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VENOMOUS SALIVA OF NON-HAEMATOPHAGOUS REDUVIID BUGS (HETEROPTERA: REDUVIIDAE): A REVIEW

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ABSTRACT

While reduviids are a modestly well characterized group of insects, especially the blood sucking triatominae due to the medical implications of the Chagas disease, which is mainly transmitted by the infected bugs whose excrement contains *Trypanosoma cruzi* that enters the body through bruises or cuts in the skin of humans, their non-haematophagus counterparts are a forgotten lot and have not been thoroughly investigated. The venom in the saliva of the non-haematophagus reduviids has come into the spotlight in the last couple of decades due to the voracious predatory lifestyle that enable them to be used as biological control agents in subduing pests. But the biochemistry of reduviid venom, its action and subsequent effect on the prey, toxicity, enzymes, peptides present in the venom and their significance, the role of extra oral digestion facilitated by the venom for its predatory lifestyle have not been given much consideration. This review aims to summarize the existing body of literature regarding the venomous saliva of non-haematophagous reduviid bugs for the first time.

Key words : reduviid bugs, enzymes, peptides, toxin, venomous saliva.

INTRODUCTION

Reduviid bugs constitute one of the largest and most successful groups of predacious insects, cosmopolitan in occurrence with approximately 6800 species (Hwang and Weirauch, 2012; Maldonado, 1990) and ecological specializations with diverse, highly evolved prey capture strategies (Soley *et al.,* 2011; Jacobson, 1911; Zhang and Weirauch, 2011; Wignall and Taylor, 2011; Ferero *et al.,* 2011) and the description and redescription of many species is being constantly incorporated at the species, generic and subfamily levels to the already existing exhaustive list (Ambrose, 1999; 2004). Most assassin bugs exhibit generalist predation wherein they prey on other arthropods, while others show specialist predation by preferring to feed on a certain group of prey organisms such as termites, ants and diplopods (Jacobson, 1911; Louis, 1974; Cobben, 1978; Weirauch and Cassis, 2006, McMahan, 1983a; 1983b; Miller, 1953). The salivary secretions of these bugs play a pivotal role in feeding as they can only ingest liquid food. It is at this juncture that the saliva begins to assay the function of a venom thus enabling reduviids to extensively exploit their predatory behavior and evolve innovative methods of predation (Edwards, 1961). Hence, the saliva of reduviid bugs is often called venomous saliva.

Venoms are natural toxins with a cocktail of complex compounds that can serve as a great

source of novel bioactive substances with unrealized potential application in the field of drug discovery, medicine and agriculture. Besides the extensive work on the blood sucking triatominae [Anderson *et al.,* 2003; Amino *et al.,* 2002; Noeske-Jungblut *et al.,* 1994; Goodchild, 1955; Wigglesworth, 1943; Schofield, 2000; Teo and Cheah, 1973; Schofield, 1994; Sandoval *et al.,* 2000; Ryckman, 1951; Patterson, 1999; Miles, 1981; Lent and Wygodzinsky, 1979; Guerenstein and Guerin, 2001], the venomous saliva of predatory reduviid bugs has not been given due consideration.

Studies on the biochemistry and composition of reduviid bug venoms are meager and have been confined to the following species: *Acanthaspis pedestris* Stal [Morrison, 1989], *Peirates turpis* Walker, *Agriosphodrus dohrni* Stal and *Isyndus obscurus* Dallas [Gerardo *et al.,* 2001], *Peirates affinis* Serville [Edwards, 1960], *Platymeris rhadamanthus* Gerstaecker [Edwards, 1961], *Holotrichius innesi* Horrvath [Zerarchia *et al.,* 1973], *Zelus renardii* Kolenati [Cohen, 1993], *Haematorrhophus nigroviolaceus* Reuter [Haridass amd Ananthakrishnan, 1981], *Catamirus brevipennis* Serville [Sahayaraj *et al.,* 2007] and *Rhynocoris marginatus* Fabricius [Sahayaraj *et al.,* 2013]. It is the purpose of this paper to review and summarize the existing body of literature on the venomous saliva of non-haematophagous reduviid bugs.

REVIEW

Effect of venomous saliva on the prey:

Reduviid bugs have an elongated head with a distinct narrow neck, long legs and a prominent segmented rostrum for feeding (Hilty, 2013; Ambrose, 1999). After capturing the prey, these bugs use the long rostrum to inject toxic saliva that liquefies the insides of the prey which are later sucked out (Jacobson, 1911; Edwards, 1961; Sahayaraj, 1994; Haridass and Ananthakrishnan, 1980; Cohen, 1990). The bite of the reduviid bug causes intense localized pain and swelling and leaves a long-standing discolored or blackened pit at the point of insertion of the rostral stylets. The dried saliva powder of these bugs exhibits an irritant activity with respect to eye and nose membranes, and induces oedema, vasodilation, increased mucous secretion and respiratory disturbances similar to those caused by snake venom and aids in the initiation of various allergic reactions (Stanic, 1956). The saliva which enters the body of the prey after the painful bite contains enzymes that digest the tissues the predators swallow, a process known as extra-oral digestion. This phenomenon is found to be ecologically important as it allows relatively small predators to consume large prey that cannot be swallowed or ingested as a whole. This characteristic of the saliva renders the bug highly effective at killing prey much larger than itself (Haridass and Ananthakrishnan, 1980; Cohen, 1990).

