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Anti-aggregation and Cytolytic Behaviour of Venomous Saliva of *Rhynocoris fuscipes* (Fab.) (Hemiptera: Reduviidae) in Response to Its Prey Hemocytes

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ABSTRACT

Venomous saliva (VS) from *Rhynocoris fuscipes* (Fab.) was analyzed for immunosuppressive activity toward two prey species, *Spodoptera litura* (Fab.) and *Helicoverpa armigera* (Hubner). Immunosuppression was assessed by examining changes in total hemocyte counts and hemocyte behaviors (aggregation and spreading). Newly ecdysed fifth stadium larvae displayed total hemocyte counts of $1.285x10^7$ cells/ml and $1.397x10^7$ cells/ml for *S. litura* and *H. armigera*, respectively. When isolated hemocytes were exposed to saliva from *R. fuscipes*, a concentration and time dependent decline in hemocyte numbers was observed for both prey species. The decrease was attributed to cell death presumed to involve both cytolytic and apoptotic pathways. Saliva also altered the behavior of hemocytes from both lepidopteron species. Plasmatocytes and granular cells incubated with saline (PBS) or untreated formed aggregations within a few minutes after introduction into 96-well plates, and also attached and then spread over the plastic surfaces. By contrast, aggregation by both cell types, as well as the ability to spread were reduced following exposure to reduviid saliva, and these events were both concentration and time dependent. The possible significance of inducing prey immunosuppression during feeding by *R. fuscipes* is discussed.

Key words: Anti-aggregation, cell lysis, hemocytes, cell death, venomous saliva.

INTRODUCTION

Arthropod venoms are complex mixtures containing a variety of biologically active substances such as proteinaceous and non-proteinaceous toxins, enzymes, nucleotides, lipids, biogenic amines and other unknown substances (Jackson and Parks, 1989; Rash and Hodgson, 2002). Based on specificity, arthropod toxins have been broadly classified as mammalian, insect, and/or crustacean toxins (Gordon *et al.,* 1998; Corzo *et al.,* 2001; Altuntaş *et al.,* 2010). The salivary toxins produced by an array of reduviid predators fall under the category of insect toxins. Reduviid predators subdue their insect prey by injecting venomous saliva (VS) (Ambrose, 1988; Cohen, 2000; Haridass and Anathakrishnan, 1981; Morrison, 1989; Sahayaraj *et al.*, 2010). The VS functions to immobilize the prey through induction of paralysis and also contributes to external digestion of the food source (Cohen, 1990; Muthukumar, 2011; Sahayaraj *et al.,* 2010).

Though VS appears to contain an arsenal of chemical weapons to disable and digest prey, the insect 'food' is not passive to attack by reduviid predators. Insects in general rely on a potent immune system, comprised of cellular and humoral defenses (Beckage, 1998; Rosales, 2011; Strand and Pech, 1995; Gillespie *et al.,* 1997). Cellular defenses include phagocytosis, nodulation, and encapsulation, as well as hemocyte-mediated immune responses (Muthukumar, 2011; Lavine and Strand, 2002; Sahayaraj and Muthukumar, 2011; Jiravanichpaisal *et al.,* 2006). Several species of parasitic insects are known to uses venoms, viruses and other agents to target host hemocytes as a key step to yield immuno-compromised hosts (Richards and Parkinson, 2000; Marris *et al.,* 1999; Parkinson *et al.,* 2002; Zhang *et al.,* 2005; Li *et al.,* 2009). By contrast, virtually nothing is known about the immunosuppressive properties of salivary toxins used by reduviid predators.

Muthukumar (2011) has reported that saliva from the reduviid *Rhynocoris marginatus* inhibits aggregation and induces cell death in hemocytes from *S. litura*. Details about the mode of action of the venom toxins or which salivary components are involved have not been determined. The fact that VS from *Rhynocoris* species may suppress the immune responses of *S. litura* warrants further investigation as the tobacco armyworm is a serious pest in Asia, attacking several agricultural and horticultural crops (Ranga Rao *et al.,* 1993; Malarvannan *et al.,* 2008). Use of reduviid predators for the biological control of *S. litura* and other closely related pests have relied almost exclusively on innoculative release strategies, and to our knowledge, no attempts have been made to use the venomous saliva of *R. marginatus* or *R. fuscipes* in the development of bio rationale insecticides. For successful predation, hunter reduviids need venomous saliva, which is injected with their prey at the time of predation. Venomous saliva induces melanization and structural damage to host hemocytes, and inhibits hemocyte aggregation and spreading behavior (Sahayaraj and Muthukumar, 2011).

As a first step toward the goal of developing biorationale insecticides using VS, we investigated the immunosuppressive properties of VS from *R. fuscipes*. Moreover, *R. fuscipes* has been distributed in many agro-ecosystems and predating upon more than 35 insect pests belongs to various orders. This prompted us to investigate the impact of VS of *R. fuscipes* against *S. litura* and American bollworm, *H. armigera*. *In vitro* assays were used to examine the impact of isolated saliva on total hemocyte counts and aggregation and spreading behavior of cells from two Asian pest species, *S. litura* and *H. armigera*.

