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Comparative Profile of Free Ions in Venom and Heamolymph of Citrus Spiders From Pakistan*

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Abstract.- Five most common families of spiders were selected for the determination of free ions in their venoms and hemolymphs or body fluids. Significant differences were found in ionic content of venoms of different families. Venom had higher concentration of K^+ than in the body fluid. Ca^{2+} and Na+ showed non-significant differences between venom and hemolymph. In some cases however, Na was lower in the venom than in the body fluids. These differences and variations were mostly species specific. Similarly Cl^- ion concentration was significantly (p>0.00) higher in the body fluids than that in the venoms. Among the two sexes females had lower concentrations of K^+ and Ca^{++} than their male counterpart. Females of the genus *Myrmarachne* had a low concentration of K^+ and Na^+ while *Sparassidae* sp. had the significantly highest K^+ content when compared with their males.

Keywords: Spider venom, body fluid, free ions, citrus orchards.

INTRODUCTION

Spider venoms are the most diverse and important due to their neurotoxic (Corzo *et al.,* 2000; Gwee *et al.,* 2002), cytotoxic (Corzo *et al.,* 2003**)** and necrotic (Richardson, 2007; Goddard, 2007) effects and have attracted great interest because of their potential applications in pharmaceutical, toxicological and biological aspects. Only a few species of spiders have been characterized for mineral components of venom and hemolymph or body fluid. Venoms and body fluids contain both neurotoxic and cytotoxic components which are linked with glucose, nucleic and free acids, inorganic ions such as Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- and neurotransmitters (Escoubas *et al.,* 2000; Wullschleger *et al*., 2005; Pukala *et al*., 2007). These ions play a vital role in prey immobilization and enhance venomous properties. Many studies have been done on the effect of spider venom on ion channels in victims (Vieira *et al*., 2007). The venom of Brazilian wondering spider *Phoneutria nigriventer* have various neurotoxins that are active in the presence of Na^+ (Matavel *et al*., 2002), K^+ (Carneiro *et al*., 2003) and Ca^{2+}, (Santos *et al*., 2002, 2006; Gomez *et al*., 2002) ion channels. Wullschleger *et al*. (2004) reported that the mortality rate of *Drosophila* and other test animals increased when neurotoxins CSTX-1, CSTX-9 or CSTX-13 were used in combination with KCl. These findings indicate that increased K^+ concentration in the venom showed synergistic effect in enhancing the potency of CSTX-13 venom of *Cupiennius salei* and facilitated the neurotoxic activity on *Drosophila* except in the case of Cupiennin-1a (Wullschleger *et al*., 2004). The high concentration of K^+ ion could also enhance nerve depolarization in order to affect Ca^{2+} channels that are inhibited by CSTX-1 (Elrick and Charlton, 1999). Marfa *et al*. (2001) suggested that α-LTX of BSWV might have both Ca^{2+} influx-dependent and independent actions with reference to toxin concentration and this additional component in whole BSWV stimulated Ca^{2+} independent effects on crustacean synapses.

Several workers (Davletov *et al*., 1995, 1996, 1998; Sugita *et al*., 1998; Lang *et al*., 1998; Volynski *et al*., 2004) investigated the role of Ca^{2+} ions of α-LTX and α-LCTX latrotoxins present in most of the black widow venoms. Moreover, K^+

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ions are abundant in venom and rare in hemolymph (Nentwig *et al*., 2004, 1994). Fresh spider hemolymph appears transparent bluish due to the presence of copper in respiratory pigment heamocyanin. Among the cations, Na^+ was predominant as compared to K^+, Ca^{2+}, Mg^{2+} and Cu^{2+} ions, while in anions, Cl^- was prevalent compared with PO_4^{2-} and SO_4^{2-}(Schartau and Leidescher, 1983). The concentration of K^+ in hemolymph and receptor-lymph was similar to some extent (Paul *et al*., 1994). Mostly in different spiders Ca^{2+} concentration of each species most probably depends on Na^+,K^+ and Mg^{2+} amount (Burton,1984).

Mineral composition of venoms and hemolymph of Pakistani spiders have rarely been explored. Therefore, there is a dire need to conduct research on characterization of spider venoms and body fluids. During present studies we investigated a complete profile of venoms and body fluids of a few locally common spider species for their ionic components.

