International Journal of Entomology Research
ISSN: 2455-4758; Impact Factor: RJIF 5.24
Received: 05-05-2020; Accepted: 07-05-2020; Published: 08-06-2020
www.entomologyjournals.com
Volume 5; Issue 3; 2020; Page No. 133-136

Bio-pesticidal potential of crude venom of *cyrtophora citricola* (Spider: Buthidae) against *brevicoryne brassicae* (Hemiptera: Aphididae)

Muhammad Shahzad[1], Muhammad Arshad[2], Sobia Kanwal[3], Zulqurnain[4], Uzma Ayaz[5], Sajjad Ali Khan[6], Nabeel Khalid Bhutta[7], Jawad Ali Shah[6], Sana Rafique[8]

[1, 2] Department of Zoology, University of education, Lower Mall Campus, Lahore, Pakistan
[3] Department of Zoology, Wildlife and fisheries University of Agriculture, Faisalabad, Pakistan
[4] Department of Zoology, GC University, Faisalabad, Punjab, Pakistan
[5] Department of Plant Breeding & Molecular Genetics University of Poonch Rawalkot Azad Jammu & Kashmir Pakistan
[6] Department of Agriculture (Entomology) University of Swabi, KP, Pakistan
[7] Department of Microbiology, University of Central Punjab, Faculty of Life Sciences, Pakistan
[8] Department of Genetics, Hazara University Mansehra, KP, Pakistan

Abstract
There is much interest in the development of eco-friendly insecticides because chemical insecticides are harmful for environment. The venoms of arthropod predators such as spiders are rich in compounds that can be used as bio-pesticides. Present study was planned to evaluate the insecticidal potential of different concentration of crude venom of *Cyrtophora citricola* (Spider: Buthidae) against *Brevicoryne brassicae* (Hemiptera: Aphididae). Dissecting method was applied to remove venom glands from spiders. Oral method was used for applying venom on model pest. Venom was applied with concentrations of 0.5ul, 1ul and 1.5ul and %age mortality was 63.3, 93.3 and 100 respectively. The values of LT50 and LT95 are 8.44 and 15.78 respectively. The values of LT50 and LT95 decrease with the increase of venom concentrations. Findings suggested that C. citricola can be used in the formation of bio-pesticides.

Keywords: *cyrtophora citricola*, *brevicoryne brassicae*, bio-pesticides, eco-friendly

Introduction

The most successful and vast group of animals are arthropods. They are estimated about 2.8–10 million global species. About 10000 species of insects are considered as pest of crops (Pimentel *et al*., 2009) [1]. Annually 14% of total worldwide crop production and 20% stored food grains are destroyed by this large group of pests (Oerke and Dehne, 2004) [2]. The estimated loss each year is about USD 100 billion (Carlini *et al*., 2002) [4]. Major loss of crop production is due to plant eating arthropods, which are known as phytophagous arthropods. Insect species, which are considered as pest, are related to Order Coleoptera (beetles), Orthroptera (locusts and grasshoppers) and Lepidoptera (moths and butterflies) (Novotny *et al*., 2002) [5].

Pest/crop management system means protection of crop from pests by using biological and chemical measures (Oerke, 2006) [2]. Unfortunately, the majority of current agrochemicals have adverse effects on human health. For example, over 250000 people die each year from suicide using insecticide (Gunnell *et al*., 2003; Gunnell, 2007). Insecticides gradually accumulate in food materials and ultimately cause severe disease in humans like cancer, kidney failure and genetic disorders (Owain *et al*., 2008; Ambethger, 2009) [30].

All these synthetic pesticides also have adverse effects on beneficial insects, pollinators (bees and butterflies), and birds, aquatic and marine life. So, about 169 insecticides were banned from January 2005 to December 2009, and only 9 new insecticides were registered during the same period (Windly M. *et al*., 2012).

The use of biological product to control pest is an effective method as compared to use synthetic insecticide (Nauen & Bretschneider, 2002) [7]. Aphid (Hemiptera: aphididiae) is epidemic pest of agriculture in temperate areas. They suck sap from phloem by inserting their stylet, which is needle like syringe and released honey dew at their posterior end. This honey dew provides medium for growth of dangerous sooty molds, which is a form of fungus. Sooty molds disturb the photosynthetic activity of plants. Aphids are also a vector of many plant viruses (Sarwar 2013) [31].