MATERIALS AND METHODS

Insect rearing

Rhynocoris fuscipes, S. litura and H. armigera were collected from the agro-ecosystems of Tirunelveli district, Tamil Nadu, India (N 08º43' 06.5"; E 077º44' 14.5"). The collected insects were reared under laboratory conditions at 30±1 ºC, 71.9± 0.4 % relative humidity and a photoperiod of 13L:11D h. Under these

conditions, *S. litura* (Srinivasamurthy *et al.,* 2006) and *H. armigera* (Ahmed *et al.,* 1998) were maintained on an artificial diet for two generations. The reduviid predators were fed *ad libitum* fourth and fifth stadium larvae of *Corcyra cephalonica. Corcyra cephalonica* was maintained using crushed wheat as per the method of Sahayaraj *et al.* (2001). Ten-day old adults of *R. fuscipes* were used to collect saliva. Newly ecdysed (within 12 h of molting) fifth stadium larvae of *S. litura* (317.24 ± 0.12 mg; 38.12 ± 0.12 mm; n = 10) and *H. armigera* (342.11±0.05 mg; 34.41±0.15 mm; n = 10; Mean ± SE) were used to isolate hemocytes.

Venomous saliva collection and preparation

Saliva was prepared from adults (males and females) of *R. fuscipes* that were deprived of food for 3-days following a period of 7 days of *ad libitum* feeding. To collect saliva, adults were milked as described previously (Sahayaraj and Vinoth Kanna, 2009; Sahayaraj *et al.,* 2006) and the isolated venomous saliva (VS) was pooled from 75 adults. The concentration of VS used in the in vitro assays was adjusted to 200-1000 ppm (v/v) using 0.2 M sodium phosphate buffer (PB) (pH 7.2) and then stored at -4 ºC until use.

Preparation of prey hemocytes

Hemocytes for in vitro assays were collected from newly ecdysed fifth stadium larvae of *S. litura* and *H. armigera.* After a brief period of starvation (6 h), larvae were immersed in sterile distilled water at 4ºC for 10 min. The larvae were blotted dry with paper towels and then the distal surface of a proleg base segment was swabbed with 70% ethanol, allowed to air dry, and then pierced with a sterile needle (13 gauge). The hemolymph was collected into a sterile micro centrifuge tube (1.5 ml), pooled (n=10) and diluted 1:10 in ice cold anticoagulant solution (Yu *et al.,* 2007).

Total hemocytes counts (THC)

Total hemocyte counts in *S. litura* and *H. armigera* were performed essentially as described by Cai *et al.* (2004). In brief, 50 µl aliquots of hemolymph/anti- coagulant solution containing hemocytes were incubated with either 5 µl PB or VS for various time intervals at 25ºC. Following incubation, the hemocytes were stained with 0.5 µl of Giemsa stain (Nice, India) and incubated for 5 sec. Hemocytes counts were performed using a Neubauer haemocytometer under a light microscope (Everest Optic, Binocular Spectrum-S2, India) and total hemocytes were calculated using the method of Jones (1962).

Hemocyte aggregation assay

The ability of saliva to prevent aggregation by plasmatocytes and granular cells was examined using the procedure described by Richard and Dani (2008). One hundred microliters of hemocyte suspension in anticoagulation solution was pipetted into each well of a 96-well plate (Himedia, India) so that the hemocyte concentration was approximately $2x10^5$ cells/ well. Assays were initiated by adding 20 µl aliquots of different saliva concentrations (prepared in PB) to the hemocyte suspensions and

incubating at 20 °C and 65% RH for up to 2 h. After the incubation period, cells were stained with 0.15% (w/v) Commassive brilliant blue G250 (HiMedia, Mumbai, India), followed by destaining in 50% (v/v) methanol and 10% (v/v) acetic acid. The hemocyte monolayers were observed by phase contrast microscopy (Olympus CX41, Japan) at a magnification of 10x and 40x. Cell images were captured digitally using a Nikon D200 camera. Three replications were made for each treatment at each time point.

Hemocytes spreading assay

A modified version of the method described by Yu *et al.* (2007) was used to assess the impact of VS on prey hemocyte spreading behavior. Hemocytes were seeded into 96-well plates as detailed for the aggregation assays to yield a cell concentration of $1x10^5$ cells/ml. A one µl venom sample diluted to 100-fold was added to a 50-µl hemocyte suspension, which was transferred by pipetting into each well of a 96-well plate containing 199 µl PBS with 10% bovine serum albumin per well. Hemocytes were incubated with either PB or VS at 20 °C and 65% RH for up to 2 h. After incubation, the cells were stained with 0.15% (w/v) Coomassie brilliant blue G250 and then destained using 50% (v/v) methanol and 10% (v/v) acetic acid. Stained hemocytes were observed by phase contrast microscopy (Olympus CX41, Japan) at a magnification of 10x and 40x. Plasmatocytes (PC) and granular cells (GC) were identified and spreading characterized using the criteria of Gupta (1979) using three randomly chosen fields of view at 40x magnification. Approximately 80 cells were counted in each field of view.

The spreading percentage and spreading inhibitory ratio of PL and GC, respectively (Sahayaraj and Muthukumar, 2011) were calculated as follows:

% Spreading= (No. of spreading PL or GC observed/Total No. of spreading and non-spreading PL or GC observed)x100

Spreading inhibitory ratio=[(% of spreading of PL or GC in controls - % of spreading of PL or GC with venom treatment)/% spreading of PC or GC in controls]x100

Five wells were evaluated for each venom concentration in three replicates.

Statistical analysis

Aggregation and spreading behavior of hemocytes were subjected to Student‘t’ tests with alpha = 0.05. Total hemocyte counts were compared using one way ANOVA and DMRT post hoc test (SPSS statistical package v.11.5).