The venomous saliva paralyzes the prey within a short duration of time, after which the bug uses its fore legs to hold the prey and suck its bodily juices. The first instar larva of *Rhinocoris carmelita* Stal is able to paralyze a final instar larva of *Ephestia kuehniella*, over 400 times its own weight within a limited duration of 10 seconds and *Platymeris rhadamanthus* Gerstaecker immobilizes the cockroach *Periplaneta americana* Linnaeus within 3-5 seconds and stalls its struggling (Edwards, 1961). In the case of successful insertion of rostral stylets and injection of salivary toxins, larger prey such as millipedes, caterpillars and beetles become completely paralyzed within 20-30 seconds (Haridass, 1985). In view of the rapid neurotoxin induced death of the prey as seen in the above mentioned examples, it is interesting to note the observations made by Sahayaraj and Vinothkanna, 2011, wherein they state that reduviid bug venoms cause long term, non lethal paralytic effects on their prey.

The salivary fluids are usually injected into the prey, with the favored site of injection being the legs, neck and antennae (Haridass and Ananthakrishnan, 1980; Cohen, 1990). The movement of the prey's stylet causes the mixing of the prey tissue and the predator's saliva after which the mixture is sucked out. This behavior was termed as lacerate-and-flush feeding by Miles, 1972. This technique enables the predators to intake large amounts of digestible

prey material without ingesting non-digestible matter. Very often, only a portion of the nutritional parts of the prey are ingested, a process referred to as partial consumption of prey, commonly observed in various heteropterans including reduviid bugs (Lucas, 1985). Although the prey are only partially consumed, these predators are highly efficient at prey extraction, ingesting more than 80% of the prey nutrients and absorbing more than 90% of what they ingest (Cohen, 1989). Thus, due to external digestion facilitated by the toxic saliva, removal of 40 to 60% of the prey's live weight, at the rate of 1.5 to 2 mg per minute is effected (Edwards, 1961) with one complete feeding session with an immobilized prey lasting for about 90-120 minutes (Haridass and Ananthakrishnan, 1980; Cohen, 1990). Injecting venom into the prey, which is a process of prey preparation, expands the predatory scope or the effective size range of prey that can be exploited (Hespenheide, 1973). This renders reduviid bugs as ideal candidates for biological control agents in places where a wide variety of insect pests need to be subdued. Their distinct character of indiscriminate killing wherein they kill more prey than they need to satiate themselves also reinstates their yet to be tapped, immense potential as effective biological control agents.

Collection of venomous saliva from Reduviid bugs:

Edwards in 1961 successfully used the method of intimidation with an unknown object to extract venom. He used a seeker to molest the insect by tapping its thorax through the break in the petri dish. This induced the bug to spit saliva from its rostrum onto the glass above. This saliva when dry is scraped from the surface using a steel blade. Venom was also obtained by holding the abdomen of the predator between the thumb and the index finger and by gently pressing the abdomen. This action stimulates the insect to eject venom which is then collected in a capillary tube that was inserted in the rostral tip. The insect on subjection to an electric stimulus of about 180-230 volts also ejects venom (Sahayaraj *et al.,* 2006). The whole body extracts of the insect and dissection of venom or salivary gland also yields venom. But these methods are rarely used as they give a very crude preparation (Ambrose and Maran, 1999). The salivary secretions can also be gathered by placing the labium of the predator in a capillary tube and injecting the insect with 2 µl of a 0.5% solution of pilocarpine which causes excessive and spontaneous salivation (Cohen, 1990).

Role of enzymes in EOD:

Digestion, in non-haematophagous reduviids is a highly efficient process that is categorized into two phases: pre-oral digestion that takes place outside the body and actual digestion in the gut of the insects. The process begins with pre-oral digestion of the prey's internal structures whose venom enabled liquefaction permits ingestion followed by further digestive processing of prey within the predators gut. In reduviids and all true bugs, enzymes which are produced in specialized glands are forced into the prey and ingested into the predator's gut, where they remain until digestion is complete. This pre-oral digestion is a form of food preparation (Kaspari, 1990) that helps in the intake and eventual consumption of prey which are too large to digest. Nutrient intake in the form of EOD is a cyclical and incremental process, i.e. after the prey has been brought under the control of the predator, a series of injections of digestive fluids in successive bouts interspersed by regular intervals is pumped into the prey followed by a mechanical pause after which ingestion of the disgorged fluids and portions of the liquefied prey take place (Cohen, 1993). This method of cycling increases the efficiency of the process of digestion by maximizing the concentration of hydrolytic enzymes in proportion to the volume of prey to be liquefied (Cohen, 1984, 1989; Baptist, 1941).

EOD is completely facilitated by digestive enzymes but the origin of these enzymes has been a matter of debate for many years. Even though studies have reported the presence of proteinase activity in the salivary secretions of many insects including reduviids (Edwards, 1961; Rastogi, 1962; Rees and Offord, 1969) there is a general view that salivary secretions are of have no significance outside of the predators' digestive system. Baptist in 1941,

