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Insecticidal activity of venomous saliva from *Rhynocoris fuscipes* (Reduviidae) against *Spodoptera litura* and *Helicoverpa armigera* by microinjection and oral administration

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Abstract: *Rhynocoris fuscipes* is a potential predator of many economically important pests in India. In the present study, its venomous saliva (VS) was collected by milking and diluted with HPLC grade water to different concentrations (200, 400, 600, 800 and 1000 ppm). Microinjection of *Rhynocoris fuscipes* VS was more toxic than its oral administration in *Helicoverpa armigera* (cotton bollworm) and *Spodoptera litura* (tobacco cutworm). Thus, *R. fuscipes* VS was found to be toxic to third instar *S. litura* and *H. armigera* with respective LD_{50}s of 846.35 and 861.60 ppm/larva at 96 hours after microinjection. The current results showed that VS of *Rhynocoris fuscipes* caused mortality of *H. armigera* and *S. litura*. Active peptides from VS may be isolated, identified and assessed for their impact in order to ascertain how they alter the physiology of these pests, information that could be applicable in pest management programs.

Key words: salivary venom, microinjection, oral administration, mortality, *Rhynocoris fuscipes*, biological control.

INTRODUCTION

Arthropod venom has attracted considerable interest as a potential source of bioactive substances. Their biological properties and proteinaceous nature render them useful in biological pest management as previously suggested (1-5). The venom of poisonous predators has novel peptides that have been isolated from snakes, scorpions, marine cone snails, spider and other animals including predatory insects. In arthropods, copious information is available on spiders and parasitoids. Among the predatory hemipterans, reduviids constitute an important predator on account of presenting worldwide distribution and having been utilized in the biological control of cotton, soybean, groundnut and coconut pests. Venoms of reduviid predators are known to possess long-term, non-lethal paralytic effects on their prey. The immobilized or partially digested prey is then used as food by the reduviid predator (6-8). Such unique paralytic activity was due to the presence, in the venom of reduviid predators, of novel neurotoxic compounds, only a few of which have been isolated and characterized to date (2, 9-12).

The tobacco caterpillar, *Spodoptera litura* (Fabricius) is one of the most destructive pests and consists of about 120 species of plants belonging to 44 families (13, 14). The chemical control of *S. litura* presents limitations due to its resistance against many insecticides including pyrethroids (15, 16). The cotton bollworm, *Helicoverpa armigera* (Hübner), one of the most important pests, affects crop production globally (17). Insecticidal resistance of this pest has also been reported in the literature (18, 19).

The reduviid predator, *Rhynocoris fuscipes* Fab. (Hemiptera: Reduviidae) is an entomophagous insect distributed in many agroecosystems and

feeds on more than 40 economically important insect pests in India (8). The potential of *R. fuscipes* as a biological control agent under laboratory and field conditions has been described previously (20-24). Maran (23) studied the paralytic potential of *R. fuscipes* salivary gland extract against selected pests. However, no one has studied the toxicological, physiological and immunological activities of this reduviid salivary venom on any pests. In the present work, we evaluated for the first time the biological activities of adult *R. fuscipes* salivary venom against *H. armigera* and *S. litura* third instar larvae.

MATERIALS AND METHODS

Insect Collection and Rearing

Laboratory colonies of the host species, *H. armigera* and *S. litura*, and the reduviid predator were established from individuals that were collected from cotton fields in Tamil Nadu, India. *R. fuscipes* were reared on the larvae of the host, *S. litura* at 30.0 ± 1°C and 70-80% relative humidity with a photoperiod of 11/13 hours darkness/light. The host colonies were maintained on fresh cotton leaves up to second instars, and then transferred to the freshly prepared artificial diet for further rearing.