RESULTS

Hemocyte types

In *Spodoptera litura*, at the normal condition, the hemolymph clumped together by the adhesive nature. This results in the attachment of the cell and aggregates to form a pack of cells (Fig. 1a), which has been inhibited by the VS (Fig. 1b). When the exposure of VS reacts with the aggregation of hemocytes which may lead to the disintegration

of hemocytes which was observed during the incubation periods (Fig. 1b). The VS reacted with the hemocytes and removed the adhesive nature of hemocytes. When considering the morphology of the circulating hemocytes such as plasmatocytes (PL) and granulocytes (GC), it was observed that they were much more affected by the VS addition. The PL was the major one affected by the VS showing a filopodial like projections (Fig. 1c). Where else, a capsule like formation (Figs. 1d, e) was observed on the outer surface of the PL and it was enlarged, which leads to the bursting of the cell and causes the cell death (Figs. 1f, g). Other than the PL, GC was also affected by the VS which may cause a pore formation on the outer cell wall followed by the oozing out of the inner cellular contents and lead to the cell death (Fig. 1h).

In *H. armigera*, the formation of aggregation is very dense (Fig. 2a), which has been inhibited by the VS (Fig. 2b). This may be due to the sticky nature of the hemolymph. The addition of VS may lead to the disintegration of hemocytes. The spreading of hemocytes was very low when compared to that of the hemocytes of *S. litura*. The morphological features of the circulating hemocytes such as PL and GC were also having their significant changes were observed. Generally, the PL was spindle shaped (Fig. 2c). The addition of VS had taken its action on the morphology and lead to the disintegration of the PL morphology was observed showing the deformed conditions of the PL (Figs. 2d, e). Simultaneously, the GC were also being affected due to the VS showing the projections on the cell surface (Fig. 2f), formation of pore and oozing of cytoplasmic inclusions (Fig. 2g) and also the filopodial like structure projected on the outer surface of GC (Fig. 2h).

Total hemocyte counts

When hemocyte suspensions in anticoagulant solution were exposed to PB or untreated, a small but statistically insignificant reduction in total hemocytes was observed by one hour post-treatment for the cells of *S. litura* (Fig. 3; $F=14.03$; $df=1,4$; $p>0.05$) and *H. armigera* (Fig. 4; $F=225.63$; $df=1,4$, $p>0.05$). By contrast, when hemocytes from either species were incubated with VS, the total cell population for *S. litura* (Fig. 3) and *H. armigera* (Fig. 4) gradually declined in a concentration and time dependent manner. The decrease in hemocyte numbers when incubated with saliva appeared to be due to cell death as there was evidence of cell debris and hemocytes undergoing hemolysis which were found to evident from the Fig. 1 and Fig. 2. Cytolysis was evident for hemocytes from both species of prey tested.

Hemocytes aggregation assay

When hemocytes from *S. litura* were plated in 96-well plates in anticoagulant buffer, more than 65% of plasmatocytes and granular cells formed aggregations by 30 min (Fig. 5). Additional incubation up to 2 h did not increase the incidence of plasmatocyte aggregations but did for granular cells (Fig. 5). By contrast, incubation of either hemocyte type with VS resulted in fewer cell aggregations. This reduction in hemocyte aggregations was dependent on saliva concentration, but not length of exposure (Fig. 5). The ability to aggregate was not restored in plasmatocytes or granular cells when saliva was removed from the culture media (data not shown).

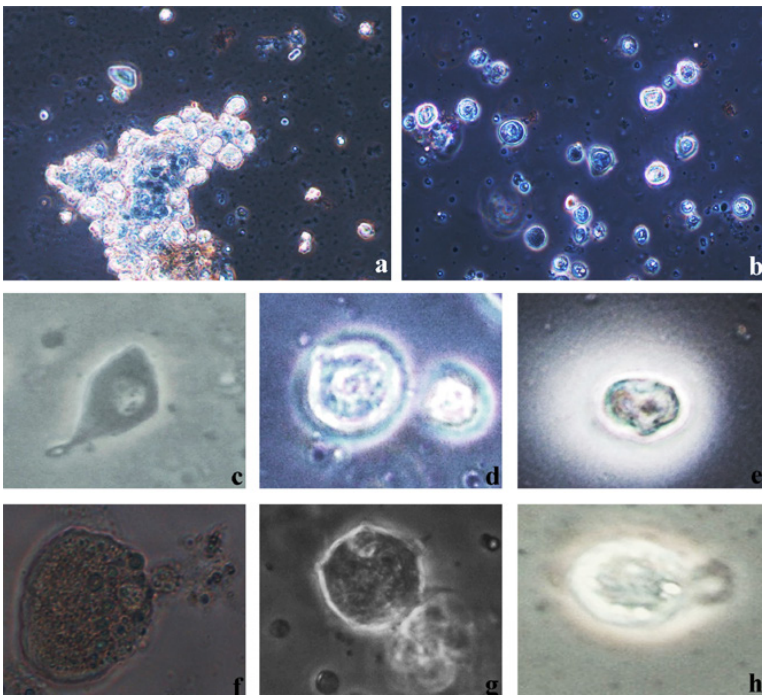

Fig. 1. Phase contrast micrographs of *Spodoptera litura* hemocytes (40x and 100x) exposed to the venomous saliva of *Rhynocoris fuscipes* and subsequently stained with Commassive Brilliant Blue G250. Aggregated (a) and spreaded hemocytes (b), also note the presence of deformed cytoplasmic extrusions (c), and capsule like formation (d, e). Note the damage on the outer cell membrane of hemocytes (f, g) and presence of elongation of capsule (h).