recorded the presence of proteinase in the salivary secretions of predaceous heteropterans, but claimed that these enzymes worked too slowly to be of any importance. Many including Law *et al.,* 1977 have expressed doubt and concern in relation to the salivary glands being considered as the source of proteolytic enzymes injected by carnivorous insects into their prey. These doubts may have arisen from the fact that in certain predatory arthropods that use pre-oral digestion, like the carabid beetles, the source of enzymes is the gut and not the salivary glands (Cheeseman and Gillott, 1987). The work done by Cohen in 1990, wherein radio labelled inulin was used to trace the origin of the enzymes, demonstrated that the gut was not the source of disgorged digestive enzymes. Moreover, a large amount of identified routine digestive enzymes that were present in the guts of the predators never reached the prey. Preliminary work on the digestive enzyme elastase, an alkaline proteinase that complements the actions of trypsin and chymotrypsin, also reveals that reduviid predators have this enzyme present in their salivary system (Cohen, 1998). In addition, Haridass and Ananthakrishnan in 1981, dissected the anterior and posterior lobes of the salivary glands from certain specific reduviid bugs such as *Haematorrhophus nigroviolaceus* Reuter, *Guionius nigripennis* Fabricius, *Ectrychotes pilicornis* Fabricius, *Pirates affinis* Serville, *Ectomocoris vishnu* Distant, *Catamiarus brevipennis* Serville, *Triatoma rubrofasciata* De Geer, *Linshcosteus costalis* Ghouri, *Acanthaspis siva* Distant, *Acanthaspis pedestris* Stal, *Acanthaspis quinquespinosa* Fabricius, *Lizarda annulosa* Stal, *Petalochirus indicus* Reuter, *Rhaphidosoma atkinsoni* Bergroth, *Sycanus collaris* Fabricius, *Sphedenolestes bowringi* Distant and tested the efficacy of their homogenates on prospective prey. Immediate and rapid paralysis of the prey proves the zootoxic effects of the salivary secretions. These experiments clearly show that the gut is not the source of digestive enzymes injected into the prey which facilitate external digestion. In fact, not all secretions of the salivary gland are toxic. The anterior lobe secretes neurotoxic substances, while the posterior lobes secrete digestive enzymes. The accessory glands present in the salivary gland complex function as water recapturing agents (Miles, 1972; Goodchild, 1966). The enzyme profile of some reduviid bug salivary secretions is given in table 1.

The biochemical differences between venoms and digestive secretions are difficult to resolve, especially since many venoms are seen to originate from digestive system structures (Scmidt, 1982). The most abundant enzymes that were present in the salivary secretion include proteinases, amylase which hydrolyzes starch to form maltose and is useful in the digestion of glycogen, invertase which hydrolyses sucrose to form fructose and glucose, lipase which splits fats into fatty acids and glycerol, pepsin and trypsin which break down complex proteins into peptones (Swingle, 1925). Proteinases are the most important liquefaction enzymes for predators (Cohen, 1993; Miles, 1972; Rees and Offord, 1969). Proteinases are classified into endopeptidases and exopeptidases. Endopeptidases attack protein molecules from within, reducing insoluble structures into water-soluble subunits. Trypsin like enzymes attack proteins at their basic amino acid sites, cleaving the proteins at lysine and arginine residues (Law *et al.,* 1977). Liquefaction results from the endopeptidase activity of the saliva. Chymotrypsin like enzymes attack proteins at their aromatic sites (Gilmour, 1961, Cohen, 1993). Phospholipase found in the saliva digests phospholipids in cell membranes, disrupting neurons and muscle cells (Schmidt, 1982). Hyaluronidase is a well known spreading factor for venoms that aids in quick paralysis of the prey (Mommsen, 1978; Foelix, 1982; Schmidt, 1982).

The enzymes are an indispensable resource that must be given approximate time to liquefy prey and must be recovered for further use in the gut if the predator is to exploit their full value. These enzymes cannot be immediately replaced if are lost or emitted unnecessarily. The specificity of these enzymes determines that only certain structures are liquefied and made available for ingestion. As a direct resultant action of the selective nature of these enzymes, the predator ingests only the inner contents of the prey and

thus, they have the advantage of selecting nutrient rich food unburdened by indigestible and potentially damaging cuticular structures (Hespenheide, 1973).

Peptides in reduviid bug saliva:

Reduviid bug venomous saliva is known to contain a complex mixture of proteins (Morrison, 1989; Maran, 2000) peptides

Table 1. Enzyme profile of reduviid bug saliva

	Platymeris rhadamanthus	*Rhynocoris marginatus*	*Catamiarus brevepennis*	*Zelus renardii*	*Sinea confusa*
Proteinase	+	+	+	+	+
Hyaluronidase	+	+	+	*	*
Lipase	-	+	+	+	+
Esterase	-	+	+	*	*
Phospholipase	+	+	+	+	+
Adenosine triphosphate	+	+	+	+	+
Amylase	+	+	+	+	+
Invertase	+	+	+	*	*
Trypsin	+	+	+	*	*
Pepsin	+	+	+	*	*
Acid phosphatase	+	+	+	*	*

+ Present; - Absent; * Not known

Table 2. Toxicity of reduviid bug salivary gland homogenates

Species	**Prey**	**Action**
Platymeris rhadamanthus	*Periplaneta americana*	Immediate cessation in systole; general contracture
Pirates affinis	*Omphora pilosa*	Immediate stoppage of all body movements and total paralysis
Rhynocoris carmelia	*Ephestia kuhniella*	Immediate cessation in systole; general contracture
Reduvius personatus	*Periplaneta americana*	Immediate cessation in systole; general contracture
Naucoris cimicoides	*Periplaneta americana*	Immediate cessation in systole; general contracture
Oncopeltus fasciatus	*Periplaneta americana*	Slow decrease in amplitude; slight increase in rate, cessation after some minutes
Pentatoma rufipes	*Periplaneta americana*	Slow decrease in amplitude; slight increase in rate, cessation after some minutes
Haematorrhophus nigroviolaceus	*Xenobolus carnifex*	Quick stoppage of antennal and leg movements and total paralysis
Platymeris rhadamanthus	*Periplaneta americana*	Immediate cessation in systole; general contracture
Pirates affinis	*Omphora pilosa*	Immediate stoppage of all body movements and total paralysis
Rhynocoris carmelia	*Ephestia kuhniella*	Immediate cessation in systole; general contracture

(Gerardo *et al.*, 2001) and enzymes (Sahayaraj *et al.*, 2007; 2011; Edwards, 1961). MALDI-TOF screening of the salivary secretion of the reduviid bugs *Peirates turpis, Agriosphodrus dohrni* Stal and *Isyndus obscurus* Dallas, followed by HPLC fractionation yielded components having molecular mass from 2 kDa to 30 kDa. Upon further purification, 3 peptides designated as Ptu1, Ado1 and Iob1 with molecular masses of 3615.3, 3781.2 and 3938.6 Da were identified from *Peirates turpis, Agriosphodrus dohrni* Stal and *Isyndus obscurus* Dallas respectively. Although the three peptides were from different reduviid bugs, their amino acid sequence motif was well conserved with some point mutations and was relatively homologus to the Conotoxins (Gerardo *et al.*, 2001).

