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Insect-specific peptides in the venom of wolf spiders (Araneae: Lycosidae)

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Abstract: The venom of two dominant species of wolf spiders, *Pardosa sumatrana* Thorell and *Pardosa birmanica* Simon, was extracted and characterized. Insecticidal potential of crude venom and selected peptide fractions (i.e. 35-kDa fraction of both spiders) was evaluated in the laboratory using *Rhopalosiphum padi* (Linnaeus) (Homoptera: Aphididae) as a model pest. Results of the study showed that both the crude venom and the protein fractions caused significantly higher mortality in treated aphids compared to the control. It is concluded that both the crude venom and the protein fractions possess insecticidal potential.

Key words: Venom, peptides, spiders, aphids, insecticide

Spiders use their venom to kill or paralyze their prey (Corzo and Escoubas, 2003; Tedford et al., 2004). Their venoms contain salts, small organic molecules, peptides, and proteins (Schroeder et al., 2008). Low-molecular-weight peptides of their venom are categorized into three groups: cytolytic peptides, neurotoxic peptides of intermediate size (3–6 kDa), and polypeptides of large size (6–9 kDa) (King, 2004).

About 10 million biologically active peptides are predicted in spider venom (Saez et al., 2010), but only 800 are fully characterized, including 136 insect-selective peptides (Vetter et al., 2011). The protein fractions of spider venom could be a safer alternative to chemical pesticides (Leng et al., 2011). Peptides of spiders are highly specific against target insects (Glare et al., 2012). Utilization of spider venom as a biopesticide has been documented in the available literature (Nauen and Bretschneider, 2002; Qaim and Zilberman, 2003; Nicholson, 2007).

Wolf spiders are active and the most abundant ground spiders in agroecosystems. They are active hunters and use their venom as a chemical weapon to immobilize their prey. The venom of two wolf spiders, *Pardosa sumatrana* (Thorell, 1890) and *Pardosa birmanica* Simon, 1884, was partially characterized in the laboratory and insecticidal potential was evaluated using *Rhopalosiphum padi* (Linnaeus) (aphid) as a model pest.

Live spiders were captured with a suction device (Siemens VK 20C01) from citrus orchards of Sargodha, Punjab, Pakistan. The venom from spiders (n = 200 for each species) was collected according to the method described by Frontali et al. (1976). Crude venom was partially characterized following the methods described by Sambrook and Russell (2001) with a few modifications. Molecular weights of peptide fractions were determined by comparison with standard protein markers.

To evaluate insecticidal potential of crude venom and selected protein fractions of *P. birmanica* (35 kDa) and *Pardosa sumatrana* (35 kDa) we used *R. padi* (aphid) as a model pest. We used 35-kDa fractions for the bioassay experiment as this protein fraction is common in the venom of both spiders and shows prominent bands on gel. Bioassay experiments (three replicates) in the laboratory were performed according to the method described by Zahra (2015). The venom (0.5 µL, 0.75 µL, or 1 µL) was injected into the bodies of insects using a microinjector. Probit analysis was used to compute LT_{50} and LT_{95} values in MINITAB 13.3. LT_{50} and LT_{95} values were calculated only against the venom dose of 0.5 µL. Mortality data in the control and experimental groups were compared using one-way analysis of variance followed by Tukey's test (SPSS 13).

The venom of *P. birmanica* was resolved into five protein bands. The heaviest band was of 105 kDa, followed by 100 kDa, 95 kDa, 68 kDa, and 35 kDa. Among all bands, the band

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of 105 kDa was most prominent. The venom extracted from *P. sumatrana* yielded six protein bands (110 kDa, 95 kDa, 90 kDa, 80 kDa, 65 kDa, and 35 kDa). Bands of 110 kDa and 65 kDa were more prominent and broad among all bands, while bands of 80 kDa and 35 kDa were of less intensity.

Crude venom and protein fractions of both wolf spiders caused significantly higher mortality at 16 h after treatment than that in the control group (Figure; $F_{4,10} = 135$; $P < 0.001$). Results of Tukey's test showed that although the mortality in treated groups was higher than that in the control group, all treated groups differed nonsignificantly from each other. Calculated LT_{50} and LT_{95} values are given in the Table. LT_{50} and LT_{95} values for *R. padi* were 0.39 ± 0.0.06 and 0.49 ± 0.07, respectively.

