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Potential Use of Venom of *Odontobuthus odonturus* (Arachnida: Buthidae) as Bio-pesticide Against *Rhopalosiphum erysimi* (Homoptera: Aphididae)

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Abstract.- Present study was designed to test the effectiveness of different concentrations of crude venom of *Odontobuthus odonturus* Pocock, 1897 (Scorpiones: Buthidae) against *Rhopalosiphum erysimi* (Hemiptera: Aphididae). Scorpions were collected from Sargodha district of Punjab (Pakistan) using portable ultra-violet (UV) lamps. The venom of the scorpions was collected in 1.5 ml plastic collection tubes and frozen at –20ºC. The bioassay tests were performed against 0.5, 0.75 and 1 dose of scorpion venom in the laboratory. The percent mortality after 12 h was 68.3, 71.65 and 83.3 against 0.5, 0.75 and 1 µl concentrations, respectively. The mortality was significantly higher in the treated groups compared to the control. The LT_{50} and LT_{95} values were decreased as the venom concentrations were increased. The calculated LD_{50} and LD_{95} values for *R. erysimi* were 0.44 µl and 1.31 µl, respectively. It is concluded that venom of *O. odonturus* contains toxins which could be used as potential bio-pesticide candidates.

Keywords: Scorpions, venom, *Odontobuthus odonturus*, bio-pesticides.

INTRODUCTION

Scorpions (Arachnida: Scorpiones) are distributed all over the world except for Greenland and Antarctica (Cao *et al.,* 2014). They inhabit a wide range of habitats including Savanna and snow covered mountains (Petricevich, 2010). Scorpions can survive in heat, drought, freezing and deserts conditions. They can also survive for months without food. Due to extraordinary power of acclimation, scorpions have an unbroken continuity in adverse climatic conditions (Bawaskar and Bawaskar, 2012).

The scorpions' family Buthidae includes some medically important species. Researchers have isolated about 400 toxic peptides from the venom of Buthids (Tan *et al*., 2006) which are toxic to insects (Loret and Hammock, 2001; Zlotkin, 2005). Scorpion's venom contains multiple low molecular weight proteinaceous neurotoxins which percolate into the brain of insects and damage their nervous system resulting in paralysis and finally death (Gurevitz, 2010). The scorpion toxins can be used in the synthesis of eco-friendly insecticides (Leng *et al*., 2011), cancer treatment (Bianchi *et al*., 1998) and protein engineering scaffolds (Fabiano *et al*., 2008). Antimicrobial activity of scorpion toxins against bacteria has also been confirmed (Corzo *et al*., 2001; Torres-Larious *et al*., 2000). The genes of scorpion toxin are also being used to kill insect by creating hyper virulent fungus in the insect (Blake, 2007).

Aphids constitute the destructive group of insect pests in many countries all over the world. They significantly reduce the yield of harvested crops (Irshad, 2001; Aslam *et al*., 2005). They not only suck plant sap but also are the carrier of many viruses that transmit diseases in plants (Soomro *et al*., 1992; Agarwal, 2007). Aphid population increases rapidly as they have short generation times and reproduce parthenogenetically (Hales *et al*., 1997).

Although the use of insecticides is an effective solution for the control of insect pests, it has many side effects. Many agri-scientists are trying to introduce such compounds in agro-ecosystems for the management of the insect pests

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which are effective, cheaper and environment friendly. Present study was undertaken to test the effectiveness of crude venom of *Odontobuthus odonturus* against *Rhopalosiphum erysimi*, which is a major insect pest in the study area. The venom of *O. odonturus* was selected as these buthid scorpions are very common in the sandy area near Sargodha district of Pakistan. The outcome of the study will provide a baseline data for the production of eco-friendly bio-pesticides in the country.

MATERIALS AND METHODS

Scorpion collection

The study was conducted at June 2012 to September 2012. The scorpions were collected from Doda, 36 km East of Sargodha, by portable UV lamps. Scorpions were collected during good moon period (when there is complete dark during night).

Test organism sampling

Rhopalosiphum erysimi (Hemiptera: Aphididae) was used as the model test organism. Field populations of *R. erysimi* were collected from the citrus orchards of the College of Agriculture, University of Sargodha. Infected leaves were kept in plastic cages and maintained at 28±4ºC. Only the adult *R. erysimi* were used in the experiment.

Extraction of scorpion venom

Crude venom was extracted mechanically by cutting the venom gland as a whole than crushing it by dissolving it in sodium phosphate buffer. The mixture was than centrifuged at 6000 rpm for 10 minutes to separate the mucous material from the venom. The supernatant was collected in 1.5 ml plastic collection tubes and frozen at –20 ºC.

Insecticidal potential of crude venom against R. erysimi

To test the insecticidal potential of the crude scorpion venom against *R. erysimi*, 80 aphids were taken and divided into the experimental (n=60) and the control group (n=20). Aphids of the experimental group were divided into three subgroups (n= 20 for each) as group I, II and III, respectively. Each individual of group I was treated topically with 0.5µl dose using micropipette. The group II and group III were treated with 0.75µl and 1µl of venom respectively. Each aphid of control group was treated 0.5µl sodium phosphate buffer. The mortality was assessed in all groups after every 4 h till 12 h. Each experiment was repeated thrice to get the concordant readings.