In parallel experiments, hemocytes from *H. armigera* were treated with either VS or PB. Hemocytes exposed to PB behaved similarly as observed for *S. litura*, the majority of plasmatocytes and granular cells formed aggregations within 30 min after seeded into 96-well plates. However, longer incubation periods did not yield additional increases in the percentage of cells forming aggregations (Fig. 5). Also similar to hemocytes from *S. litura*, incubation with increasing concentration of VS decreased the ability of plasmatocytes and granular cells to form aggregations, but longer exposure to saliva did not result in further suppression of aggregation (Fig. 5). Inhibition of hemocyte aggregation was maintained even if VS was removed from the media (data not shown).

Hemocytes spreading assay

Similar to hemocyte aggregation behavior, venomous saliva from *R. fuscipes* inhibited hemocyte spreading of both species in a concentration and time dependent manner (Fig. 6). There were insignificant differences between species in terms of the responses of plasmatocytes ($F=2.98$; $df=1,22$; $p<0.05$) and granular cells (F=

2.77; dF=1,22; $p<0.05$) to VS in terms of concentration or time dependent effects. Hemocytes did not regain the ability to spread before cell death.

Within 30 min after spreading, venom-treated granular cells from *S. litura* begin to show signs of injury in the form of invaginations along the plasma membrane (Fig. 1f). Degranulation of these cells followed, presumably due to loss of membrane integrity as ultimately granular cells died by cytolysis (Fig. 1h). Plasmatocytes responded to venom exposure by rounding (Fig. 1a), forming blebs on the plasma membrane (Fig. 1f), swelling (Fig. 1e) and then the plasma membrane lysed (Fig. 1f). The morphological changes were evident in plasmatocytes within 30 min after addition of saliva and the majority of the cells were dead by 2 h post-treatment.

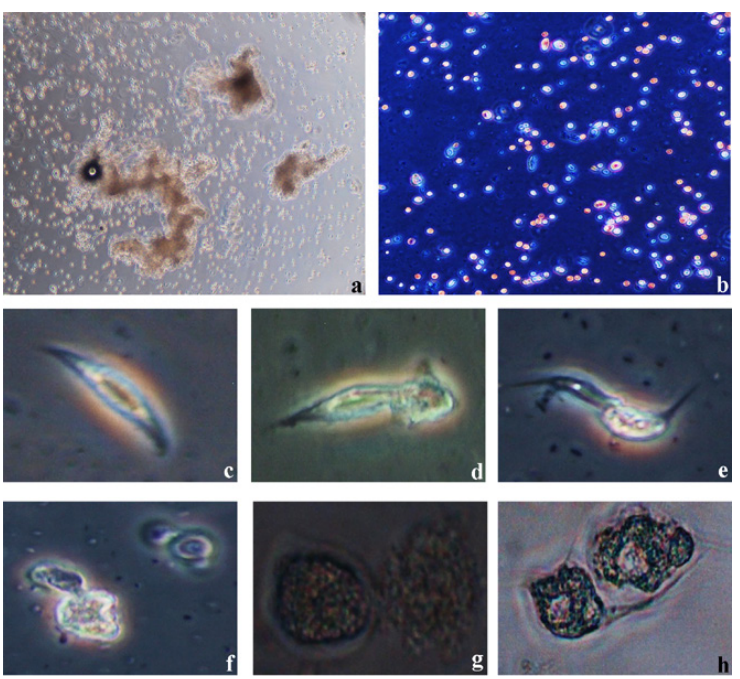

Fig. 2. Phase contrast micrographs of *Helicoverpa armigera* hemocytes (40x and 100x) exposed to the venomous saliva of *Rhynocoris fuscipes* stained with Commassive Brilliant blue (G250). Aggregated (a) and spreaded hemocytes (b), normal plasmatocyte (c), deformed plasmatocytes (d, e). The presence of deformed granulocytes (f), oozing out of cellular contents (g) and filopodial elongation (h).

Granular cells from *H. armigera* displayed a similar sensitivity to salivary venom as they displayed rupturing of the plasma membrane (Fig. 2a) within 2 h following treatment, with the cells reforming closed membranes. This yielded hemocytes with amorphous cell shapes and numerous invaginations of the plasma membrane (Fig. 2c). Plasmatocytes appeared to be injured by a mechanism that did not yield rounded or swollen cells (Figs. 2d, e). Several venom-treated plasmatocytes were observed to lyse with no evidence of swelling. Simultaneously as like *S. litura*, the granulocytes also found to be get damaged due to the venomous saliva having invaginations which

lead to the ooze out of the cellular contents which were shown in the Figs. 2f, g and finally looks its deformed condition lead to the cell death (Fig. 2h)