Among the many taxes of animal, which are venomous in nature arthropods are most successful in utilizing their venom against predators and paralyzing their prey (King, 2011) [9]. In the arthropods, spiders and scorpions are terrestrial predators (Windley *et al*., 2012) [10]. Evolutionary period of spiders is Carboniferous period and their evolution started about 300 million years ago along with their predatory nature. Spiders use their venom to paralyze insect (Platnick, 2012) [3]. Disulphide rich peptides of spider's venom damage the neuronal ion channel of insect (King and Hardy, 2013) [12]. Similarly, the method of spiders to capture their pray by web formation and using their fangs to inject venom to paralyze the pray, makes them most successful terrestrial predator (Vollrath and Selden, 2007) [13]. Many researchers are interested to use peptides of spider's venom as an insecticide in agriculture, to ensure eco-friendly environment.

The present study was planned to evaluate potency of crude spider venom (*Cyrtophora citricola*) to control aphid

(*Brevicoryne brassicae*) which is most destructive pest throughout the world. The venom of spider venom belonging to family Buthidae is selected for study.

Materials and Method

Spider Collection & Maintenance

Collection of spiders (C. citricola) was done from leaves of old trees and orchards by Jerking and Hand-picking method from Jallu Park, Lahore, Pakistan. Spiders were kept in the laboratory at room temperature in plastic jars. For proper aeration jars were covered with mesh cloth and placed near the window. Food source provided to spiders was live flies, per week. For maintaining humidity, water was sprinkled on jars from time to time.

Pest Collection

The tested insect was aphid (B. brassicae). Aphids were collected from the mustard field of Theeng Mor, Qasur district. Their collection was done from November to March. The insects were calculated by Hand picking method. Then insects were kept in the laboratory at a temperature of 25^0 C and 60% humidity.

Venom Extraction from Spiders

Specimens were immobilized by placing in refrigerator 5-7 minutes. For obtaining venom gland, the specimens were dissected under dissecting microscope. Tip of forceps were used to separate venom gland by pulling out chelicerae of specimens. Glands were mixed with 50ul cold 0.05 M Tris-HCL buffers at pH 7.6. The homogenate was centrifuged for 10 minutes at 15000 rpm. The resulting material was stored and diluted with Tris-HCl.

Insecticidal Potential of Venom against Aphids

For evaluation of pesticides potential of Arachnid's venom against aphid oral method was selected. Forty aphids were removed from the jar and placed in two groups (n=40). Control group contain 10 aphids. Group that was made for experiment was further subdivided into three subgroups which were represented by I, II and III. Each subgroup contains 10 aphids. Aphids of control group were treated with 0.5ul of solvent only. Concentration of venom was 0.5ul, 1ul, and 1.5ul. Following the oral method, venom was mixed with food of aphids and spread on Petri plate. After every six hours, mortality of individuals was observed in each subgroup. Experiment on each subgroup was repeated thrice to obtain accurate results.

Statistical Analysis

ONE WAY ANOVA test was selected to calculate the mortality in given groups. SPSS version 16 (Statistical package for social sciences) was applied for obtaining result. Software mini tab (14.1) was applied to get values of LT50 and LT95.

Results

Figure 4 explained the results of %age mortality of B. brassicae at 6 hours of treatment with various concentrations (0.5ul, 1ul and 1.5ul) of spider venom. Non-treated group was taken as control for comparison. %age mortality with 0.5ul was observed 3.33±3.33. With 1ul %age mortality was 16.67±3.33 and with 1.5ul was 36.67±3.33. A concentration dependent effect was observed for the spider insecticidal activity on B. brassicae. When compare with control, all the groups showed significant difference (f=33.222, p=0.000)

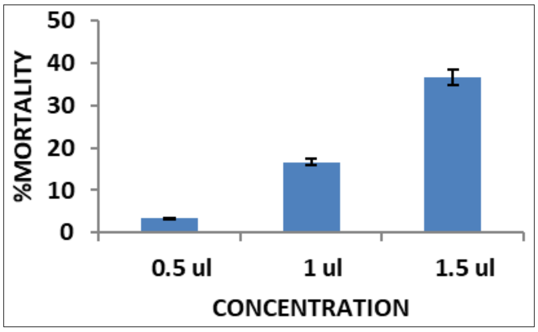

Fig 4: Insecticidal effects (%age mortality) of different concentrations of C. citricola on B. brassicae at 6 hours.