Conotoxins are venom ejected by *Conus* species to help hunt their prey. A single injection can cause the fish to be immobilized within 1 or 2 seconds. Total paralysis was effected a few seconds later. It has been observed that the biologically active small peptides in the venom contribute to its potency (Olivera *et al.*, 1991). These paralytic elements could well be those responsible for the action of reduviid venom. Similarly mass spectrometric analysis of *Rhynocoris marginatus* saliva contained components with molecular masses ranging from 3 kDa to 50 kDa. Three peptides namely RmIT-1, RmIT-2 and RmIT-3 with a molecular mass of 3.79, 7.5 and 10.94 kDa were identified (Sahayaraj *et al.,* 2013). Peptides identified from *Rhynocoris fuscipes* include RfIT1 and RfIT2 with 2358 and 3423 Da molecular mass respectively (Sahayaraj, 2013).

pH of venomous saliva:

The freshly secreted venomous saliva of the reduviid bugs has pH ranging from 6.6 to 6.8 with that of adult females slightly alkaline when isolated from recently prey fed individuals. However, the saliva is found to become gradually neutral after three days of food deprivation and continues to become more acidic with increasing length of the starvation period (Sahayaraj *et al.,* 2013; Edwards, 1961). The pH of the prey was found to be between 7.0 to 7.2 during the initial stages of feeding and continued to increase till 8.5 after which it kept fluctuating till the end of feeding but never touched below 7.5 (Cohen, 1993).

Action of venomous saliva on whole animals, organs and tissues:

The venomous saliva of reduviid bugs have proved to be toxic to a wide range of insects representing seven orders but are immune to their own species. Application of saliva obtained from the adult insects on the heart of the younger instars of the same species show no marked alteration in their rhythmic contractions. 5 to 15 % of the saliva application on the heart dorsum of an arthropod prey brings about immediate cessation of the systolic movements followed by a general contracture. 5 to 10 % of saliva when applied on the abdominal nerve causes an increase in electrical activity for a few seconds which terminates abruptly after which the nerve cord ceases to conduct. With regard to muscles, the saliva caused an immediate strong coiling followed by slow uncoiling with concurrent lysis of tubule cells at higher concentrations. The contractions become irregular, with movements eventually ceasing either in the coiled or extended state.

The lytic activity of the venomous saliva is highly pronounced with breakdown of fat bodies being the first effect after the paralysis of the prey. Major changes in the appearance and mechanical properties of the tissue are apparent and observable within a short time of immersion in insect saliva (Smith and Wigglesworth, 1959; Wigglesworth, 1957). The responses of innervated and non-innervated muscle, intact and isolated nerve to treatment with the venomous saliva indicate that the mechanism of paralysis does not involve a specific site of action. Rather, it has been observed that the saliva attacks and disrupts the cell membranes on which the functioning of the excitable tissue depends. The subsequent lysis that occurs is an extension of the initial membrane breakdown that causes paralysis. In other words, the paralysis caused by the reduviid bug saliva is a special function of external digestion (Edwards, 1961).

Toxicity of reduviid bug venomous saliva:

The salivary system of reduviid bugs is very complex (Southwood, 1955, Haridass, 1978; Baptist, 1941). The anterior lobes of the main glands are concerned with the secretion of neurotoxic substances involved in the paralysis and death of the prey while the posterior lobes secrete the digestive enzymes. The venomous saliva in the dried state retains toxicity for at least three years but declines slowly in potency in aqueous solution. The saliva of *Pirates affinis* and *Haematorrhophus nigroviolaceus* exhibit pronounced zootoxic effects. The salivary gland homogenates of *P. affinis* and *H. nigroviolaceus* cause total paralysis and complete stoppage of all twitching movements of the carabid beetle in 12-16 seconds. Similar effects were seen with the homogenates of *H. nigroviolaceus* on the movements of the millipede in 48-52 seconds (Haridass and Ananthakrishnanm 1981). In table 2, the toxicity of some bugs and their resultant effect on the prey are tabulated.

C. brevepennis venom shows antibacterial activity against the pathogens *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Proteus vulgaris, Enterobacter aerogenes, Staphylococus aureus, Bacillus sphaericus* and *Salmonella typhimurium,* while *R. marginatus* venom shows the same activity against only *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Streptococcus pyogenes* and *Salmonella typhimurium* (Sahayaraj *et al.,* 2006). The saliva of *R. fuscipes* was found to be venomous to *Helicoverpa armigera* and *Spodoptera litura* when the larvae were treated orally or by injection. It has also been noted that the crude venom has more impact than purified peptides and that the toxic nature of venomous saliva is due to its protein content (Sahayaraj and Vinothkanna, 2011). The protein content of the saliva varies with respect to males and females. In *R. marginatus*, the protein content was 1.16 ± 0.03 mg/100 mg body weight for males and 0.92 ± 0.02 mg/100 mg body weight for females (Sahayaraj *et al.*, 2013). This implies the existence of difference in salivary production and action with respect to male and female reduviid bugs. Records of the female bug paralyzing the prey more rapidly than the male reinstates the fact that the toxicity of the saliva is also seen to vary in adult male and female insects (Sahayaraj *et al.*, 2007).