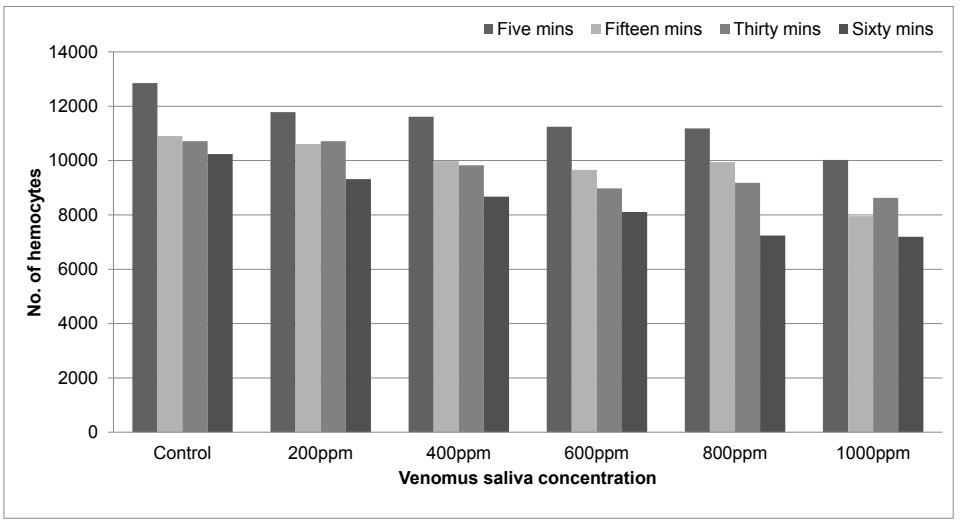

Fig. 3. Box plot represents the total hemocyte count (x 10^5 cells/ml) of *S. litura* fifth instar larvae treated with various concentrations of *R. fuscipes* venomous saliva. Hemocytes counted after 5, 15, 30 and 60 mins of incubation at 25 °C with venomous saliva of different concentrations.

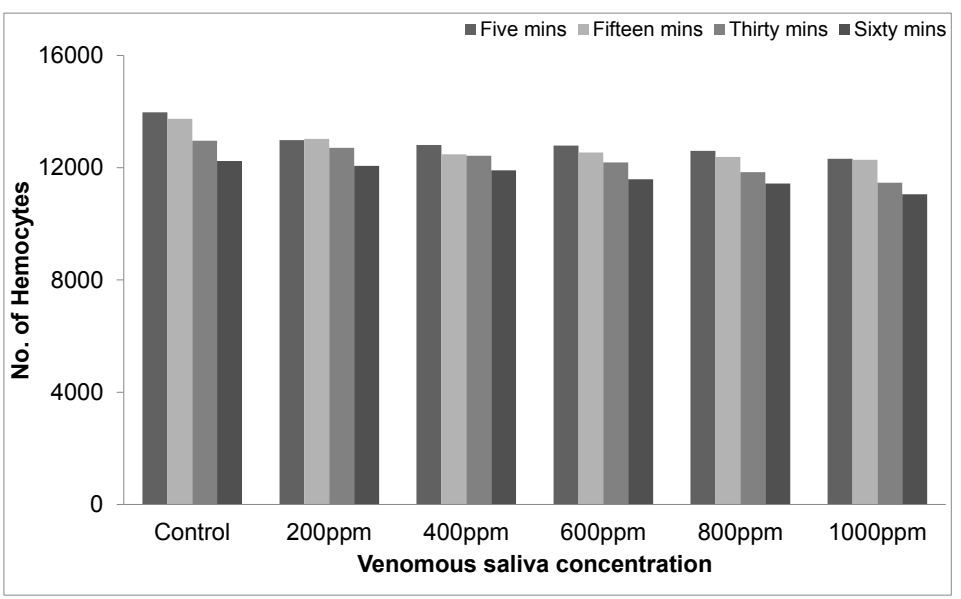

Fig. 4. Box plot represents the total hemocyte count (x 105 cells/ml) of *H. armigera* fifth instar larvae treated with various concentrations of *R. fuscipes* venomous saliva. Hemocytes counted after 5, 15, 30 and 60 mins of incubation at 25°C with venomous saliva of different concentrations.

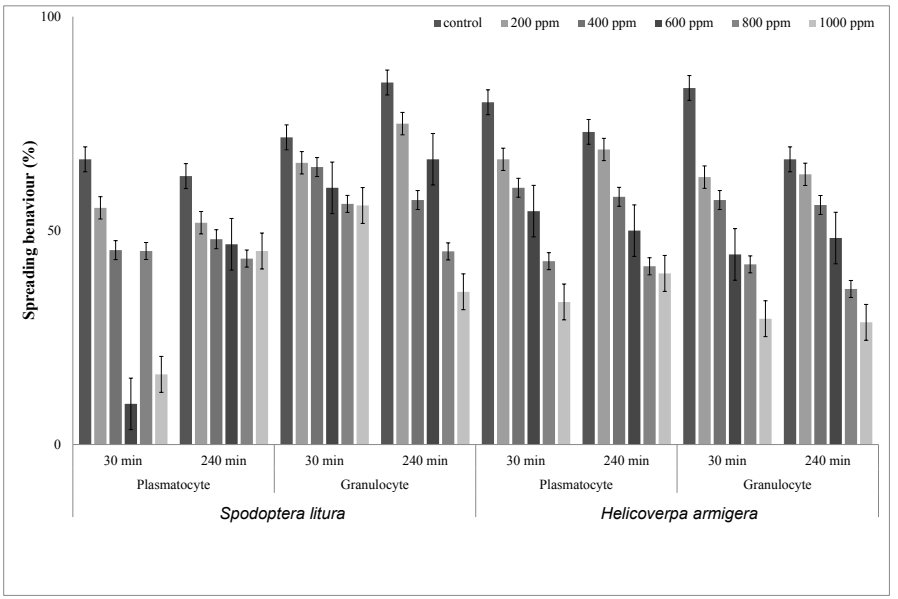

Fig. 5. Aggregation of hemocytes of *Spodoptera litura* and *Helicoverpa armigera* on addition of venomous saliva of *Rhynocoris fuscipes* during the 30 min and 240 min incubation periods. Error bars refer to standard error mean (SEM).

