INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0532/201x/00–0–000–000
DOI: 10.17957/IJAB/15.0785
http://www.fspublishers.org

Full Length Article

Evaluation of Venom Peptides of Two Jumping Spider Species (Araneae: Salticidae) as Insecticide Potential

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Abstract

Protein fractions in crude venom preparation of two species of the jumping spiders, *Plexippus paykulli* (Audouin) and *Theyne imperialis* (Rossi), were evaluated in the laboratory for their effectiveness against field-collected live *Rhopalosiphum erysimi* (Hemiptera: Aphididae). Venom glands of spiders were removed, and protein fractions from the venom were separated by SDS-PAGE. A common and dominant protein fraction (~29 kDa) in venom of each spider was selected for bioassay. The observed mortality rate of *R. erysimi* was 79.5% and 90% respectively against the tested protein fractions of *P. paykulli* and *T. imperialis.* Based on the results, it is concluded that selected protein fractions of *P. paykulli* and *T. imperialis* have bio-insecticide potential. © 2018 Friends Science Publishers

Keywords: Bio-Insecticide, Pesticides; Spiders; Biological Control; Insect Pests

Introduction

Synthetic and natural insecticides, since the 1940s, are in use for managing insect pest populations (Casida, 2009), however, repeated use of these insecticides over the years has decreased their effectiveness against insect pests (Damalas and Eleftherohorinos, 2011). Biological pesticides or bioactive compounds have been documented as the best alternative to synthetic insecticides due to their specificity to target pest species (Copping and Menn, 2000; Glare *et al*., 2012).

Spiders like many predatory insects use their venom to immobilize prey (Sebastian and Peter, 2009) and an estimated 10 million biologically active peptides are present in spiders venom (Escoubas and King, 2009; Saez *et al*., 2010) including 38 insect specific peptides (Vetter *et al*., 2011). Mostly cellular targets of spider-venom peptides are voltage-gated potassium (KV) (Swartz and MacKinnon, 1997), calcium (CaV) (Adams, 2004; King, 2007), or sodium (NaV) (Adams, 2004; King *et al*., 2008) channels. Some spider toxins are highly selective against specific insect groups. This selective toxicity of spider venom could be the basis for developing a new generation of insecticides (Nicholson, 2007).

At present, development of bio-pesticides is on the rise due to negative impacts of chemical insecticides such as their persistence within the environments, the development of resistance in target insect species and broad activity spectrum across non-target organisms (Nauen and Bretschneider, 2002). Furthermore, presently bio-pesticides are playing an important role in promoting the efficiency of pest management programs (Wratten, 2009).

Most important purpose to introduce bio-pesticides is to reduce environmental problems associated with repeated use of wide spectrum chemical insecticides (Nauen and Bretschneider, 2002). In the agriculture, these have attained a very important place because they neither create any severe harm to ecological chain nor do they produce any environmental pollution (Leng *et al*., 2011). Utilization of spider venom as a bio-pesticide has been documented in the available relevant scientific literature. It is already known that there are three main constituents of spider venom: μ-AGTX-Aa1d, U1-AGTX-Ta1a and μ-DGTX-Dc1d (Prikhodko *et al*., 1998).

Spider venoms are rich source of highly stable insecticidal proteins that cause insect mortality through the modulation of ion channels, receptors and enzyme. The mini-peptides of spider venom could be potential source of bio-insecticides and are expected to limit the need for treatments with chemical insecticides to enhance yield levels (Qaim and Zilberman, 2003; Windley *et al*., 2012).

To cite this paper: Tahir, H.M., K. Zahra, R. Yaqoob, A.A. Khan, A. Butt, Z. Hassan and S.Y. Khan, 201x. Evaluation of venom peptides of two jumping spider species (araneae: salticidae) for use as insecticide. *Int. J. Agric. Biol*. 00: 000-000

There is no doubt that synthetic chemistries provide efficient control of pest insects, however, they simultaneously result in a variety of devastating effects against the ecosystem. Experts strongly desire investigation and introduction of low-cost and safe alternatives to chemical insecticides. In light of this, present study was designed and aimed to extract venom from two spider species inhabiting citrus orchards of District Sargodha, Punjab, Pakistan. In this context use of spider venom seems promising and should be further investigated for its bio-pesticidal potential. The present study was therefore conducted to evaluate the potential use of selected protein fractions of ~29 kDa of two local commonly available spiders.