CONCLUSION

Reduviid bug salivary venom has long been hypothesized to facilitate external digestion following the immobilization of the prey. Research developments have clearly established that the enzymes that bring about external digestion are being produced and secreted from the lobes of the salivary gland rather than the gut. Evidence for the digestive role of the venoms is provided by the extensive tissue damage that occurs in the prey after a bite. Thus it can be stated that either salivary venoms were evolutionarily selected to complement the digestive process or normal saliva could have evolved into its venomous counterpart as an adaptation towards a more successful predatory technique or prey capture strategy. Either way, the venomous saliva in non-haematophagous reduviid bugs is proving to be highly effective in using these bugs as biocontrol agents (Anand *et al.,* 2010; Imamura *et al.,* 2008; George and Ambrose, 2001; Claver *et al.,* 2002; 2003; Grundy, 2007; Vennison and Ambrose, 1992; Wignall and Taylor, 2011; Edwards, 1962; Nagarajan and Ambrose, 2013; Ambrose and Kumaraswami, 1990; Lakkundi, 1989; Evangelin *et al.,* 2012; Claver *et al.,* 2004; Rocha and Redaelli, 2004). While extra oral digestion employed by the reduviid bugs increases the maximum size of the prey that a given predator can handle, it does not compromise the predator's ability to handle prey at the smaller end of its prey range (Nentwig and Wissel, 1986). Due to this typical characteristic, reduviids may not be useful as predators on specific pests, but are valuable predators in situations where a variety of insect pests occur (Schaefer, 1988). They can be efficiently mass reared and disseminated in the pest infested fields with ease. This process can also be suitably customized as per individual requirements and incorporated into integrated pest management strategies. With their large and diverse size range in addition with their

fascinating specialized habits aided by venomous saliva, reduviid bugs have considerable but unrealized potential as biological control agents.

Despite the apparent lack of literature on the venomous saliva of non-haematophagus reduviid bugs, we should be extremely cautious with claims regarding their primary biological utility and applicative purposes. More work along these lines can open up new avenues aimed at understanding the toxinology and evolutionary aspects of predatory venoms and can effectively aid in developing novel agrochemicals and pharmaceuticals.

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REFERENCES

1. **Ambrose, D.P.** (1999). Assassin bugs. New Delhi, India: Oxford and IBH Publ. Co. Pvt. Ltd.
2. **Ambrose, D.P.** (2004). The status of biosystematics of Indian Reduviidae (Hemiptera: Heteroptera). In: Perspectives on biosystematics and biodiversity. Rajmohana, K., Sudheer, K., Girish, P., Kumar, Santhosh, S., (Eds.). Harvest Media Services, Calicut, 441-459.
3. **Ambrose, D.P. and Kumaraswami, N.S.** (1990). Functional response of the reduviid predator *Rhinocoris marginatus* Fabr. on the cotton stainer *Dysdercus cingulatus* Fabr. Journal of Biological Control. 4(1): 22-24.
4. **Ambrose, D.P. and Maran, S.P.M.** (1999). Quantification of protein content and paralytic potential of saliva of fed and prey deprived reduviid Acanthapsis pedestris Stal. (Heteroptera: Reduviidae: Reduviinae). Indian Journal of Environmental Science. 3(1): 11-16.
5. **Amino, R., Martins, R.M., Procopio, J., Hirata, I.Y., Juliano, M.A. and Schenkman, S.** (2002). Trialysin, a Novel Pore-forming Protein from Saliva of Hematophagous Insects Activated by Limited Proteolysis. The Journal of Biological Chemistry. 277(8): 6207-6213.
6. **Anand, G.B., Rizwana, F.A. and Prakash, S.** (2010). Ecofriendly technology for the management of Brinjal pest using reduviids. International Journal on Applied Bioengineering. 4(2):15-18.
7. **Andersen, J.F., Francischetti, I.M.B., Jesus, G., Valenzuela, Schuck, P. and Ribeiro, J.M.C.** (2003). Inhibition of Hemostasis by a High Affinity Biogenic Amine-binding Protein from the Saliva of a Blood-feeding Insect. J. Biol. Chem. 278: 4611-4617.
8. **Baptist, B.A.** (1941). The morphology and physiology of the salivary glands of Hemiptera-Heteroptera. Quart. J. Micros. Sci. 83: 91-139.
9. **Cheeseman, M.T. and Gillott, C.** (1987). Organization of protein digestion in *Calosoma calidum* (Coleoptera: Carabidae). J. Insect Physiol. 33:1-8.
10. **Claver, M.A., Muthu, M.S.A., Ravichandran, B. and Ambrose, D.P.** (2004). Behaviour, prey preference and functional response of *Coranus spiniscutis* Reuter, a potential predator of tomato insect pests. Pest Management in Horticultural Ecosystems. 10:19-27.
11. **Claver, M.A., Ramasubbu, G., Ravichandran, B. and Ambrose, D.P.** (2002). Searching behaviour and functional response of *Rhynocoris longifrons* (Stål) (Heteroptera: Reduviidae), a key predator of pod sucking bug, *Clavigralla gibbosa* Spinola. Entomon. 27:339-346.
12. **Claver, M.A., Ravichandran, B., Khan, M.M. and Ambrose, D.P.** (2003). Impact of cypermethrin on the functional response, predatory and mating behaviour of a non-target potential biological control agent *Acanthaspis pedestris* (Stål) (Het., Reduviidae). Journal of Applied Entomology. 127:18-22.
13. **Cobben, R.H.** (1978). Evolutionary trends in Heteroptera: mouthparts, structure and feeding strategies. Mede, part 2.
14. **Cohen, A.C.** (1984). Food consumption, food utilization and metabolic rates of *Geocoris punctipes* (Het.: Lygaeidae) fed