Figure 5 explained the results of %age mortality of B. brassicae at 12 hours of treatment with various concentrations (0.5ul, 1ul and 1.5ul) of spider venom. Non-treated group was taken as control for comparison. %age mortality with 0.5ul was observed 26.67±3.33. With 1ul % age mortality was 43.33±0.00 and with 1.5ul was 73.33±3.33. A concentration dependent effect was observed for the spider insecticidal activity on B. brassicae. When compare with control, all the groups showed significant difference (f=113.222, p=0.000)

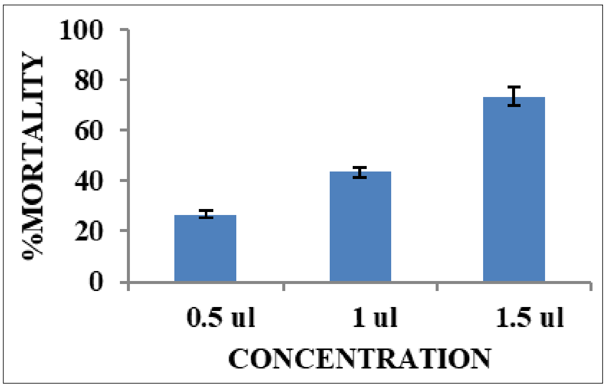

Fig 5: Insecticidal effects (%age mortality) of different concentrations of C. citricola on B. brassicae at 12 hours.

Figure 6 explained the results of %age mortality of B. brassicae at 18 hours of treatment with various concentrations (0.5ul, 1ul and 1.5ul) of spider venom. Non-treated group was taken as control for comparison. %age mortality with 0.5ul was observed 63.33±0.00. With 1ul %age mortality was 93.33±3.33 and with 1.5ul was 100±0.00. A concentration dependent effect was observed for the spider insecticidal activity on B. brassicae. When compare with control, all the groups showed significant difference (f=151.133, p=0.000)

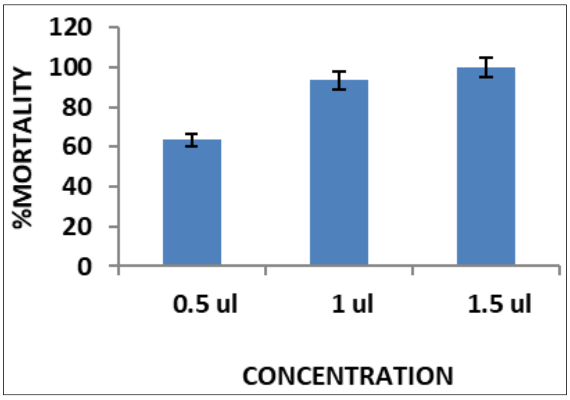

Fig 6: The number of *Brevicoryne brassicae* died at 18 h exposed to different doses of crude venom of *Cyrtophora citricola*

The calculated LT50 and LT95 values for B. brassicae are given in table.1. The highest LT50 and LT95 values were recorded for the dose 0.5 ul. LT50 and LT95 values decreased with the increase of venom concentration. The calculated LT50 and LT95 values for B. brassicae were (8.4±1.2 and (15.5±2.2) with 1.5ul venom concentration respectively.

Table 1: Calculated LT50 and LT95 for *Brevicoryne brassicae* exposed to different doses of crude venom of *Cyrtophora citricola*.

	0.5ul	1ul	1.5ul
LT50	14.8±1.7	12.02±1.3	8.4±1.2
LT95	24.9±4.1	20.8±2.9	15.5±2.2

Note: Time in the table is in hours

Discussion

The most effective and dominant method of controlling insects is use of chemical insecticides (Smith *et al*., 2013). Investment of one dollar on the crop in the form of insecticide to protect them from pests returns 4 dollars in the form of increase of crop yield (Pimentel, 2009) [1]. On the other hand, uncontrolled use of insecticides creates many problems such as harmful effect on organisms that are beneficial for environment. They are responsible for development of resistance in insect against insecticides

Due to all these adverse effects of insecticides, bio-pesticides are considered as a safer method of controlling insects rather than chemical pesticides. Bio-pesticides do not cause any problem to environment (Leng *et al*., 2011) [14]. Bio-pesticides are derived from natural products. They are environment friendly and not pose any threat to life of beneficial organisms. These bio-pesticides can be formed by using proteins of organisms, which are insecticidal (Khan *et al*., 2006; Cao C-W *et al*., 2010) [16]. For example, Cry proteins are endotoxins which were obtained from the bacterium B. thuriengiensis. Cry proteins destroy mid gut of insects and kill them (Soberon *et al*., 2007). Due to its capability of killing pest, it was introduced into many crops such as cotton, corn, potato and many more (Qaim M. 2010) [18]. But it has narrow host range and in some cases resistance has also been reported in some pests (Tabashnik *et al*., 2011; Bravo *et al*., 2011) [19, 17]. Similarly, spider venom is also a source of many insecticidal toxins (Windley *et al*., 2012) [10]. Its venom contains more than thousand peptides, which are toxic for many insects (Escoubas *et al*., 2006) [22].