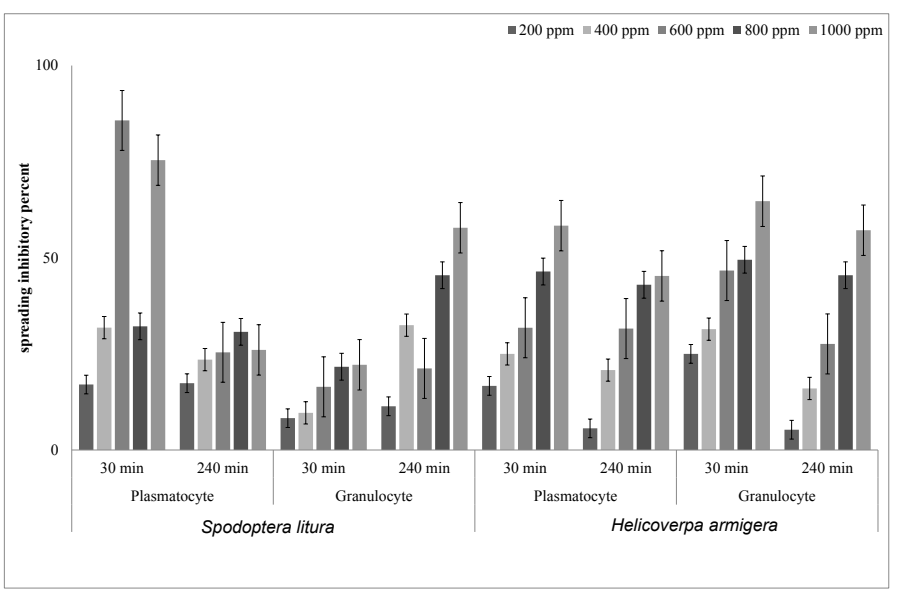

Fig. 6. Spreading behavior of the plasmatocytes and granulocyte of *Spodoptera litura* and *Helicoverpa armigera* on addition of venomous saliva of *Rhynocoris fuscipes* during the 30 min and 240 min incubation periods. Error bars refer to SEM.

DISCUSSION

Predatory reduviids like *R. fuscipes* attack larval stages of various lepidopterans to use as a source of prey. The fact that the prey are mobile poses a potential injury threat and mortality risk to adult reduviids, and thus, such predators often rely on salivary venoms to subdue the food (Sahayaraj and Muthukumar, 2011). *Rhynocoris fuscipes* produces venomous saliva, that when injected into larvae of *S. litura*, *H. armigera*, and several other species of lepidopterans cause the prey to become sessile and are eventually consumed following the digestive action of enzymes pumped into larvae with the saliva.

Sahayaraj and Muthukumar (2011) demonstrated that saliva from the closely related reduviid *R. marginatus* also induces immunosuppression in their prey, apparently by targeting larval plasmatocytes and granular cells. In this study, we demonstrated that VS from *R. fuscipes* has a similar effect on hemocytes from both *S. litura* and *H. armigera*: the ability of plasmatocytes and granular cells to form aggregations and spread were inhibited in a concentration and time dependent manner. The inhibition was permanent as both cell types died following exposure to saliva if the concentration was sufficiently high. The fact that venomous saliva did not induce death non-discriminately and targeted prey hemocytes within minutes of injection argues that suppression of key cellular-mediated prey defenses is a necessary first step in subduing the food source prior to consumption.

Saliva induced reductions in hemocyte cell numbers and inhibition of aggregation and spreading is consistent with the action of venoms from several species of endo and ectoparasitic wasps that must suppress the host immune system to ensure either successful development of progeny (endoparasitic species) and/or host feeding by adults (ectoparasitoids) (Beckage, 1998; Richards and Dani, 2008; Richards and Edwards, 1999). The latter situation would also be especially critical to fluid feeding reduviids that need to obtain prey hemolymph without disruption by host cellular immune responses (Cohen, 1990). Injection of saliva into prey larvae appears to target the two main hemocyte types (plasmatocytes and granular cells) involved in wound healing, hemolymph precipitation, and cell recruitment (Lavine and Strand, 2002; Jiravanichpaisal *et al.,* 2006).

The fact that these cells did not recover after exposure to saliva and other cell types did not appear affected by salivary toxins suggests that plasmatocytes and granular cells are specifically targeted and that the mode of action of venomous saliva is receptor-mediated rather than non-discriminate. Further investigation is needed to determine which constituents in saliva are responsible for immunosuppression and to decipher their mode of action.

The permanent suppression of hemocyte immune responses was evident by the induction of cell death. Plasmatocytes and granular cells from *S. litura*, as well as granular cells from *H. armigera*, displayed morphological changes following incubation with saliva that were consistent with oncosis, a form of cell death typically characterized by a loss of membrane integrity leading to swelling and ultimately lysis.

Retraction of pseudopodia and cell rounding were evident within 30 min, and swelling and lysis occurred by 2 h post-treatment with saliva. Hemolytic activity has been reported for numerous parasitic wasp venoms (Asgari and Rivers, 2011; Rivers *et al.,* 2009; Er *et al.,* 2011) and has been speculated to occur via receptor regulated signal transduction pathways rather than non-discriminate pore formation (Asgari and Rivers, 2011).