Material and Methods

Collection of Spiders and Tested Insects

In order to analyze the insecticide properties of the spider venom, two dominant jumping spiders, *Plexippus paykulli* (Audouin) and *Theyne imperialis* (Rossi), were collected from Citrus orchards of Sargodha (32.1506°N 72.6454°E.), Punjab Province, Pakistan, with the help of a battery-operated suction device and were brought in the laboratory to be maintained at 25±2°C, until utilized for the study. Aphids were also collected from different citrus seedlings raised for purpose of collecting aphids simulating pesticide free habitat.

Venom Extraction

Field-collected spiders were kept in individual containers (8.5 cm diameter ×7 cm height, and 250 cm^3 volume) without food for 2-3 days. Spiders were immobilized by keeping them in the freezer at -20°C for 5-7 min. Thereafter, adult spiders were dissected; their cephalothoraxes were separated (Frontali *et al*., 1976) and venom glands were removed (Guerrero *et al*., 2010). To obtain venom from each spider species, venom glands from 200 to 300 spiders were homogenized manually in 4 - 4.5 mL cold 0.05 M Tris-HCl buffer, pH 8.2 (Frontali *et al*., 1976). The homogenate was centrifuged for 15 minutes at 15,000 rpm in a refrigerated centrifuge at -5°C (Frontali *et al*., 1976). Supernatant was frozen at -20°C and used for fractionation (Frontali *et al*., 1976). Supernatant (15 μL) and loading buffer (15 μL) were mixed in an eppendorf and this mixture was heated at 100°C for 3-4 min, later centrifuged for 20 sec at 12000 rpm.

Characterization of Venom on Gel Electrophoresis: Gel Preparation

To identify and characterize spider venom fractions, Polyacrylamide Gel Electrophoresis (PAGE) was performed for each spider species (Sambrook and Russle, 2001) with slight modifications for analysis of its protein fractions. Clean gel plates were assembled after placing the spacers. Separating gel (10 mL, 10%) was prepared by mixing 4 mL distilled water, 3.3 mL acrylamide mix (30%), 2.5 mL Tris-HCl (pH 8.8, 1.5 M), 100 uL SDS (10%), 3 mL ammonium per sulphate (10%) and 28 μL TEMED. This solution was poured in space between two glass plates. Staking gel (3 mL, 5%) was prepared by mixing 2.1 mL distilled water, 500 μL acrylamide (30%), 380 μL Tris buffer (pH 6.8, 1 M), 30 μL SDS (10%), 90 μL ammonium per sulphate (10%) and 21 μL TEMED. Staking gel was poured over separating gel and a comb was inserted into staking gel to make the wells. Comb was removed after polymerization of gel. Wells were rinsed with distilled water. To characterize spider venom fraction on PAGE, Gel plates were adjusted in vertical position to the gel apparatus and electrode buffer was added in both upper and lower tanks of the gel apparatus. Samples (15 μL) were loaded into wells by using micropipette. In first well, ladder was loaded for reference. After loading all the samples safely, lid was placed on the unit which was attached to power supply. The gel was run at 60 volts for one hour and then voltage was increased to about 75 volts until the dye reached the lower edge of the gel. Power supply was disconnected after 4 h and gel was taken out of the plates. In order to visualize the spider venom protein fractions, gel was transferred to a plastic tray containing the staining solution. The gel was stained for 45 min in Coomassie Blue solution and then de-stained to remove the extra stain. Photographs of the stained gels were captured. Approximate molecular weights of proteins in the venom were estimated by comparing them with ladder (reference protein standards). In order to utilize the spider venoms for bioassay, the eppendorf containing elution buffer was placed in incubator at room temperature for 24 h and then centrifuged in refrigerated centrifuge at 12000 rpm for 15 minutes. Supernatant was used for bioassays.