MATERIALS AND METHODS

Fifty to hundred live specimens of each spiders species were captured from Citrus Orchard, Department of Horticulture and other orchards, brought to the Araneae Laboratory, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. Spiders were identified as *Elaver* sp. (n=762), *S. sarasinorum* Karsch (n=1934), *C. mildei* C. L. Koch (n=802), *T. imperialis* Rossi (n=2998), *M. japonica* Karsch (n=396), *M. maratha* Tikader (n=396), *M. orientales* Tikader (n=396), *M. bengalensis* Tikader (n=396), *M. laetus* Thorell (n=396), *P. workmani* Peckhman & Peckhman (n=1050), *P. paykulli* Audouin (n=595), *P. castriana* Grube (n=1277), *P. vercounda* Chamberlin & Gertisch (n=743), *H. kandiana* Pocock (n=2076), *O. lutescens* Thorell, *O. flavidus,* O. P. Cambridge and *O. giganticus* (n=3000). Females proved a potent source of venom and were subjected to venom collection. For future sampling these spiders were individually kept in cages under controlled conditions and used when needed. Samples were divided into venom and body fluid extracts and analyzed for the estimation of ion concentration.

Venom extraction

Live spiders were anesthetized with alcohol and fangs were simply brushed under microscope. Low voltage current was applied across the chelicerae and venom was collected directly into capillary jet from fangs as described earlier by Escoubas *et al.* (2000). For preparation of desired samples, 40-80 spiders were milked, aspirated by the capillary jet and transferred into a prior frozen buffer in micro-sampling vial. The venom yield per spider gland was measured in ppm.

Determination of free K^+, Na^+, Ca^{2+} and Cl^- ions

The concentration of K^+, Na^+, and Ca^{2+} ions were detected on flame photometer (Jenway PFP 7) while Cl^- was determined by titration (Bomsztyk *et al*., 1988).

RESULTS AND DISCUSSION

Comparative chemical quantification of these ions showed that venom samples had higher concentration of potassium ions as compared to those of calcium, sodium and chloride (Table I). In *P. workmani* calcium ions concentration was greater while it was lower in the rest of the species, *P. versicunda* showed higher concentration of sodium ions as compared to than present in rest of the species. Conversely, the haemolymph had lower concentration of potassium ions as compared to those of others. The comparison of ion concentration in males and females' samples indicated that calcium ions concentration was higher in the females as compared to the males. The pooled species of family *Sparassidae* had lower concentration of potassium ions while *P. versicunda* showed their higher concentration. The results for free ions indicated that Na^+ ($p \geq 0.377$), K^+ ($p \geq 0.439$) and Cl^- ($p \geq 0.751$) concentration was lower in the females as compared to their males, while the concentrations of Na^+, Ca^{2+} and Cl^- ions were lower in venoms as compared to the body fluids and vice versa. Similar trend in the concentration of Na^+, Ca^{2+} and K^+ has been observed previously by Nentwig *et al*. (2004). According to Schartau and Leidescher (1983) among the cations, Na^+ was predominant as compared to K^+, Ca^{2+}, Mg^{2+} and Cu^{2+} ions, while among anions, Cl^- was prevalent

Table I.- **A comparative profile of free ions (in ppm) Cl^-, Ca^{2+}, K^+, Na^+ from venom and heamolymph of citrus spiders.**

Species	Sex	Cl^- contents in		Ca^{2+} contents in		K^+ contents in		Na^+ contents in	
		Venom	Heamolymph	Venom	Heamolymph	Venom	Heamolymph	Venom	Heamolymph
Thyen imperialis	♀	76.02±64.65	103.28±25.67	0.01±0.02	0.04±0.03	0.39±0.21	0.03±0.02	3.25±0.43	3.21±2.49
	♂	8.05±7.48	119.66±39.58	0.10±0.01	0.04±0.03	0.63±0.28	0.03±0.02	3.25±0.4 3	.33±2.79
Cheiracanthium	♀	8.05±7.48	119.66±39.58	0.01±0.00	0.04±0.04	0.50±0.15	0.03±0.02	3.13±2.01	1.64±0.13
mildei	♂	59.80±44.46	120.37±38.36	0.03±0.04	0.05±0.04	0.43±0.17	0.03±0.02	6.14±4.03	5.02±5.66
Elaver sp.	♀	21.49±15.41	101.85±26.71	0.01±0.01	0.05±0.04	0.50±0.15	0.04±0.01	3.30±0.51	2.95±2.84
	♂	52.10±43.83	129.63±8.64	0.01±0.01	0.04±0.03	0.50±0.15	0.04±0.01	3.49±2.62	2.43±0.59
Stegodyphus sarasinorum	♀	5.91±5.29	99.71±48.08	0.00±0.00	0.25±0.37	0.23±0.08	0.04±0.01	0.31±0.0	63.35±2.73
Phidippus workmani	♀	61.39±40.69	65.53±19.74	0.00±0.00	0.03±0.02	0.23±0.08	0.05±0.03	8.87±7.58	5.63±7.42
Phintella sp.	♀	69.71±51.45	118.95±16.04	0.00±0.00	0.04±0.03	0.67±0.23	0.07±0.02	7.73±6.09	5.87±6.47
Plexippus paykulli	♀	41.17±33.43	93.30±30.24	0.00±0.00	0.06±0.04	0.67±0.23	0.05±0.03	3.36±2.46	3.84±2.78
Phintella castriesiana	♀	55.93±43.98	79.77±60.45	0.00±0.00	0.07±0.05	0.67±0.23	0.04±0.03	4.10±2.92	6.56±0.75
Myrmarachne spp. *	♀	27.60±22.61	95.44±28.45	0.00±0.00	0.02±.02	0.19±0.19	0.01±0.00	1.09±0.91	1.94±0.078
Sparassidae spp.*	♀	63.30±47.56	106.12±63.11	0.00±0.00	0.07±0.05	0.75±0.11	0.05±0.01	8.83±5.38	13.42±1.20
Phelegrina verecunda	♀	48.34±42.59	46.30±6.17	0.00±0.00	0.04±0.03	0.57±0.05	0.05±0.01	1.44±0.92	2.19±0.12
Average	♀	46.53±40.40	89.11±37.94	0.00±0.01	0.07±0.13	0.45±0.23	0.04±0.02	3.81±4.09	4.54±4.34
	♂	46.17±39.82	116.38±27.60	0.10±0.02	0.04±0.03	0.59±0.21	0.05±0.02	4.79±3.61	4.01±3.79

