extracts separately. The predators chose either test chamber or control chamber, or none of them. If the predator chose the test chamber, it was recorded as a positive choice. If it was found in the control chamber, it was recorded as a negative choice. If the predator chose neither of the chambers, it was considered making no choice. The approaching behaviour of the predator between the water-soluble fraction of the pest extracts and water was analyzed by χ^2 test and the significance was expressed at 5% level. The predatory behaviour was observed in terms of approaching and sucking time. From these the handling time was calculated by summing up both (COHEN, 2000). Correlation was observed between the approaching behaviour and handling time for each pest separately (both control and test separately). One way ANOVA and Tukey Honest Significance Difference (HST) test were performed to determine the differences in the handling time between the control (water) and test (water fraction of the pest hexane extracts).

Results

When the predators were released into the central chamber of the olfactometer, *R. marginatus* oriented towards the odour source present in the Whatmann paper with antenna facing the odour source. After getting a perfect orientation position, the reduviid palpated its antennae, then aroused and subsequently showed the other behavioural responses. Once the predators entered the test chamber, they exhibited exploration and probing behaviour by showing head lifting, approaching, rostrum protrusion and sucking and post-predatory behaviour like rostrum cleaning and antenna cleaning.

R. marginatus showed maximum response (Table 1) to water fraction of *H. armigera* as compared to that of *M. pustulata* and *S. litura.* With respect to *H. armigera* extract, 70, 80, 60 and 80% of II, III, IV and V instars of *R. marginatus* showed positive response, respectively. The preference of *R. marginatus* decreased for *M. pustulata* (20, 40, 20, and 10% of II, III, IV and V instar of *R. marginatus*, respectively) and further decreased response was observed with *S. litura* extract (10% for II and V instars). In chi square test, both the II and V instar results were significant for *M. pustulata* and only II instar nymphs showed significant response to *S. litura* extract whereas in *H. armigera,* II, III and V instar results showed significant results at 5% level (Table 1).

The feeding behaviour of *R. marginatus* (in terms of handling time) on three pest extracts is shown in Table 2. Second instar *R. marginatus* exhibited maximum handling time in *S. litura* extract (40.62 ± 0 min) followed by *H. armigera* (13.44 ± 0.85 min) and *M. pustulata* (1.30 ± 0.16 min). The third instar nymphs spent more time with *M. pustulata* extract (12.72 ± 2.11min) when compared to *H. armigera* (6.85 ± 1.24 min) extract. However, III and IV instar nymphs of *R. marginatus* showed no response to *S. litura* extract. The IV and V nymphal instars showed maximum handling time (17.81 ± 1.77 min and 30.18 min respectively) towards *H. armigera* extract followed by *M. pustulata* extract (3.71 ± 1.66 min and 10.04 ± 1.08 min respectively). One way ANOVA and Tukey Honest Significance Difference (HST) test were performed. The tests showed there is no significance between the water fraction of the pests extracts and water.

Table 1. Approaching Behaviour of *R.marginatus* to water soluble fraction of three pests.

Response	Life stages of predator			
	II	III	IV	V
		M. pustulata		
Positive choice	2	4	2	1
Negative choice	1	1	3	1
No choice	7	5	5	8
χ^2	6.205	2.601	1.400	9.809
Significance	*	-	-	*
		S. litura		
Positive choice	1	0	0	1
Negative choice	7	4	3	1
No choice	0	2	3	4
χ^2	8.092	4.000	3.000	3.000
Significance	*	-	-	-
		H. armigera		
Positive choice	7	8	6	8
Negative choice	1	2	2	1
No choice	2	0	2	1
χ^2	6.206	10.410	3.203	9.809
Significance	*	*	-	*

* Significant, (-) not significant at 5% level.

Table 2. Handling time (in minutes) of *R. marginatus* (N ± SE) to the water fractions of three pests.

Predator life stages	Handling time					
	M. pustulata		*S. litura*		*H. armigera*	
	Control	Test	Control	Test	Control	Test
II	08.64 ± 0.64	01.30 ± 0.16	5.53 ± 0.18	40.62 ± 0	26.92 ± 0	13.44 ± 0.85
III	00.67 ± 0	12.72 ± 2.11	16.84 ± 1.05	-	12.53 ± 0	06.85 ± 1.24
IV	03.92 ± 1.05	3.71 ± 1.66	03.81 ± 0.26	-	08.49 ±	17.81 ± 1.77
V	27.69 ± 2.66	10.04 ± 1.08	04.66 ± 0.19	06.25 ± 0	21.76 ±	30.18 ± 2.24

Discussion and Conclusion

The predatory behavioural pattern of reduviid is arousal–approach–rostral probing-injecting toxic saliva and paralyzing-sucking-post predatory behaviour. Harpactorine reduviids oriented towards the prey with facing antenna, after getting a perfect orientation position, the reduviid palpated its antennae, then aroused and subsequently showed the other behavioural responses (AMBROSE 1999) as observed in this harpactorine reduviid. The handling time of *R. marginatus* life stages on the info-chemicals from the three pests studied could be supported by the findings of SAHAYARAJ & PAULRAJ (2001) in reduviids and by those of YASUDA & WAKAMURA (1996); YASUDA (1997) and JHANSILAKSHMI *et al.* (2000) in other predatory insects. When we compare the approaching behaviour with handling time by correlation, the results were significant ($P < 0.05$). It was suggested that the predators identify the volatile chemicals emitted by the prey body and that this which-elicit their prey searching. The volatiles emitted by the faeces of the pests may also act as attractants in prey-predator interactions. The presence of such volatiles in the feces may be attributed to the digestion of the plant products by the pests and other processes involving micro-organisms present in the feces. It has been reported that there are instar specific blends of chemicals emitted by feces of second or fourth instar larvae of *Pieris* species (ANANTHAKRISHNAN, 2002). In the present work, the pests put in hexane immediately exuded fecal matter which was immiscible in the solvent. The immiscible portion was fractionated with water and used for this study. Hence we presumed that the volatiles present in the feces not only act as attractants but also elicit an intense host searching behaviour. The present study showed that the kairomones of the pest extract on the filter paper elicited the usual host seeking behaviour in *R. marginatus*. This was clearly noticed in all the instars against *H. armigera* extract. However, rostrum protrusion and sucking were not observed in the II, III and V instars against *M. pustulata* extract. *S. litura* extract failed to elicit such host seeking behaviour in III and IV instar nymphs. Similar study was carried out by USHA RANI & WAKAMURA (1993), who showed that the feces of *S. litura* stimulated the host-seeking behaviour of *Eocanthecona furcellata*. The variation in the behaviour of *R. marginatus* to the three pests might be due to the different volatile chemicals of the pests, as explained by ANANTHAKRISHNAN (2002). In other words, the behavioural change could be ascribed to the specificity of the predator to a prey species. Both fourth and fifth instar nymphs of the predator preferred *H. armigera* whereas second and third instar nymphs, preferred *H. armigera* and *M. pustulata*. These findings could be used in the application of *R. marginatus* as a biocontrol agent in the management of groundnut pests.

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