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Original Article

Effect of *Hottentotta tamulus* (Scorpiones: Buthidae) crude venom on *Rhopalosiphum erysimi* (Hemiptera: Aphididae) in the laboratory

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Abstract
The present study was conducted to find out the effectiveness of venom extracted from *Hottentotta tamulus* (Scorpiones: Buthidae) against *Rhopalosiphum erysimi* (Hemiptera: Aphididae) in the laboratory. *R. erysimi* were treated orally as well as topically with different concentrations of the crude venom. All aphids died within 6 hr with oral treatment at 1.5µl of the venom dose. However, at the same venom dose, 100% mortality was achieved at 8 hr post-treatment when applied topically. Similarly, oral administration of 1µl and 0.5µl venom caused 100% mortality at 8 hr post-treatment compared to 10 hr and 12 hr post-treatment of topical application, respectively. It is concluded that oral treatment of venom was more efficient compared to its topical treatment with same doses of treatment.
Key words: *Hottentotta tamulus,* venom, aphids, *Rhopalosiphum erysimi,* scorpions.

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INTRODUCTION

Agricultural crops are destroyed by a variety of insect pests leading to decreased crop yields (Gupta and Dikshit, 2010). It is estimated that agricultural pests annually destroy 35 % of total yield worldwide (Krishna *et al*., 2013). While secondary yield loss was recorded upto 38% due to insect pests (Cerda *et al*., 2017). Although, chemical pesticides effectively reduce insect pest problems in most situations, they, however, simultaneously generate many environmental problems (Gupta and Dikshit, 2010). Pesticides not only destroy the insect pests but also eliminate natural predators of the pests, reduce soil fertility as well as pose health problems (Kandpal, 2014).

Therefore, exploration of alternative pest control possibilities that destroy the harmful pests but are not injurious to the beneficial organisms is desirable. In this context, use of bio-pesticides is considered to be the best option for managing insect pests because they are eco-friendly and relatively safe to the non-target organisms as well (Gupta and Dikshit, 2010; Kandpal, 2014; Ortiz and Possani, 2015).Additionally, bio-pesticides target specific pests and are less harmful to humans (Gupta and Dikshit, 2010).

Scorpion venom because of its specific insectotoxins has become the striking candidate for the development of novel insecticides (Gurevitz, 2010; Leng *et al.,* 2011; Suze *et al.,* 2004; Tahir *et al*., 2015). Scorpion venom is a rich source of unique and biologically active neurotoxins that specifically affect insects (Bertazzi *et al.*, 2003). Among these neurotoxins, specific peptides of 3-9 KDa are of great value according to theoretical as well as in applied research (Possani *et al*., 1999a, b, 2000; Srinivasan *et al*., 2002a; Srairi-Abid *et al*., 2005). These peptides target the major ion channels, such as Na^+, K^+, Ca^+ and Cl^- (Choung *et al*., 1998; Possani *et al.,* 2000; Joseph and George, 2012) by binding at the surface of excitable cells to modify their normal functioning (Zlotkin *et al*., 1991; Radha, 2014).

The present study was designed to evaluate effectiveness of crude scorpion venom against *R. erysimi* aphid, common insect pest of agricultural crops in Sargodha District of Punjab, Pakistan. The venom of *H. tamulus* scorpion

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belonging to Buthidae family was selected for the study. This scorpion species was selected being most common in the study area. Results of the present study will be helpful discover and design new environmentally friendly pesticides from scorpion venom for the population management of aphids in agricultural crops in the study area and perhaps elsewhere.

MATERIALS AND METHODS

The present study was conducted during May to September, 2015 and 2016. For the study, 50 scorpions were field collected from muddy houses of Sargodha District by using battery-operated portable ultra-violet (UV) lights. The field-collected scorpions were brought to the laboratory and kept in especially designed boxes and maintained under laboratory condition (Yaqoob *et al.,* 2016). Scorpions were fed with cockroaches, grasshoppers and locusts in the laboratory. The aphids for the experiment were provided by the Agricultural Department of Sargodha District.

The venom from the field-collected scorpions was extracted by the method of Ozkan and Filazi (2004). For this purpose, scorpions were kept unfed 4-5 days before the venom extraction. Venom from each scorpions was extracted by electrically stimulating the base of its telson. Venom was collected in graduated capillary tubes and preserved in a freezer at -20ºC till further use.