In the given study, venom obtained from C. citricola was applied on B. brassicae to test the effectiveness of crude venom of spider against aphids. Results of our study show that venom of spider is toxic to aphids and more than 60% mortality was observed after 18 hours even with 0.5ul concentration of venom. King and Hardy (2013) [12] worked on spider-venom peptides and reported that these peptides can be used in the formation of bio-pesticides and can be introduced in the plant as trans-genes. Such crops will be able to synthesis their own pesticides against their pests.

Lin *et al*., (2017) reported that Nc1a peptide of Nephila clavata spider venom is a novel insecticidal toxin for cockroach. It has ability to block the NaV and KV channels in cockroaches. So that Nc1a peptides of spider venom can be used as template for the formation of bio-pesticides. Hafiz *et al*., (2018) concluded that both crude venom and protein fractions of two wolf spiders, Pardosa sumatrana and Pardosa birmanica have ability to kill model pest R. padi. Musarrat *et al*., (2017) [25] reported that Hvt peptide of H. versuta spider shows toxic effect against two species of aphids, Aphis gossypii and Myzus persicae. Khan *et al*., 2006; Shah *et al*., 2011, Javaid *et al*., 2016 reported the oral insecticidal toxicity of the Hvt peptides of spider against aphids and bollworms.

Rachel *et al*., (2006) reported that the application of SFI1/GNA fusion protein that derived from snowdrop lectin and spider venom is responsible for slowing the developmental activity and reproducing capability of aphid (M. persicae). This fusion protein was mixed in the food of aphids. After 7 days, 100% mortality was observed due to slow rate of development of aphids.

From the above discussion, it is cleared that venom of spider is a rich source of peptides which are toxic for insects. So that these peptides can be used as bio-pesticides and can replace the use of chemical insecticides. As we know that chemical pesticides are harmful for human health. On the other hand, bio-pesticides are eco-friendly. Our next aim is to isolate the peptides which are actual toxin for insects by fraction method. If we are able in doing so, than that information will provide us a raw material for the synthesis of selective bio-pesticides at industrial level.

References

1. Pimentel D, Burgess M. Environmental and economic costs of application of pesticides primarily in the States. Integrated pest management, 2014, 47-71.
2. Oerke EC, Dehne HW. Safeguarding production—losses in major crops and the role of crop protection. Crop protection, 2004; 23(4):275-285.
3. Platnick NI. The world spider catalog, version 7.5. American Museum of Natural History, 2006; 3477:1-20.
4. Carlini CR, Grossi-DE-SÁ MF. Plant toxic proteins with insecticidal properties. Toxicon. 2002; 40(11):1515-1539.
5. Novotny V, Basset Y, Miller SE, Weiblen GD, Bremer B, Cizek L, *et al*. Low host specificity of herbivorous insects in a tropical forest. Nature, 2002; 416(6883):841.
6. Ambethgar V. Potential of entomopathogenic fungi in insecticide resistance management (IRM): A review. Journal of Biopesticides. 2009; 2(2):177-193.
7. Nauen R, Bretschneider T. New modes of action of insecticides. Pesticide Outlook. 2002; 13(6):241-245.
8. Agarwala BK, Das S, Senchowdhuri M. Biology and food relation of Micraspis discolor (F.) an

aphidophagous coccinellid in India. J Aphidology. 1988; 2(1-2):7-17.