Venom Collection and Preparation

The venomous saliva (VS) was collected from the ten-day-old freshly emerged adult reduviid as described previously (25, 26). The salivary venom collected from more than 50 reduviid predators was pooled and then stored on ice until used in our toxicity experiments within 12 hours. VS was collected from each predator only once. Concentrations of the VS (200, 400, 600, 800 and 1000 ppm) were prepared by diluting with HPLC-grade water (Qualigens, India).

Determination of Toxicity

The toxicity of *R. fuscipes* VS was evaluated against third instar larvae of *H. armigera* and *S. litura* using microinjection and oral toxicity methods (27, 28). In the microinjection method, different VS concentrations were tested for toxicity by injecting 1.0 µL of VS into each third stage *S. litura* larva of approximately 120 mg in weight. Control category larvae were injected with HPLC-grade water. Salivary venom and water injected larvae were placed individually in a plastic container (5.5 cm height x 3.8 cm diameter) and maintained in a BOD incubator on an artificial diet. Larval mortality was observed at 24 hours intervals up to 96 hours. Behavioral changes, if any, in the host insect were observed and recorded up to three hours post-injection. A soybean seed-based artificial diet was used to assay VS by oral delivery against newly hatched third stage S. *litura* larvae (starved for six hours prior to exposure to diet) (29).

For each treatment, six larvae were maintained in a sterilized plastic container containing moist filter paper to prevent diet desiccation. For the oral toxicity bioassay, 1 mL of VS of different concentrations (200, 400, 600, 800 and 1000 ppm) was blended thoroughly with 100 mg of artificial diet separately and provided to the larvae. Control diets contained an equal amount of HPLC-grade water. Survival was monitored daily up to 96 hours. A similar procedure was used for *H. armigera* third instar larvae.

Statistical Analysis

The LD_{50} value was calculated by the method of Finney (29). Control animal data were compared with different VS concentrations. All data were subjected to one-way ANOVA and *post hoc* Tukey's test using the statistical software SPSS (Version 11.5). The significance level was set at 5 or 1%.

RESULTS

Individual *H. armigera* and *S. litura* injected with minimum concentrations (200 and 400 ppm) exhibited no initial response; but within 90 minutes the following sequence was observed: wriggling, restless movement, rapid mastication of the mandible, lateral fall and, finally, motionlessness. The onset of these symptoms seemed to occurring faster (30 to 40 minutes) with increasing concentrations. None of the control injections of 1.0 µL HPLC-grade water resulted in fatality or symptoms of envenomation within 96 hours, the maximum period of observation.

Microinjection Toxicity

Spodoptera litura third instar larvae injected with VS, only 26.67% larvae died within 24 hours ($F = 9.42$; df1,18; $p < 0.01$). However, at 96 hours, 64.29% of *S. litura* larvae had died ($F=19.43$; df1,18; $P<0.05$) and showed an LD_{50} value of 861.60 ppm/larva (Table 1). However, during the

Table 1. Microinjection and oral administration of venomous saliva of *R. fuscipes* on the corrected mortality (%) at different exposure moments (24, 48, 72 and 96 hours), $LC_{30,}$ LC_{50} and LC_{90} (ppm) of *Spodoptera litura* and *Helicoverpa armigera* third instar larvae