Heliothis virescens (Lep.: Noctuidae) eggs. Entamophaga. 29: 361-367.
15. **Cohen, A.C.** (1989). Ingestion and food consumption efficiency in a predacious hemipteran. Ann. Entomol. Soc. Am. 82:495-499.
16. **Cohen, A.C.** (1990). Feeding adaptations of some predateous hemiptera. Ann. Entomol. Soc. Am. 83(6):1215-1223.
17. **Cohen, A.C.** (1993). Organization of digestion and preliminary characterization of salivary trypsin like enzymes in a predaceous heteropteran, *Zelus renardii.* J. Insect Physiol. 39: 823-829.
18. **Cohen, A.C.** (1998). Biochemical and morphological dynamics and predatory feeding habits in terrestrial heteroptera. In Predatory Feeding Habits in Terrestrial Heteroptera, J.R. Ruberson and M. Coll. (Ed.) Thomas say pubs., Phoenix, Arizona. 21-32.
19. **Edwards, J.S.** (1960). Spitting as a defensive mechanism in a predatory reduviid. In Proceeding of International Congress of Entomology, Vienna. 259-263.
20. **Edwards, J.S.** (1961). The action and composition of the saliva of an assassin bug *Platymeris rhadamanthus* Gaerst. (Hemiptera, Reduviidae). J. Exp. Biology. 38: 61-77.
21. **Edwards, J.S.** (1962). Observations on the development and predatory habit of two reduviid heteroptera, *Rhinocoris carmelita* Stäl and *Platymeris rhadamanthus* Gerst. In Proceedings of the Royal Entomological Society of London. Series A, General Entomology. 3(7): 89–98.
22. **Evangelin, G., Bertrand, H., Muthupandi, M. and John William.** (2012). Bioefficacy of *Rhynocoris kumarii* on the hemipteran pests of cotton (abstract). In Proceedings of the National conference on Climate change – a challenge to sustainable development, Andhra Pradesh, India, BEITR 22, 23.
23. **Foelix, R.F.** (1982). Biology of Spiders. Cambridge, MA: Harvard Univ. Press.
24. **Forero, D., Choe, D.H. and Weirauch, C.** (2011). Resin Gathering in Neotropical Resin Bugs (Insecta: Hemiptera: Reduviidae): Functional and Comparative Morphology. Journal of Morphology. 272: 204–229.
25. **George, P.J.E. and Ambrose, D.P.** (2001). Polymorphic adaptive insecticidal resistance i*n Rhynocoris marginatus* (Fabr.) (Het., Reduviidae) a non-target biocontrol agent. Journal of Applied Entomology. 125(4): 207-209.
26. **Gerardo, C., Salumi, A., Akahane, T.W., Yoshihisa, K. and Tomni, W.** (2001). Novel peptides from assassin bugs (Hemiptera: Reduviidae): isolation, chemical and biological characterization. FEBS Lett. 499: 256-261.
27. **Gilmour, D.** (1961). The biochemistry of insects. New York, Academic.
28. **Goodchild, A.J.P.** (1955). Some observations on growth and egg production of the blood-sucking reduviids, *Rhodnius proxilus* and T*riatoma infestans.* In Proceedings of the Royal Entomological Society of London. 30(10-12): 137–144.
29. **Goodchild, A.J.P.** (1966). Evolution of the alimentary canal in the hemiptera. Biol. Rev. 41: 97-140.
30. **Grundy, P.R.** (2007). Utilizing the assassin bug, *Pristhesancus plagipennis* (Hemiptera: Reduviidae), as a biological control agent within an integrated pest management programme for Helicoverpa spp. (Lepidoptera: Noctuidae) and Creontiades spp. (Hemiptera: Miridae) in cotton. Bull Entomol Res. 97(3): 281-90.
31. **Guerenstein, P.G. and Guerin, P.M.** (2001). Olfactory and behavioural responses of the blood-sucking bug *Triatoma infestans* to odours of vertebrate hosts. The Journal of Experimental Biology. 204: 585–597.
32. **Haridass, E.T.** (1978). Biological and ethological studies on some South Indian Reduviids (Hemiptera:Reduviidae). Ph.D. thesis. University of Madras, India.
33. **Haridass, E.T.** (1985). Feeding and ovipositional behavior in some reduviids (Insecta- Heteroptera). In Proc. Indian Acad. Sci. (Animal. Sci.) 94:239-247.
34. **Haridass, E.T. and Ananthakrishnan, T.N.** (1980). Models for the predatory behavior of some reduviids from Southern India (Insecta-