The precise death pathways targeted by saliva have not been examined but operate by mechanisms similar to parasitic wasp venoms. Interestingly, plasmatocytes from *H. armigera* died but apparently by a pathway unique from the other hemocytes examined. Hemolysis was induced but the cells did not round or swell prior to lysis. This cell morphology is more consistent with a non- apoptotic form of programmed cell death rather than oncosis. The observations also suggest that saliva from *R. fuscipes* contains multiple agents capable of inducing multiple forms of cell death. Such speculation has also been indicated for venoms from endo and ectoparasitic wasps (Asgari and Rivers, 2011; Rivers *et al.,* 2010).

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REFERENCES

Ahmed, K., Khalique, F. Malik, B. A., 1998, Modified artificial diet for mass rearing of chickpea pod borer, *Helicoverpa armigera* (H.). *Pakistan Journal of Biological Science,* 1: 183-187.

Altuntaş, H., Kiliç, A. Y., Zeytinoğlu, H. S., 2010, The effects of parasitism by the ectoparasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) on host hemolymph proteins in the Mediterranean lour moth *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae). *Turkish Journal of Zoology,* 34(3): 409-416.

Ambrose, D. P., 1988, *Biological control of insect pests by augmenting assassin bugs (Insecta: Heteroptera: Reduviidae)*. *In:* Ananthasubramanian, K. S., Venkatesan, P., Sivaraman, S. (Eds.). Bicoyas. Loyola College, Madras, 25-40.

Asgari, S., Rivers, D. B., 2011, Venom proteins from endoparasitoid wasps and their role in host-parasite interactions. *Annual Review of Entomology,* 56: 313-335.

Beckage, N. E., 1998, Modulation of immune responses to parasitoids by polydnaviruses. *Parasitology,* 116: 57-64.

Cai, J., Ye, G., Hu, C., 2004, Parasitism of *Pieris rapae* (Lepidoptera, Pieridae) by a pupal endoparasitoid, *Pteromalus puparum* (Hymenoptera, Pteromalidae), effects of parasitization and venom on host hemocytes. *Journal of Insect Physiology,* 50(4): 315-322.

Cohen, A. C., 1990, Feeding adaptations of some predaceous heteropterans. *Annals of Entomological Society of America,* 83(6): 1215-1223.

Cohen, A. C., 2000, Feeding fitness and quality of domesticated and feral predators, effects of long-term rearing on artificial diet. *Biological Control,* 17(1): 50-54.

Corzo, G., Adachi-Akahane, S., Nagao, T., Kusui, Y., Nakajima, T., 2001, Novel peptides from assassin bugs (Hemiptera: Reduviidae): isolation, chemical and biological characterization. *FEBS Letters* 499(3): 256-261.

Er, A., Uckan, F., Rivers, D. B., Sak, O., 2011, Cytotoxic effects of parasitism and application of venom from the endoparasitoid *Pimpla turionellae* on hemocytes of the host *Galleria mellonella*. *Journal of Applied Entomology,* 135(3): 225-236.

Gillespie, J. P., Kanost, M. R., Trenczek, T., 1997, Biological mediators of insect immunity. *Annual Review of Entomology,* 42(1): 611-643.

Gordon, D., Savarin, P., Gurevitz, M., Zinn-Justin, S., 1998, Functional anatomy of scorpion toxins affecting sodium channels. *Toxin Review,* 17(2): 131-159.

Gupta, A. P., 1979, *Insect Hemocytes, Development, Forms, Functions and Techniques*. Cambridge University Press, London, Cambridge, UK, 614 pp.

Haridass, E. T., Ananthakrishnan, T. N., 1981, Functional morphology of salivary system in some Reduviidae (Insecta: Heteroptera). *Proceedings of Indian Academy of Science,* 90(2): 145-160.

Jackson, H., Parks, T. N., 1989, Spider toxins, recent applications in neurobiology. *Annual Review of Neuroscience,* 12(1): 405-414.

Jiravanichpaisal, P., Lee, B. L., Söderhall, K., 2006, Cell-mediated immunity in arthropods, hematopoiesis, coagulation, melanization and opsonization. *Immunobiology,* 211(4): 213-236.

Jones, J. A., 1962, A phase contrast study of the blood cells of the adult cockroach *Periplaneta americana. Analytical Research,* 128: 571.

Lavine, M. D., Strand, M. R., 2002, Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology,* 32: 1295-1309.

Li, Q., Alan Sun, Y., Wang, G., ShengLiu, X., 2009, Effects of the mermithid nematode *Ovomermis sinensis* on the hemocytes of its host *Helicoverpa armigera. Journal of Insect Physiology,* 55(1): 47-50.

Malarvannan, S., Giridharan, S., Sekar, S., Prabavathy, V. R., Sudha, N., 2008, Bioefficacy of crude and fraction of *Argemone mexicana* against tobacco caterpillar, *Spodoptera litura* Fab. (Noctuidae: Lepidoptera). *Journal of Biopesticides,* 1: 55-62.

Marris, G. C., Bell, A. B., Naylor, J. M., Edwards, J. P., 1999, The role of *Pimpla hypochondriaca venom* in the suppression of pupal Noctuid host immunity. *Entomologica Expermentalis et Applicata,* 93(3): 289-296.