Separation of Biologically Active Protein Fractions

For the separation of biologically active protein fractions, native Polyacrylamide Gel Electrophoresis was used. The part of gel containing bands of low molecular weight protein fractions (~29 kD) were excised from the gel and chopped into little pieces. These pieces were transferred in an ependorf containing 100 μL Tris-Cl elution buffer (0.25 M, pH 6.8) and vortexed for 30 seconds.

Evaluation of Insecticide Potential of Crude Venom Protein Fraction

To evaluate paralytic activity of crude insect venom, adults of *Rhopalosiphum erysimi* (Homoptera: Aphididae) were divided into control and treatment groups (n = 20 in each group). *R. erysimi* of experimental groups were treated topically with 0.5 μL crude venom or protein fractions of spiders using micropipette. Aphids were immobilized for topical treatment by placing them in a refrigerator for few

Table 1: Calculated LT_{50} and LT_{95} for the field-collected aphid, *Rhopalosiphum erysimi*, exposed to crude venom and protein fractions of two species of field-collected jumping spiders, *Plexippus paykulli and Theyne imperialis.* Unit of LT is hours and level of confidence interval was 95%. (CVPP= crude venom of *P. paykulli;* CVTI= crude venom of *T. imperialis;* PFPP = protein fraction of *P. paykulli;* PFTI= protein fraction of *T. imperialis*)

Treatments	LT_{50} (confidence interval)	Slope	Chi- Square
CVPP	5.05 ± 0.8 (2.99 - 6.46)	-1079	4-23
CVTI	2.13 ± 1.3 (2.06 - 4.23)	-0.995	2-61
PFPP	5.15 ± 1.64 (-0.77 -7.75)	-1077	3.96
PFTI	4.28 ± 1.15 (0.99 - 6.15)	-1.62	3.15
Treatments	LT_{50} (confidence interval)	Slope	Chi- Square
CVPP	15.00 ± 1.4 (12.78 - 19.12)	0.86	1.68
CVTI	13.96 ± 1.66 (11.50 - 19.20)	0.73	1.49
PFPP	25.92 ± 5.17 (19.34 - 48.99)	1.17	3.95
PFTI	17.41 ± 2.13 (14.32 - 24.16)	0.91	1.83

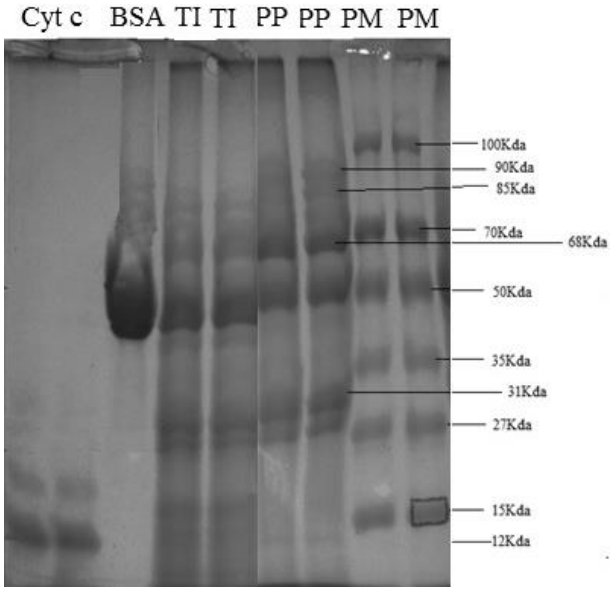

Fig. 1: Separation of proteins fractions of venom from *Plexippus paykulli* and *Theyne imperialis*. **(**PP = *Plexippus paykulli*, TI = *Theyne imperialis*, PM = Protein Marker, BSA = Bovine Serum Albumin, and Cyt c = Cytochrome C)

seconds. In control group, each *R. erysimi* received topical treatment of 0.5 μL Tris buffer. Mortality was checked after every 4 h for 24 h. Experiment was repeated thrice to get the concordant readings. LT_{50} and LT_{95} were calculated using probit analysis with the Minitab 13.3®.