Heteroptera-Reduviidae). In Proc. Indian Acad. Sci. (Animal. Sci.). 89: 387-402.
35. **Haridass, E.T. and Ananthakrishnan, T.N.** (1981). Functional morphology of the salivary system in some Reduviidae (Insecta-Heteroptera). In Proc. Indian Acad. Sci. (Animal. Sci.). 90(2): 145-160.
36. **Hespenheide, H.A.** (1973). Ecological inferences from morphological data. Rev. Sys. Ecol. 4:213-299.
37. **Hilty, J.E.** (2013). Insect Visitors of Illinois Wildflowers. (illinoiswildflowers.info, version 03).
38. **Hwang, W.S. and Weirauch, C.** (2012). Evolutionary History of Assassin Bugs (Insecta: Hemiptera: Reduviidae): Insights from Divergence Dating and Ancestral State Reconstruction. PLoS ONE. 7 (9): (e45523. doi:10.1371/journal.pone.0045523).
39. **Imamura, T., Murata, M. and Miyanoshita, A.** (2008). Biological Aspects and Predatory Abilities of Hemipterans Attacking Stored-Product Insects. Japan Agricultural Research Quarterly. 42(1):1-6.
40. **Jacobson, E.** (1911). Biological notes on the hemipteron *Ptilocerus ochraceus.* Tijdschrift voor Entomologie. 54:175–179.
41. **Kaspari, M. (**1990). Prey preparation and determinants of handling time. Anim. Behav. 40: 118-126.
42. **Lakkundi, N.H.** (1989). Assessment Of Reduviids For Their Predation And Possibilities Of Their Utilization In Biological Control. Ph.D. thesis, IARI, Division of Entomology, New Delhi.
43. **Law, J.H., Dunn, P.E. and Kramer, K.J.** (1977). Insect proteases and peptidases. Adv. Enzymol. 45: 389-425.
44. **Lent, H. and Wygodzinsky, P.** (1979). Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas disease. Bul Am Mus Nat Histo. 163:123-520.
45. **Louis, D.** (1974). Biology of Reduviidae of Cocoa farms in Ghana American midi. Nature 91:68-89.
46. **Lucas, J.R.** (1985). Partial prey consumption by antlion larvae. Anim. Behav. 33:945-958.
47. **Maldonado, J.** (1990). Systemic catalouge of the Reduviidae of the world (Insecta: Heteroptera). Carribean. Special edition, university of Puerto Rico, Mayaguez, J. Sci. 694.
48. **Maran, P.M.** (2000). Chosen reduviid predators-prey interaction: nutritional and pheromonal chemical ecology (Insecta:Heteroptera: Reduviidae). Ph.D. thesis. Manonmanium Sundaranar University, Department of Zoology, India.
49. **McMahan, E.A.** (1983a). Adaptations, feeding preferences, and biometrics of a termite-baiting assassin bug (Hemiptera, Reduviidae). Annals of the Entomological Society of America. 76:483–486.
50. **McMahan, E.A.** (1983b). Bugs angle for termites. Natural History. 92: 40-47.
51. **Miles, M.A., Souza de, A.A. and Povoa, M.** (1981). Chagas disease in the Amazon basin. III. Ecotopes of ten triatomine bug species (Hemiptera: Reduviidae) from the vicinity of Belém, Pará State, Brazil. J Med Entomol. 18: 266-278.
52. **Miles, P.W.** (1972). The saliva of Hemiptera. Adv. Insect Physiol. 9:183-256.
53. **Miller, N.C.E.** (1953). Notes on the biology of the Reduviidae of Southern Rhodesia. Trans Zool Soc London. 27: 541–672.
54. **Mommsen, T.P.** (1978). Digestive enzymes of a spider (*Tegenaria atricia* Koch), Digestion of proteins. Comp. Biochem. Physiol. 60(A): 371-375.
55. **Morrison, N.M.** (1989). Gel electrophoretic studies with reference to functional morphology of the salivary glands of *Acanthaspis pedestris* Stal. (Insecta: Heteroptera: Reduviidae). In Proc. Indian Acad. Sci. Anim. Sci. 98:167-73.
56. **Nagarajan, K. and Ambrose, D.P.** (2013). Chemically Mediated Prey-Approaching Behaviour of the Reduviid Predator *Rhynocoris fuscipes* (Fabricius) (Insecta: Heteroptera: Reduviidae) by Y-arm Olfactometer. Pakistan Journal of Biological Sciences. 16: 1363-1367.
57. **Nentwig, W. and Wissel, C.** (1986). A comparison of prey lengths among spiders. Oecologica. 68: 595-600.