Statistical analyses

One way ANOVA followed by Tukey`s test was applied to compare the mortalities among different groups. Statistical Package for Social Sciences (SPSS version 13) was used for this purpose. LT_{50} and LT_{95} and LD_{50} and LD_{95} values were calculated using statistical software Minitab (14.1).

RESULTS

The results showed that the experimental group which was exposed with 0.5µl of scorpion venom showed 13.66 ± 0.577 (68.3%) mortality after 12 h (Fig. 1). However, the mortalities in the groups dosed with 0.75µl and 1µl of scorpion venom were 14.33±0.8 (71.65%) and 16.66±0.57 (83.3%), respectively. The mortality in the control group was 5% only. The results of Analysis of variance (ANOVA) showed that the mortality was significantly higher in the experimental groups compared to the control (df = 3, 8; F = 351; $P < 0.001$).

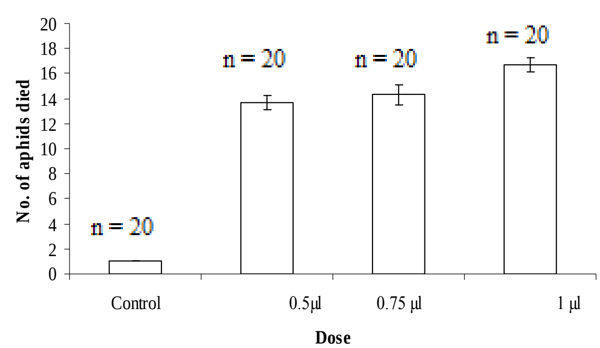

Fig. 1. The number of *Rhopalosiphum erysimi* died at 12 h exposed to different doses of crude venom of *Odontobuthus odonturus*.

The calculated LT_{50} and LT_{95} values for *R. erysimi* are given in Table I. The highest LT_{50} and LT_{95} values were recorded for the dose of 0.5µl. The

LT_{50} and LT_{95} values were decreased as the venom concentration was increased. The calculated LD_{50} and LD_{95} values for *R. erysimi* were 0.44 µl (0.31 – 5.72) and 1.31 µl (1.19 – 1.77), respectively.

Table I.- Calculated LT_{50} and LT_{95} for *Rhopalosiphum erysimi* exposed to different doses of crude venom of *Odontobuthus odonturus*.

Dose (µl)	**LT50 (Confidence Interval)**	**LT95 (Confidence Interval)**
0.5	9.03 (7.52 – 11.39)	16.48 (13.43 – 23.36)
0.75	8.62 (7.24-10.56)	15.22 (12.65 – 20.60)
1	7.02 (5.69 – 8.72)	13.81 (11.33 – 19.13)

Note: Time in the table is in hours

DISCUSSION

In agro-ecosystems of Punjab, Pakistan, aphids constitute one group of the most destructive pests which reduce the yield of crops (Irshad, 2001; Stonehouse *et al.*, 1998). Bio-pesticides are considered as safer alternative rather than chemical pesticides as they do not cause any serious harm nor create any environmental pollution (Leng *et al.*, 2011). Many scorpion venom toxins can effectively be employed in bio-pesticide engineering as they contain toxins active against insects (Gurevitz, 2010).

In the present study bioassay tests upon aphids were performed in order to evaluate the effectiveness of the crude venom extracted from *O. odonturus* against *R. merysimi*. Results of our study clearly demonstrated that venom from *O. odonturus* is effective against *R. erysimi* as even with 0.5 µl concentration the mortality was more than 60% (Fig. 1). Many researchers have already confirmed the effectiveness of the venoms extracted from different scorpions against insect pests. For example Wudayagiri *et al*. (2001) reported that venom from *Mesobuthus tamulus* is effective against *Heliothis virescens*. Moskowitz *et al*. (1998) reported the presence of a depressant insect selective toxin analog in the venom of the scorpion *Leiurus quinquestriatus hebraeus*. When they injected this venom in to blowfly larvae, it caused fast contraction paralysis (Nakagawa *et al*., 1997). Suze *et al*. (2004) have also purified a new arthropod selective toxin (ardiscretin) from the venom of the Venezuelan scorpion *Tityus discrepans*, which was found to be highly specific for invertebrates, but non-toxic to mice, at the dose they assayed.

Miyashita *et al*. (2007) confirmed in their study that the venom of *Liocheles australasiae* (Hemiscorpiidae) is toxic for the insects. Pimenta *et al*. (2001) has isolated two new toxins from the South American scorpion *Tityus bahiensis,* Toxin TbIT-I and Tb2-II. These two toxins have different biological properties. Toxin TbIT-I had almost no activity or pharmacological effects in vertebrate tissues whereas it was lethal to insects, so could be best alternative to the insecticide.

It is clear from the above discussion that scorpion's venom is a rich source of insect specific toxins which could serve as good alternative to the insecticides. *O. odonturus* is a common scorpion species in the study area. Although its venom is highly toxic to the *R. erysimi* in crude form. Our next aim is to isolate the fraction/fractions from the venom of this scorpion species which is the actual cause of insect's mortality. If we are able in doing so than this information could provide a raw material for the production of selective bio-pesticides at the industrial level.

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