9. King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. Expert opinion on biological therapy. 2011; 11(11):1469-1484.
10. Windley MJ, Herzig V, Dziemborowicz SA, Hardy MC, King GF, Nicholson GM, *et al*. Spider-venom peptides as bio-insecticides. Toxins. 2012; 4(3):191-227.
11. Platnick NI. The world spider catalog, version 7.5. American Museum of Natural History, 2006, 3477:1-20.
12. King GF, Hardy MC. Spider-venom peptides: structure, pharmacology and potential for control of insect pests. Annual review of entomology. 2013; 58(1):475-496.
13. Vollrath F, Selden P. The role of behavior in the evolution of spiders, silks, and webs. Annual Review of Ecology, Evolution, and Systematics, 2007, 38.
14. Leng P, Zhang Z, Pan G, Zhao M. Applications and development trends in biopesticides. African Journal of Biotechnology. 2011; 10(86):19864-9873.
15. Khan SA, Zafar Y, Briddon RW, Malik KA, Mukhtar Z. Spider venom toxin protects plants from insect attack. Transgenic research. 2006; 15(3):349.
16. Cao CW, Liu GF, Wang ZY, Yan SC, Ma L, Yang C. P. (2010). Response of the gypsy moth,Lymantria dispar to transgenic poplar, Populus simonii×P. nigra, expressing fusion protein gene of the spider insecticidal peptide and Bt-toxinC-peptide. Journal of Insect Science. 2006; 10(1):200.
17. Soberón M, Fernández LE, Pérez C, Gill SS, Bravo A. Mode of action of mosquitocidal Bacillus thuringiensis toxins. Toxicon. 2007; 49(5):597- 600.
18. Qaim M. Benefits of genetically modified crops for the poor: household income, nutrition and health. New Biotechnology. 2010; 27(5):552-557.
19. Tabashnik BE, Huang F, Ghimire MN, Leonard BR, Siegfried BD, Rangasamy M, *et al*. Efficacy of genetically modified Bt toxins against insects with different genetic mechanisms of resistance. Nature biotechnology. 2011; 29(12):1128.
20. Bravo A, Likitvivatanavong S, Gill SS, Soberón M. Bacillus thuringiensis: a story of a successful bioinsecticide. Insect biochemistry and molecular biology. 2011; 41(7):423-431.
21. Windley MJ, Herzig V, Dziemborowicz SA, Hardy MC, King GF, *et al*. Spider-venom peptides as bio-insecticides. Toxins. 2012; 4(3):191-227.
22. Escoubas P, Sollod B, King GF. Venom landscapes: mining the complexity of spider venoms via a combined cDNA and mass spectrometric approach. Toxicon. 2006; 47(6):650-663.
23. Tahir HM, Zahra K, KHAN AA. Insect-specific peptides in the venom of wolf spiders (Araneae: Lycosidae). Turkish Journal of Zoology. 2018; 42(5):614-616.
24. Jin L, Fang M, Chen M, Zhou C, Ombati R, Hakim MA, *et al*. An insecticidal toxin from nephila clavata spider venom. Amino acids. 2017; 49(7):1237-1245.
25. Shaheen M. Use of a Plant Virus Vector for Screening of Gene Constructswith Potential Insecticidal Activity Musarrat Shaheen. Unpublished phD thesis. Doctoral dissertation, Pakistan Institute of Engineering and Applied Sciences, 2017.
26. Khan SA, Zafar Y, Briddon RW, Malik KA, Mukhtar Z. Spider venom toxin protects plants from insect attack. Transgenic research. 2006; 15(3):349.
27. Shah AD, Ahmed M, Mukhtar Z, Khan SA, Habib I, Malik ZA, *et al*. Spider toxin (Hvt) gene cloned under phloem specific RSs1 and RolC promoters provides resistance against American bollworm (Heliothisarmigera). Biotechnology letters. 2011; 33(7):1457-1463.
28. Javaid S, Amin I, Jander G, Mukhtar Z, Saeed NA, Mansoor S, *et al*. A transgenic approach to control hemipteran insects by expressing insecticidal genes under phloem-specific promoters. Scientific reports, 2016, 6:34706.
29. Down RE, Fitches EC, Wiles DP, Corti P, Bell HA, Gatehouse JA, *et al*. Insecticidal spider venom toxin fused to snowdrop lectin is toxic to the peach- potato aphid, Myzus persicae (Hemiptera: Aphididae) and the rice brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Pest Management Science: formerly Pesticide Science. 2006; 62(1):77-85.
30. Owain ER, Franzmann B, Thackray D, MICIC S. Insecticide resistance and implications for future aphid management in Australian grains and pastures: A review. Australian Journal of Experimental Agriculture, 2008, 48:1523.
31. Sarwar M. Comparative suitability of soil and foliar applied insecticides against the aphid Myzus persicae (sulzer) (Aphididae: Hemiptera) in canola Brassica napus L. International Journal of Scientific Research in Environmental Sciences. 2013; (1):138-143.
32. Windley MJ, Herzig V, Dziemborowicz SA, Hardy MC, King GF, Nicholson GM. Spider-venom peptides as bioinsecticides, Toxins, 2012; 4:191-227.