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Zootoxic effects of reduviid *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) venomous saliva on *Spodoptera litura* (Fab.)

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ABSTRACT

Rhynocoris marginatus is a predominant and potential reduviid predator of many economically important pests in India. The venomous saliva (VS) was collected by milking method and diluted with HPLC grade water to prepare different concentrations (200, 400, 600, 800 and 1000 ppm). The VS from *R. marginatus* was found to be toxic and the LD_{50} of the VS in *Spodoptera litura* third instar were 768 and 929 ppm at 48 and 96 h for microinjection and oral toxicity studies, respectively. Level of hydrolase and detoxification enzymes significantly decreased in a dose-dependent manner after treating the host with VS for 96 h. A decrease in carbohydrate (21%) and lipid (46%) contents and an increase in the protein content (50%) were prominent in the experimental category. The VS reduced the relative growth rate, approximate digestibility, efficiency of conversion of ingested and digested food of *S. litura* in the oral toxicity study. Salivary venom inhibits the haemocytes from aggregation and affects spreading behavior of haemocytes separated from the fifth stadium larvae of *S. litura*. The result showed that VS toxins caused mortality, changed the nutritional indices, and altered the levels of macromolecule quantity and digestive enzymes of *S. litura*. We concluded that the VS of *R. marginatus* is venomous to a prey species, *S. litura*.

1. Introduction

The venom of arthropods 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 suggested early in the 1990s (Maeda et al., 1991; Mc Cutchen et al., 1991; Stewart et al., 1991; Tomalski and Miller, 1991; Hammock et al., 1993). The venom of poisonous predators has novel peptides which have been isolated from snakes, scorpions, marine cone snails, spiders and other animals including predatory insects. In arthropods, enormous information is available about the insecticidal activity for spiders, scorpions and parasitoids. Among the predatory hemipterans, reduviids constitute an important predator, distributed worldwide and have 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 (Blum, 1978; Cohen, 1990; Sahayaraj, 2007) prey are then used to feed by the reduviid predator. Such unique paralytic activity (Edwards, 1961; Haridass and Ananthakrishnan, 1981; Mc Mahan, 1983; Maran and Ambrose, 2000) was due to the presence of novel neurotoxin compounds in the venom of reduviid predators (Corzo et al., 2001). To date, only few neurotoxin compounds have been isolated and characterized from reduviid predators (Corzo et al., 2001).

Tobacco caterpillar, *Spodoptera litura* (Fabricius) is one of the most destructive pests of about 120 species of plants belonging to 44 families (Qin et al., 2004; Nandagobal and Gunathilagaraj, 2008). The use of insecticides for the

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control of *S. litura* has limitations due to its resistance against many insecticides (Kodandaram and Dhingra, 2007) including pyrethroids (Munir et al., 2009). The reduviid predator, *Rhynocoris marginatus* (Fab.) is an entomophagous insect distributed in many agroecosystems and feeding on more than twenty economically important insect pests in India (Sahayaraj, 2007). The potential of *R. marginatus* as a biocontrol agent under laboratory (Sahayaraj, 2000; Sahayaraj and Balasubramanian, 2009; Sahayaraj et al., 2003, 2004) and field conditions (Sahayaraj, 1999; Sahayaraj and Martin, 2003; Sahayaraj and Ravi, 2007) has been reported earlier. Maran (2000) studied the paralytic potential of *R. marginatus* salivary gland extract against selected pests. In our previous study, the antimicrobial activity of *R. marginatus* salivary venom against selected human pathogens has been recorded (Sahayaraj et al., 2006a). However, none of them has studied the toxicological, physiological and immunological activities of this reduviid venomous saliva on any pests.