In the present study we recorded protein bands that ranged from 110 to 35 kDa in the venom of two common wolf spiders (*P. birmanica* and *P. sumatrana*) of citrus orchards. Low-molecular-weight peptides of spider venom are target-specific and recommended as safer alternatives to chemical pesticides (Glare et al., 2012). Tahir et al. (2015) also recoded higher mortality in *Rhopalosiphum erysimi* against crude venom of *Odontobuthus odonturus* (Arachnida: Buthidae). Many researchers have reported the presence of low-molecular-weight compounds in spider venom (Schroeder et al., 2008). Chaim et al. (2011) recorded proteins of 5–40 kDa in brown spider (genus *Loxosceles*) venom. Similarly, Castro et al. (2004) reported three insecticidal toxins (LiTx1, LiTx2, and LiTx3) from the venom of *Loxosceles intermedia*.

Toxicity of venom from wolf spiders (Lycosidae) was examined by Quistad et al. (1992). Devaraja et al. (2010a, 2010b) isolated two serine proteases (16.3 and 28.7 kDa) from the lycosid *Hippasa agelenoides* that disturb hemostasis. Antipest effects of different peptide constituents of spider venom occur in several different ways. Delta-atracotoxin (delta-ACTX) was separated by Parodi et al. (2010) from Australian funnel-web spiders. It caused overstimulation, resulting in various muscular and heart problems. In the venom of black widow spiders a-latrotoxin (a-LTX) of

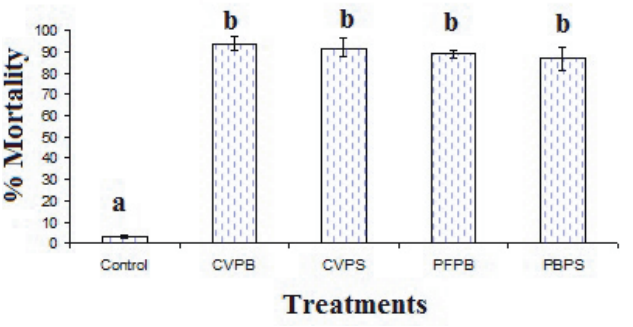

Figure. Percent mortality of *R. padi* treated with crude venom of *P. birmanica* (CVPB), crude venom of *P. sumatrana* (CVPS), protein fraction of *P. birmanica* venom (PFPB), and protein fraction of *P. sumatrana* venom (PFPS). Bars with the same letters represent nonsignificant differences.

molecular weight 132 kDa is present. In target organisms it changes in a way that causes increased permeability of membranes for Ca^{++} at nerve terminals, causing overstimulation resulting in many muscle disorders.

We performed bioassay tests in the laboratory against *R. padi* to assess the effectiveness of the crude venom and selected protein fractions extracted from *P. sumatrana* and *P. birmanica* on aphids. Many researchers have already indicated that spider venom is effective against insect pests. Richardson et al. (2006) in a study on house flies 72 h old evaluated toxic effects of every constituent peptide of spider venom. They observed that these proteins resulted in increased production of saliva, shivering, and immobility, which finally led to death.

In agroecosystems of Punjab, Pakistan, aphids represent one group of the most harmful pests that decrease the yield of crops (Irshad, 2001). Results of the present study clearly indicate that both the crude venom and protein fractions of the two selected spider species exhibit strong bioinsecticidal effects against *R. padi* (aphid).

Table. Calculated LT_{50} and LT_{95} values for *R. padi* exposed to crude venom and protein fractions of *P. birmanica* and *P. sumatrana*. Venom dose used was 0.5 µL.

Treatments	LT_{50} (confidence interval)	LT_{95} (confidence interval)
CVPB	2.61 ± 1.03 (–0.40 to 4.24)	11.74 ± 1.31 (9.77 to 15.75)
PFPB	1.79 ± 1.10 (–1.65 to 3.46)	9.98 ± 1.20 (8.20 to 13.81)
CVPS	2.39 ± 0.97 (–0.46 to 3.91)	10.39 ± 1.17 (8.63 to 13.98)
PFPS	3.28 ± 0.84 (0.99 to 4.66)	11.37 ± 1.18 (9.58 to 14.84)

CVPB: Crude venom of *P. birmanica*; PFPB: protein fraction of *P. birmanica*, CVPS: crude venom of *P. sumatrana*; PFPS: protein fraction of *P. sumatrana*.

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