Results

The resolved venom protein bands were compared to protein bands of known molecular weight, separated on same gel. Electrophoretic analysis of venom extracted from *P. paykulli* revealed presence of 7 different protein bands. It contained bands of 83 kDa, 68 kDa, 50 kDa, 29 kDa, 27 kDa, 16 kDa, and 12 kDa. Band of 50 kDa was denser and broader among all bands indicating higher concentration of this peptide. Band of 83 kDa was the heaviest, followed by 68 kDa, 50 kDa, 29 kDa, 27 kDa, 16 kDa, and 12 kDa. Bands of 83kDa, and 68kDa were less dense (Fig. 1).

Venom extracted from *T. imperialis* was also resolved into 5 bands. Heaviest band was of 68 kDa, followed by 50 kDa, 29 kDa, 27 kDa, and 17 kDa. Band of 50 kDa was more conspicuous as compared to others. All other bands appeared almost the same as far as their strength was concerned. Bands of 29 kDa and 27 kDa were sharp. Results of bioassay revealed that treatment group of *R. erysimi* exposed to crude venom and protein fractions of *P. paykulli*, and *T. imperialis*, showed out of 20 aphids, 19±1 (95%), 18.5±0.5 (92%), 15.5±0.7 (77%), and 18±2 (90%) died, respectively, at 16 h post treatment period (Fig. 2). Number of dead aphids in control group (1.5±0.8) was significantly lower than experimental groups (Fig. 2). Calculated LT_{50} and LT_{95} values for *R. erysimi* are given in Table 1. The highest LT_{50} and LT_{95} values were recorded for the protein fractions of *P. paykulli*.

Discussion

Bio-insecticides offer better alternative to pest control than chemical pesticides since the latter pose enormous environmental risk and related health hazards (Leng *et al*., 2011). In this study, we have separated different venom protein fractions (~29 kDa) of two jumping spiders and evaluated their bio-insecticidal potential (~29 kDa) against *R. erysimi* aphid. Present study showed that mortality of *R. erysimi* was significantly higher in groups treated with crude venom or protein fractions of both tested spiders compared to corresponding control group. This resonates the efficacy of spider venoms against targeted insects. Our results are in accordance with the various previous studies where spider venom has been found effective against controlling insect pests. Furthermore, these venoms were not found to be non-targeted and their unwanted affects are limited. Previously, many researchers have identified insect selective toxin in spider venom (Schroeder *et al*., 2008). Richardson *et al*. (2006) evaluated the toxic effect of peptides of spider venom against house flies and found that protein fractions caused unusual sensitivity to stimulation, increased production of saliva, shiverness, and immobility which finally lead to end of life of the targeted flies. Our results are also in line with another study where higher mortality in houseflies was also recorded when treated with P11a/GNA (Macedo *et al*., 2015). When *Acyrthosiphon pisum* nymphs were offered food mixed with Pl1a/GNA, death of all nymphs occurred.

It was also found that Pl1a/GNA binds with alimentary canal of aphid and remains there even after

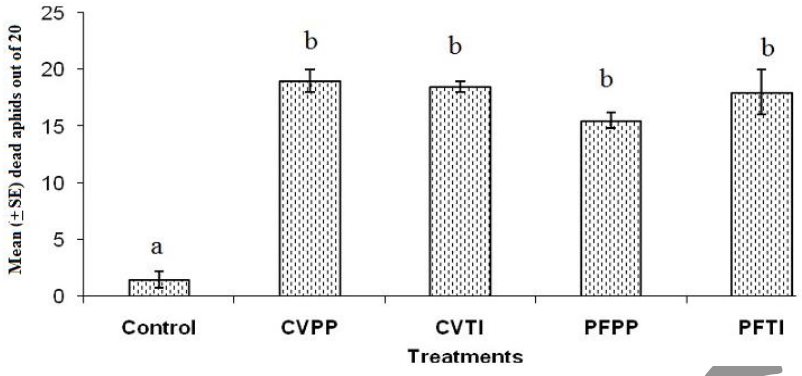

Fig. 2: Mean number (±SE) of dead *Rhopalosiphum erysimi* exposed to crude venom and protein fractions of *Plexippus paykulli* and *Theyne imperialis.* (CVPP = crude venom of *P. paykulli;* CVTI = crude venom of *T. imperialis;* PFPP = protein fraction of *P. paykulli;* PFTI = protein fraction of *T. imperialis*)

48 h. All these studies support rationale of present studies that spider venoms are efficient in killing targeted insects.