58. **Noeske-Jungblut, C., Kratzschmar, J., Haendler, B., Alagon, A., Possani, L., Verhallen, P., Donner, P. and Schleuning, W.D.** (1994). An Inhibitor of Collagen-induced Platelet Aggregation from the Saliva of *Triatoma pallidipennis*. The Journal of Biological Chemistry. 269(7)*:* 5050-5053.
59. **Olivera, B.M., Rivier, J., Scott, J.K., Hillyard, D.R. and Cruz, L.J.** (1991). Conotoxins. The Journal of Biological Chemistry. 266(33):22067-22070.
60. **Patterson, J.** (1999). A Morphometric Investigation of the Relationships between Triatoma rubrofasciata (Hemiptera: Reduviidae: Triatominae), Old World Triatoma and Representatives of the New World Species. Ph.D. thesis, LSHTM, London.
61. **Rastogi, S.C.** (1962). The salivary enzymes of some phytophagous and predaceous heteropterans. Sci. Cult. 28:479-480.
62. **Rees, A.R. and Offord, R.E.** (1969). Studies on the protease and other enzymes from venom of *Lethocerus cordofanus.* Nature. 221:665-667.
63. **Rocha, L. and Redaelli, L.R.** (2004). Functional response of *Cosmoclopius nigroannulatus* (Hem.: Reduviidae) to different densities of *Spartocera dentiventris* (Hem.: Coreidae) nymphae. Braz. J. Biol. 64(2):309-316.
64. **Ryckman, R.E.** (1951). Recent observations of cannibalism in *Triatoma* (Hemiptera: Reduviidae). J Parasitol. 37: 433-434.
65. **Sahayaraj, K.** (1994). Capturing success by reduviid predators *Rhinocoris kumarii* and *Rhinocoris marginatus* on different age groups of *Spodoptera litura*, a polyphagous pest (Heteroptera: Reduviidae). J. Ecobiol. 6(3): 221-224.
66. **Sahayaraj, K.** (2013). Threrapeutic biomolecules of venomous arthropods (abstract). Proc. Bioavailability and Bioequivalence: Pharmaceutical R&D Summit, Beijing, China.
67. **Sahayaraj, K. and Vinothkanna, A.** (2011). Insecticidal activity of venomous saliva from *Rhynocoris fuscipes* (Reduviidae) against *Spodoptera litura* and *Helicoverpa armigera* by microinjection and oral administration. The Journal of Venomous Animals and Toxins including Tropical Diseases. 17(4): 486-490.
68. **Sahayaraj, K., Borgio, J.F., Muthukumar, S. and Anandh G.P.** (2006). Antibacterial activity of *Rhynocoris marginatus* (fab.) and *Catamirus brevipennis* (serville) (hemiptera: reduviidae) venoms against human pathogens. J. Venom. Anim. Toxins incl. Trop. Dis. 12(3): 487-496.
69. **Sahayaraj, K., Kumara Sankaralinkam, S. and Balasubramaniam, R.** (2007). Prey influence on the salivary gland and gut enzymes qualitative profile of *Rhynocoris marginatus* (Fab.) and *Catamirus brevipennis* (Serville) (Heteropetera: Reduviidae). Journal of Entomology. 4(4):331-336.
70. **Sahayaraj, K., Muthukumar, S. and Anandh, G.P.** (2006). Evaluation of milking and electric shock methods for venom collection from hunter reduviids. Entomon. 31(1): 65-68.
71. **Sahayaraj, K., Muthukumar, S. and Rivers, D.** (2013). Biochemical and electrophoretic analyses of saliva from the predatory reduviid species *Rhynocoris marginatus* (Fab.). Acta Biochimica Polonica. 60(1): 91-97.
72. **Sandoval, C.M., Joya, M.I., Gutierez, R. and Angullo, V.M.** (2000). Cleptohaematophagy of the triatomine bug *Belminus herreri*. Med Vet Entomol. 14: 100-101.
73. **Schaefer, C.W.** (1988). Reduviidae (Hemiptera: Heteroptera) as agents of biological control. In Bicovas, K.S. Ananthasubramanian, P. Venkatesan and S. Sivaraman (Ed.), Loyola College, Madras. 27-33.
74. **Schmidt, J.O.** (1982). Biochemistry of insect venoms. Annu. Rev. Entomol. 27:239-268.
75. **Schofield, C.J.** (1994). *Triatominae - Biology & Control.* West Sussex, UK: Eurocommunica Publications;
76. **Schofield, C.J.** (2000). *Trypanosoma cruzi* - The Vector-parasite Paradox. Mem. Inst. Oswaldo Cruz, Rio de Janeiro. 95(4): 535-544.

77. **Smith, D.S. and Wigglesworth, V.B.** (1959). Collagen in the perilemma of insect nerve. Nature. 183: 127.
78. **Soley, F.G., Jackson, R.R. and Taylor, P.W.** (2011). Biology of *Stenolemus giraffa* (Hemiptera: Reduviidae), a web invading, araneophagic assassin bug from Australia. New Zealand Journal of Zoology. 38: 297–316.
79. **Southwood, T.R.E.** (1955). The morphology of the salivary glands of terrestrial Heteroptera (Geocorisae) and its bearing on classification. Tijdschr., Entomol. 98:77-84.
80. **Stanic, M.** (1956). Allergenic properties of venom hyper-sensitiveness in man and animals. In Venoms, Edited by Buckley, E.E. & Proges, N. Washington.
81. **Swingle, H.S.** (1925). Digestive enzymes of an insect. The Ohio Journal of Science. 25(5): 209-218.
82. **Teo, S.K. and Cheah, J.S.** (1973). Severe reaction to the bite of the triatomine bug (*Triatoma rubrofasciata*) in Singapore. J Trop Med Hyg. 76: 161-162.
83. **Vennison, S.J. and Ambrose, D.P.** (1992). Biology, Behaviour and Biocontrol Efficiency of a Reduviid Predator, *Sycanus reclinatus* Dohrn (Heteroptera: Reduviidae) from Southern India. Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologisches Museum und Institut für Spezielle Zoologie (Berlin). 68 (1):143–156.
84. **Weirauch, C. and Cassis, G.** (2006). Attracting ants: the trichome in Ptilocnemus lemur (Heteroptera: Reduviidae) and novel glandular area on the sternum. J. Ny Entomol. Soc. 114: 28-37.
85. **Wigglesworth, V.B.** (1943). The fate of haemoglobin in *Rhodnius prolixus* (Hemiptera) and other blood-sucking arthropods. In Proceedings of the Royal Entomological Society of London B. 131(865): 313-339.
86. **Wigglesworth, V.B.** (1957). The use of osmium in the fixation and staining of insects. In Proc. Roy. Soc. B. 147:185-199.
87. **Wignall, A.E. and Taylor, P.W.** (2011). Assassin bug uses aggressive mimicry to lure spider prey. In Proceedings of the Royal Society B-Biological Sciences. 278:1427–1433 (doi: 10.1098/rspb.2010.2060).
88. **Zerachia, T., Bergmann, F. and Shulov, A.** (1973). Pharmacological activities of the venom of the predaceous bug *Holotrichius innessi* (Heteroptera: Reduviidae). Anim. Plant toxins. 143-146.
89. **Zhang, G. and Weirauch, C.** (2011). Sticky predators: a comparative study of sticky glands in harpactorine assassin bugs (Insecta: Hemiptera: Reduviidae). Acta Zoologica (doi: 10.1111/j.1463-6395.2011.00522.x).

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