*Pooled species.

Table II.- **A subset of more interesting values.**

Ion	Species	Sex	Venom	Hemolymph
Cl^-	*Stegodyphus sarasinorum*	♀	5.91±5.29	99.71±48.08
Ca^{2+}	*Stegodyphus sarasinorum*	♀	0.00±0.00	0.25±0.37
	Sparassidae spp.*	♀	0.00±0.00	0.07±0.05
K^+	*Sparassidae* spp.*	♀	0.75±0.11	0.05±0.01
	Myrmarachne spp *	♀	0.19±0.19	0.01±0.00
Na^+	*Phidippus workmani*	♀	8.87±7.58	5.63±7.42
	Sparassidae spp.*	♀	8.83±5.38	13.42±1.20

*Pooled species.

with respect to PO_4 and SO_4^{2-}. Our results are in agreement to the result presented by other researchers (Carneiro *et al*., 2003; Santos *et al*., 2002; Gomez *et al*., 2002). The comparison between different spider species reflected some unexpected results as in case of *P. castriesiana,* the Na^+ ion concentration in venom was low while in body fluid it was high as compared to the results reported by Nentwig *et al*. (1994) who found higher concentration of K^+ (215 mM), Na^+ (8.9 mM) and Ca^{2+} (0.94 mM) in the venoms of *C. salei,* The low Ca^{2+} concentration and high K^+ concentration might be responsible for the stability and efficient functioning of the spider's venoms and may help them in dominance over prey. Moreover, K^+ ions are abundant in venoms and rare in haemolymph in other spiders as well (Nentwig *et al*., 1994) but the exact mechanism has not yet been investigated. *S. sarasinorum*, a social spider has extremly high concentration of Ca^{2+} ions that has been shown to be highly neurotoxic only in the presence of Ca^{2+} and immediately cause an effect on the nervous system of the victim (Table II).

It is concluded that venom samples possessed higher concentration of K^+ ions. Ca^{2+} ion was higher in females than that in male venoms. *P. versicunda* showed the highest concentration of Na^+. Only males of *Phenitella* showed the highest concentration of Cl^- followed by *P. versicunda* (Fig.1). This kind of study will help to deepen our insight in the screening of more potent venom for the development of anti venom drug that will be advantageous in the treatment of envenomation.

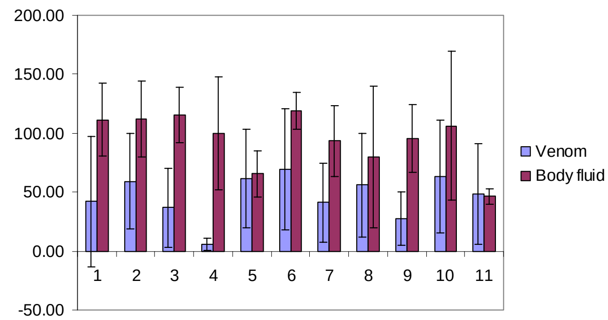

Fig. 1. Chloride ions concentration (both in venom and hemolymph of male and female)
1: *Thyen imperialis* 2: *Cheiracanthium mildei* 3: *Elaver* sp. 4: *Stegodyphus sarasinorum* 5: *Phidippus workmani* 6: *Phintella* sp. 7: *Plexippus paykulli* 8: *Phintella castriesiana* 9: *Myrmarachne* spp. 10: *Sparassidae* spp. 11: *Phelegrina verecunda*

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