To evaluate the insecticidal activity of venom, experimental adult aphids were divided into control (*n*=10) and three experimental groups (*n*=10 in each group). Each experimented group was administered with one of the three concentrations of *H. tamulus* venom (i.e., 0.5µl, 1µl and 1.5µl) orally or topically. For oral treatment, venom was mixed with the food of the aphids and this food was spread on whole Petri plate so that each aphid was exposed with food while in topical treatment venom was directly applied on the body of aphids using micropipette. Mortality was assessed in all groups at various time intervals during a 24 hr. period. The experiment was repeated at three different occasions to get the concordant readings.

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. The LT_{50} and LT_{95} values were calculated using statistical software Minitab (14.1).

RESULTS

Crude venom of *H. tumulus* caused complete mortality in aphids treated with different doses of the venom (Fig. 1). Oral treatment was found to be more effective than topical treatment. It is evident from Figure 1a that all aphids died within 6hr of oral treatment at 1.5µl of the venom dose. However, 100% mortality was achieved at 8hr post-treatment with 1µl or 0.5µl of venom administration orally. With topical application of the venom, 100% mortality of aphids was at 8, 10 and 12 hr post-treatment when treated with 1.5, 1 and 0.5µl of venom respectively (Fig. 1b).

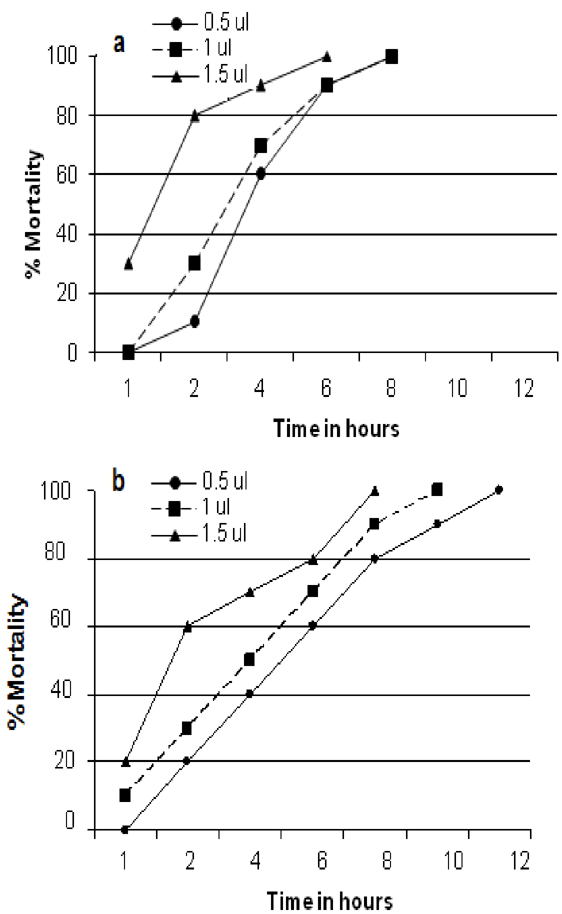

Figure 1. Effect of oral (a) and topical (b) application of crude venom extracted from *H. tamulus* scorpion on the mortality of field-collected aphids in the laboratory.

Results of ANOVA showed significant differences in mortalities among treatments; higher mortality was achieved at 1.5µl treatment rate (P<0.05). Calculated LT_{50} and LT_{95}values for the oral and topical treatments are given in Table I. It is evident from Table I that LT_{50} andLT_{95} values decreased with the increase of venom dose. It was also recorded that LT_{50} andLT_{95} values for oral treatment were lower as compared to the values obtained by topical treatment at all venom doses tested.

Table I: Calculated LT_{50} and LT_{95} values after oral (a) and topical (b) application of crude venom extracted from *H. tamulus* scorpion.