LD values / Concentration (ppm)	Microinjection				Oral toxicity			
	Hours after treatment				Hours after treatment			
	24	48	72	96	24	48	72	96
***Spodoptera litura* third instar larvae**								
200	13.33	33.33	42.86	42.86	0	0	13.33	21.43
400	6.67	6.67	14.29	35.71	0	6.67	13.33	21.43
600	13.33	40.00	35.71	50.00	6.67	20.00	26.67	35.71
800	33.33	53.33	50.00	64.29	20.00	33.33	46.67	50.00
1000	26.67	60.00	57.14	64.29	33.33	40.00	53.33	71.43
LD_{30}	–	869.50	846.05	832.43	–	–	883.67	868.29
LD_{50}	–	890.13	883.65	861.60	–	–	913.00	891.17
LD_{90}	–	929.29	915.88	900.43	–	–	941.49	913.43
***Helicoverpa armigera* third instar larvae**								
200	26.67	46.67	42.86	38.46	0.00	0.00	0.00	7.14
400	33.33	40.00	35.71	38.46	6.67	7.14	7.69	14.29
600	40.00	53.33	64.29	69.23	13.33	21.43	28.57	30.77
800	46.67	73.33	78.57	76.92	20.00	21.43	28.57	46.15
1000	53.33	80.00	78.57	84.62	20.00	42.86	50.00	69.23
LC_{30}	863.19	816.56	820.14	822.31	–	–	891.22	881.63
LD_{50}	906.24	847.29	847.13	846.35	–	–	928.12	899.91
LC_{90}	947.51	877.11	873.41	869.83	–	–	960.82	917.48

–: no results were found

same period, 84.62% of *H. armigera* larvae died with LD_{50} of 846.35 ppm/larvae. VS of *R. fuscipes* caused dose-dependent mortality in both pests.

Oral Toxicity

At 24 and 48 hours of observation, VS caused less than 50% mortality in *H. armigera* and *S. litura* larvae. Oral administration of VS provoked respective mortalities of 71.43 and 69.23% in *S. litura* (F=19.42; df1,18; P<0.05) and *H. armigera* (F=19.44; df1,18; P<0.05) larvae and LD_{50} values of 891.17 and 899.91 ppm/larva (Table 1).

DISCUSSION

In the present study, the saliva from the reduviid predator was determined to be venomous

to *H. armigera* and *S. litura* whether larvae received the treatment orally or by injection. The maximum corrected mortality of *Spodoptera litura* was observed at 96 hours with respective LD_{50}s of 861.60 and 891.17 ppm/animal for microinjection and oral administrations. For the same period and administration methods, *H. armigera* third instar larvae required more venom (Table 1), which indicates the pest is less susceptible than *S. litura.* The concentrations we administrated were biologically relevant. But it was reported previously that crude *Paracoelotes luctuosus* (Amaurobiidae) venom had an LD_{50} of 9-50 µg/g against a prey species, *S. litura* showing that reduviid crude venom is more potent than spider toxin (2). Moreover, toxins isolated from spiders and scorpions also produced lower LD_{50} values against *S. litura* (7.6, 5.1 and 14.4 µg/g for AalT, $LqhT_2$ and µAgalV, respectively).

Toxicity of three reduviids (*Peirates turpi, Agriosphodrus dohrni* and *Isyndus obsurus*) venoms were tested against *S. litura* larvae. The fact that they did not show any toxicity against the insects indicates that crude venom has more impact than such purified peptides as PtuI, AdI and IobI (2). We concluded that the toxic nature of the VS is due to its protein content. However, isolation and identification VS peptides will be required in the near future. Previous reports of the toxic nature of *Platymeris rhadamanthus*, *Peirates affinis* and *Haematorrhophus nigroviolaceous*, *Catamiarus brevipennis* salivary venoms support our findings (9, 10, 12, 25). Injection of such venoms caused wriggling and restless movement, rapid mastication action of the mandible, a lateral fall and motionlessness for 30 to 40 minutes prior to resuming its routine activities. The paralysis was due to medium and low molecular weight neurotoxin (proteins). Edwards (9) reported that the salivary proteins of *P. rhadamanthus* provoke rapid loss of nervous conduction followed by loss of muscle contraction and relaxation. The study indicates that the venomous saliva is more toxic when injected directly into the insect haemocoel, completely bypassing the gut, which might be: consistent with the neuromuscular abnormalities we observed after injection of venom, or secondary consequences of a mixture of neurotoxic components in the venomous saliva of the reduviid. It is suggested to isolate, purify and identify the insecticidal compounds from reduviid VS.

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CONFLICTS OF INTEREST

There is no conflict.

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