Morrison, N. M., 1989, Gel electrophoretic studies with reference to functional morphology of the salivary glands of *Acanthaspis pedestris* Stål. (Insecta: Heteroptera: Reduviidae). *Proceedings of Indian Academy of Science,* 98(3): 167-173.

Muthukumar, S., 2011, *Rhynocoris marginatus (Fab.) Salivary Venom Characterization Andits Zootoxic Effects on Spodoptera litura (Fab.).* Ph. D. Thesis, Manonmanium Sundaranar University, Tirunelveli, India, 15-42.

Parkinson, N. M., Richards, E. H., Conyers, C., Smith, I., Edwards, J. P., 2002, Analysis of venom constituents from the parasitoid wasp *Pimpla hypochondriaca* and cloning of cDNA encoding a venom protein. *Insect Biochemistry and Molecular Biology,* 32(7): 729-735.

Ranga Rao, G. V., Wightman, J. A., Ranga Rao, D. V., Rameswar Rao, V., Hall, D. R., 1993, Optimization of Pheromone traps for the groundnut leaf miner, *Aproarema modicella* (Deventer) (Lepidoptera, Gelechiidae). *Journal of Plant Protection Tropics,* 10: 260-266.

Rash, L. D., Hodgson, E. C., 2002, Pharmacology and biochemistry of spider venoms. *Toxicon,* 40(3): 225-254.

Richards, E. H., Edwards, J. P., 1999, Parasitization of *Lacanobia oleracea* (Lepidoptera: Noctuidae) by the ectoparasitic wasp, *Eulophus pennicornis*, effects of parasitization, venom and starvation on host hemocytes. *Journal of Insect Physiology,* 45: 1073-1083.

Richards, E. H., Dani, M. P., 2008, Biochemical isolation of an insect hemocyte anti-aggregation protein from the venom of the endoparasitic wasp, *Pimpla hypochondriaca,* and identification of its gene. *Journal of Insect Physiology,* 54(6): 1041-1049.

Richards, E. H., Parkinson, N. M., 2000, Venom from the endoparasitic wasp *Pimpla hypochondriaca* adversely affects the morphology, viability, and immune function of hemocytes from larvae of the tomato moth, *Lacanobia oleracea*. *Journal of Invertebrate Pathology,* 76(1): 33-42.

Rivers, D. B. Uckan, F., Ergin, E., Keefer, D. A., 2010, Pathological and ultra-structural changes in cultured cells induced by venom from the ectoparasitic wasp *Nasonia vitripennis* (walker) (Hymenoptera: Pteromalidae). *Journal of Insect Physiology,* 56: 1935-1948.

Rivers, D. R., Dani, M. P., Richards, E. V., 2009, The mode of action of venom from the endoparasitic wasp *Pimpla hypochondriaca* (Hymenoptera: Ichneumonidae) involves Ca^{2} -dependent cell death pathways. *Archives of Insect Biochemistry and Physiology,* 71(3): 173-190.

Rosales, C., 2011, Phagocytosis, a cellular immune response in insects. *Insect Survey Journal,* 8: 109-131.

Sahayaraj, K., Muthukumar, S., 2011, Zootoxic effects of reduviid *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) venomous saliva on *Spodoptera litura* (Fab.). *Toxicon,* 58(5): 415-425

Sahayaraj, K., Vinoth Kanna, A., 2009, Starvation impact on venom quantity of a reduviid predator, *Catamirus brevipennis* (Servile) (Hemiptera: Reduviidae). *Entomon,* 34(2): 119-121.

Sahayaraj, K., Kanna, A. V., Kumar, S. M., 2010, Gross morphology of feeding canal, salivary apparatus and digestive enzymes of salivary gland of *Catamirus brevipennis (*Servile) (Hemiptera: Reduviidae). *Journal of Entomological Research Society,* 12: 37-50.

Sahayaraj, K., Kumar, M. S., Prem Anandh, G., 2006, Evaluation of milking and electric shock methods for venom collection from hunter reduviids. *Entomon,* 31(1): 65-68.

Sahayaraj, K., Sathyamoorthi, P., Rajamanickam, K., 2001, Impact of different rearing media on *Corcyra cephalonica* Stainton biology and life table. *Shashpa,* 8(2): 125-130.

Srinivasamurthy, K., Jalai, S. K., Venkatasan, T., 2006, Development of *Spodoptera exigua* (Lepidoptera, Noctuidae) on a semi synthetic diet. *Indian Journal of Plant Protection,* 34(2): 250-251.

Strand, M. R., Pech, L. L., 1995, *Microplitis demolitor* polydnavirus induces apoptosis of a specific hemocyte morphotype in *Pseudoplusis includens*. *Journal of General Virology,* 76: 283-291.

Yu, R., Chen, Y., Chen, X., Huang, F., Lou, Y., Liu, S., 2007, Effects of venom/calyx fluid from the endoparasitic wasp, *Cotesia plutellae* on the hemocytes of its host, *Plutella xylostella* in *in vitro. Journal of Insect Physiology,* 53(1): 22-29.

Zhang, Z., Ye, G. Y., Cai, J., Hu, C., 2005, Comparative venom toxicity between *Pteromalus puparum* and *Nasonia vitripennis* (Hymenoptera, Pteromalidae) toward the hemocytes of their natural hosts, nontarget insects and cultured insect cells. *Toxicon,* 46(3): 337-349.

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