A

	Treatment rate of venom (Oral)		
	0.5 (µl)	**1 (µl)**	**1.5 (µl)**
LT_{50}	3.8±0.42 (2.75-4.71)	3.18±0.64 (1.04-4.31)	2.84±0.58 (0.75-3.88)
LT_{95}	7.13±1.06 (5.68-1.68)	6.3±0.76 (5.24-9.25)	6.13±0.94 (4.81-10.50)

B

	Treatment rate of venom (Topical)		
	0.5 (µl)	**1 (µl)**	**1.5 (µl)**
LT_{50}	7.25±0.0.62 (5.89-.62)	5.92±0.0.61 (14.51-7.18)	4.15±0.0.71 (2.13-5.44)
LT_{95}	12.61±1.33 (10.94-7.56)	10.97±1.20 (9.22-14.90)	9.34±01.22 (7.60-13.80)

Note: values that follow± in the above table represent standard error and values within bracket are values of 95% confidence interval (Cl)

DISCUSSION

Aphids are considered as serious insect pests that damage a variety of agricultural crops in Pakistan (Aslam *et al.,* 2009; Khan *et al*., 2015; Sarwar *et al.,* 2004). They can cause up to 70-80% destruction of the yield (Rohilla *et al.,* 1987; Singh *et al.,* 1987; Basavaraju *et al.,* 1995). The use of chemical pesticides to control these insect pests is becoming a topic of discussion in recent years because of their side effects on humans and the environment (Ahmad *et al.,* 2007). Thus, scorpion venom was tested as a biopesticide and as a safer alternative to chemical pesticides (Xie *et al.,* 2015).

H. tamulus belongs to the family Buthidae of scorpions. Scorpions of this family are medically significant (Baswakar and Baswakar, 2012). Scorpion venom contains insect specific toxins so it can be a source of bio-pesticide (Gurevitz, 2010). In the present study, effect of *H. tamulus* venom on aphids was evaluated by topical and oral treatment methods. The scorpion venom was found very effective against aphids. Furthermore, oral treatment was more effective than topical treatment. The results of the present study are comparable with those of Xie *et al.* (2015) who had reported significant mortality on cotton aphids with the venom of *Buthus martensi.* The specific neurotoxins present in scorpion venom enter in the nervous system of aphids and cause rapid paralysis (Ortiz and Possani, 2015). However, chlorotoxins and maurotoxins of scorpion venom result in melanization of injected aphids (Pal *et al.,* 2013).Some of the alpha toxins that are identified include LqhαIT from *Leiurus quinquestriatus hebraeus* (Eitan *et al.,* 1990), ButaIT from *Mesobuthus tumulus* (Wudayagiri *et al.,* 2001) and BjαIT from *Buthotus judaicus* (Arnon *et al.,* 2005). These toxins are highly specific for killing of insects (Gordon *et al.,* 2007). In the laboratory, upon injection of venom in to the aphid's hemocoel, aphids initially showed fast movement in petri dish but later on symptoms of toxicity appeared on treated aphids showing minimal locomotion within few hours after treatment and falling down without recovering again (Palma *et al*., 2003). Results of the present study clearly showed that oral treatment of the venom on aphids was more effective as compared to its topical application. These findings are in accordance with the results of Ortiz and Possani (2015) who also reported higher mortality of orally treated insects as compared to those treated topically. The reason might be that insect's cuticle creates hindrance for the venom to readily enter the body of the insect during topical application and degrades the venom in the environment. With oral application, venom directly passes towards the target sites of insects and cause rapid paralysis. Therefore, oral ingestion is more effective than topical treatment (Fitches *et al.,* 2002; Pham *et al.,* 2006; Bravo *et al.,* 2007). Tianpei *et al.* (2014) had studied oral ingestion of LqhIT2 protein from scorpion and reported that it affects the feeding of *Lepidopteran* larvae. Since scorpion venom contains insect specific neurotoxins, it can effectively replace chemical insecticides that are in use at present.

Previously, many scientists have isolated insect specific toxins from scorpion venom. For example, Kawachi *et al.* (2013) isolated Im3 neurotoxin from the venom of *Isometrus maculates* and found it effective against crickets. Similarly, AaIT (*Androctonus australis* Hector Insect Toxin) produced by the highly toxic scorpion *Androctonus. australis* is an excitatory long-chain insect specific toxin composed of 70 amino acids with four disulfide bridges (Zlotkin *et al.,* 1971;Darbon *et al.,* 1982). Cestele *et al.* (1997) isolatedIT2, one of the depressant toxins, from the venom of *B. arenicola* and checked its anti-insect properties in cockroach and reported that it leads to slow depressant flaccid paralysis and damages the insect brain. Thus, these toxins can be developed and commercially utilized as safer alternatives to chemical pesticides.

Conclusion

It is evident from the present study that *H. tamulus* venom is highly effective against adult *R. erysimi* aphid. Furthermore, oral treatment of the venom is more effective than its topical application.

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