The true venoms of arthropods possesses insecticidal activity against many economically important pests (Wudayagiri et al., 2001; Parkinson et al., 2002; Tedford et al., 2004; Dani et al., 2005; Ergin et al., 2006; de Lima et al., 2007; Nicholson, 2007; Chaim et al., 2011; Baeka et al., 2011). The venoms saliva of hunter reduviids possesses insecticidal activity against many crop pests (Edwards, 1961; Ambrose and Maran, 2000; Maran, 2000; Corzo et al., 2001). However, studies of the effects of venomous saliva on various gut enzymes in the insect have been seriously neglected. There is a vast literature regarding the stage and age variation of digestive enzymes in whole gut preparations of all groups of insects (see Terra et al., 1996a, b; Chapman, 1998; Guo et al., 2011). In most insects, food digestion largely occurs in the alimentary canal, in which most of the enzymes are produced and secreted, including protease, lipases, carboxylases, amylase, invertases, and maltases. Insect gut also produces a variety of detoxification enzymes which play important roles in adapting to an environment altered by endo and exogenic compounds (Zhu et al., 2011). There is, however, relatively little known about the factors controlling the release of digestive enzymes in insects. This knowledge is a prerequisite for developing methods of control of pests based on inactivation of digestive enzymes. The chief aim of this research therefore was to determine what extrinsic factors (incorporating venom into the diet) are most important in the regulation of enzyme release and food consumption indices. The second aim was to examine the action of mixture of neurotoxic components in the venomous saliva of *R. marginatus* adult's on mortality, whole body total carbohydrates, proteins and lipids and inhibition of haemocytes aggregation and spreading of *S. litura* third instar larvae.

2. Materials and methods

2.1. Insect collection and rearing

Laboratory colonies of the host species, *S. litura* and reduviid predator were established from individuals that were collected from cotton fields of Tamil Nadu, India. *R. marginatus* were reared on the larvae of the host, *S. litura* at 30 ± 2 °C, 70–80% RH and with a photoperiod of 11:13 h D:L. Host colony was maintained on fresh cotton leaves up to second instars, and then transferred in to the freshly prepared artificial diet (Mani and Rao, 1998) for further rearing.

2.2. Venom collection and preparation

The venomous saliva (VS) was collected from the 10-day old freshly emerged adult reduviid as described by Sahayaraj et al. (2006b) and Sahayaraj and Kanna (2009). The salivary venom collected from more than 50 reduviid predators were pooled and then stored on ice until their use in our toxicity experiments within 12 h VS was collected from each predator only once. Concentrations of the VS (200, 400, 600, 800 and 1000 ppm) (1 ppm = 1 μl of crude venom in 1000 ml phosphate buffer) were prepared by diluting with HPLC grade water (Qualigens, India).

2.3. Determination of toxicity

The toxicity of *R. marginatus* VS was evaluated against third instar larvae of *S. litura* using microinjection (Escoubas et al., 1995) and oral toxicity (Fitches et al., 1997) methods. In microinjection method, different concentrations of the VS were tested for toxicity by injecting 1.0 μl of VS into third stadium *S. litura* larvae 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 h × 3.8 cm d) and maintained in Biological Oxygen Demand (BOD) incubator with artificial diet. Larval mortality was observed at 24 h interval up to 96 h. Behavioral changes if any, in the host insect was observed and recorded up to 3 h post-injection. A soybean seed based artificial diet (Mani and Rao, 1998) was used to assay VS by oral delivery against newly hatched third stadium *S. litura* larvae (starved for 6 h prior to exposure to diet). For each treatment, thirty larvae were maintained in sterilized plastic container containing moist filter paper to prevent diet desiccation. For oral toxicity bioassay, 1 ml of VS of different concentrations (200, 400, 600, 800 and 1000 ppm) was blended thoroughly with the 100 mg of artificial diet separately and provided to the larvae. Diets containing an equal amount of HPLC grade water were controlled. Survival was monitored daily up to 96 h. Then, all the live insects were used for estimating macromolecules and enzyme profile analysis.

2.4. Macromolecular studies

Live insects obtained from the previous study have been used for the estimation of whole body total carbohydrates, proteins and lipids. The total carbohydrate (Sadasivam and Manickam, 1997), protein (Lowry et al., 1951) and lipids (Bragdon, 1951) were estimated using glucose, bovine serum albumin (BSA) and cholesterol as standards, respectively.

2.5. Preparation and quantification of enzymes

Third instars of VS treated *S. litura* larvae were used to quantify enzyme activities. Enzyme extracts were prepared by the method of Applebaum (1964) and Applebaum et al.