Seven and five fractions and their characterization in the venom of *P. paykulli* and *T. imperialis*, were reported respectively. In our study, we recorded 7 peptide fractions (83 to 12 kDa) in the venom of *P. paykulli* compared to 5 fractions (68 to 17 kDa) in *T. imperialis* venom. Futrell (1992) also reported a group of venomous proteins (1 to 40 kDa) in the venom of *Loxosceles* spiders. Protein of 10 kDa was identified by Liang *et al*. (2000) in *Selenocosmia huwena* spider venom. De Castro *et al.* (2004) reported three new potential insecticidal toxins named LiTx X (7.4 kDa), LiTx X 2(7.9 kDa) and LiTx X 3(5.6 kDa) from the venom of *Loxosceles intermedia*. Vassilevski *et al*. (2008) separated the Cyto-insectotoxins (8 kDa) from venom of Central Asian spider, *Lachesana tarabaevi*. Chaim *et al*. (2011) recorded proteins of 5-40 kDa in Brown recluse spider (*Loxosceles* genus) venom. All these studies exhibit characterization of various protein fractions active against targeted insects. Our results are in accordance with these studies since we have also characterized the protein fractions based on PAGE analysis.

In the present study, 7 protein bands ranging from 12 to 83 kDa molecular weight were recorded through Electrophoretic analysis of P. paykulii venom. Similarly, venom of T. imperialis resolveds into 5 bands ranging from 17 – 68 kDa. Kuhn-Nentwig (2003) found that ~3–7 kDa peptides of spiders venom are neurotoxic, whereas cytolytic peptides are typically rather small (~3 kDa). Vassilevski *et al*. (2010), purified and established full amino acid sequence (134 residues) of principal toxic component CpTx 1 (15.1 kDa) in the venom of yellow sac spider *Cheiracanthium punctorium* ((Miturgidae)). In our study we were not able to separate protein fractions of molecular weights below 12 kDa. These fractions could be separated by using HPLC.

We have found toxic effect of spider venoms against *R. erysimi*. The putative toxins present in spider venom affect neuronal ion (Ca 2+, Na+ and K+) channels and NMDA-type glutamate receptors (Parodi *et al*., 2010). Delta-atracotoxins (delta-ACTX) of 42 amino acids form present in Australian funnel-web spider binds to voltage-gated sodium channels. It stimulates release of neurotransmitter and cause muscle contraction, tremor, abnormally rapid heart rate, heavy sweating, etc. Latrotoxin (a-LTX) (132 kDa) from black widow spiders, consist of a unique N-terminal sequence and a C-terminal part. LTX exhibit tetramerization and form Ca2+-permeable pores in pre-synaptic membranes of neuron cells. As a result, muscle contraction and anxiety is caused in insects.

Two of the latroinsectoxins, αLIT-Lt1a (111kDa) and δ LIT-Lt1a (130 kDa) have been cloned and fully sequenced by Kiyatkin *et al*. (1993) and Dulubova *et al*. (1996). These toxins paralyze insect prey by inducing massive neurotransmitter release from nerve terminals (Rohou *et al*., 2007). Although we did not study the mechanism of action of ~29 kDa peptide isolated from two jumping spiders (*P. paykulli* and *T. imperialis*) but from the previous studies we can predict that said peptide is neurotoxic and similar mechanisms as described above could be responsible for insect toxicity in our targeted spiders **(**Windley *et al.*, 2012**).** It is concluded that selected protein fractions of *P. paykulli* and *T. imperialis* was effective against *R. erysimi*. Frequent use of chemical insecticides can result in heavy burden on the economy of the country and simultaneously have adverse environmental as well as health effects. Therefore, there is a dire need to explore and develop bio-pesticides specifically for the agriculture sector. However, further studies should be conducted to evaluate the effect of this fraction against other insect pests and non-target organisms.

Acknowledgements

Corresponding Author is thankful to HEC, Pakistan and

UoS, Sargodha for providing funding and facilities respectively to conduct this research.

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(Received 07 April 2018; Accepted 07 June 2018)