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Scorpion Venom Peptides for the Management of *Bactrocera zonata* (Diptera: Tephritidae)

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Abstract: A variety of toxin proteins have been used against insect pests of economic importance having rare success to control the targeted pests. Scorpion venoms are complex mixtures of toxic peptides that are target specific against wide range of crop pests. Present study was aimed to evaluate insecticidal potential of peptide fractions (3-5KD) obtained from *Odontobuthus odonturus* (Arachnida: Buthidae) venom. The peptides were used against fruit flies, *Bactrocera zonata* (Diptera: Tephritidae) in controlled conditions. Topical treatment of different concentrations was done on the dorsal part of flies. Calculated LD_{50} and LD_{95} were 6.53 µl and 18.84 µl respectively. Significantly higher mortalities were recorded in the treated groups. Findings suggested that low molecular peptide fractions are effective toxin against *B. zonata*. These peptides if formulated commercially could be the alternative to hard chemistries.

Key words: Scorpion · Fruit Fly · Biological Control · Bio-Pesticides

INTRODUCTION

The members of Buthidae family of scorpions are considered as medically important and their venoms are toxic against wide range of living organisms [1]. Scorpion venoms are divided into three groups on the basis of their molecular mass [2], (i) proteins with enzyme activity (phospholipases, sphyngomyelinases and hyaluronidases), (ii) cytolytic compounds with molecular masses lower than 10kDa and (iii) amino acids, ions, free biogenic amines, neurotransmitters, acylpolyamines, alkaloids and heterocyclic compounds [3, 4]. Crop protection is switching to develop to these toxins as alternative to broad spectrum insecticides [5] and scorpions are vigorous source of a wide range of toxins. Fruit fly (Diptera: Tephritidae) is an economically important pest of fruits and vegetables affecting yield and quality of products [6] and is distributed in most of the courtiers lacking legislative control measures. The genus *Bactrocera* with 40 species is considered as significant pest of fruits and vegetables [7]. Pakistan is bearing annual losses of US$ 200 million caused by fruit flies [6]. This led to discouraged trade of the products in international markets. *Bactrocera zonata* is widely distributed throughout citrus growing areas of district Sargodha (Punjab) and an economically important pest of the region. Synthetic insecticides with harmful residues are in practice to control this pest in orchards [8, 9] which need to be replaced with eco-friendly products saving lives. Their indiscriminate and misuse creates problems of pest resurgence, resistance and environmental pollution added with economy threats. The present study was aimed to control economically important insect pests by employing toxic peptides of scorpion venom. The finding would be helpful to develop target specific eco-friendly bio-pesticides against wide range of fruit flies and other pests.

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MATERIALS AND METHODS

Present study was conducted at Department of Biological Sciences, University of Sargodha, Pakistan during April 2012 to February 2013. The scorpions, *O. odonturus* were collected from adjoining sandy areas of Sargodha district, Pakistan, in night using ultra violet lamps in absence of moon light. *Bactrocera zonata* were obtaining from infected fruits collected from Guava orchards in surroundings of main city Sargodha. Fruits were kept in ventilated plastic box (2x2x2 ft) provided with sand as media for pupation of flies. A laboratory colony for *B. zonata* was maintained in growth chamber (Percival Scientific Inc. Perry, Iowa, USA) at photoperiod of 14L: 10D, relative humidity 58±3% and at 26±3°C temperature. Fifty adult scorpions of same size and weight were milked to collect the venom. Telson of each scorpion was electrically stimulated and extracted venom was collected in 1.5ml ependroff tubes. From the venom peptide fractions were separated using High Performance Liquid Chromatography (HPLC). It was not possible to use only one fraction due to insufficient quantity, so a mixture of four low molecular weight peptide fractions (3-5kDa) was used in the experiment. Mixture of 50µl was than diluted with 100 µl phosphate buffer and for each experiment fresh mixture was used. For the evaluation of pesticidal potential of the venom against *B. zonata,* 40 flies were divided into treated (n=30, with further division in three subgroups n=10/each) and untreated group (n=10). For temporarily inactivating flies were put into refrigerator for 5 minutes at -4°C. Treatments were done tropically on dorsal part of flies; 0.5µl, 0.75µl and 1.00µl mixture respectively for each sub-group. Mortality was recorded after 6 and 12 hours of exposure and experiment was repeated thrice. One way ANOVA succeeded by Tukey`s test was applied in order to analyze difference in mortality among control and experimental groups. LT_{50} and LT_{95} and LD_{50} and LD_{95} were calculated applying Probit-Analysis.

RESULTS

Bactrocera zonata found susceptible to scorpion peptide fractions. The group treated with 0.5µl of scorpion venom showed 70% mean mortality after 12 hours. The mean mortalities in the groups treated with 0.75µl and 1µl of scorpion venom were 76.6% and 96.6% respectively, while untreated group showed 6.6% mortality (Fig. 1). The mortality of *B. zonata* in the treated groups was significantly higher compared to untreated

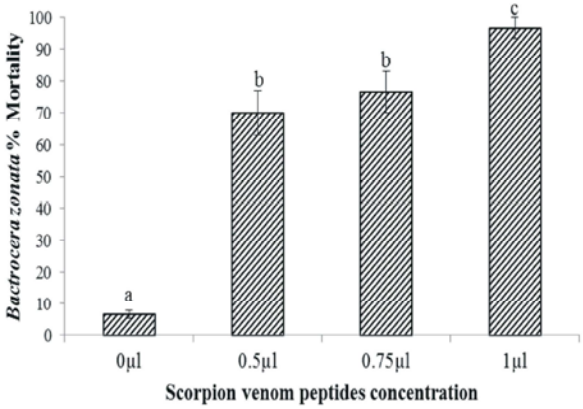

Fig. 1: Percent mortality of *B. zonata* at 12 hours exposure of scorpion peptides. A significant more mortality observed at the concentration (0.5 µl, 0.75 µl and 1.00 µl) as compared to 0 µl. The 0.5 µl and 0.75 µl were insignificant to each author with 70-80% mortality.

Table 1: Results of Tukey's test showing differences in mortality of *B. zonata* at different doses and time intervals.

Treatment	Mortality at 6 hours (n =10)	Mortality at 12 hours (n =10)
0 µl (Control)	0.00 ± 0.00^{a}	0.66 ± 0.33^{a}
0.5µl	2.33 ± 0.33^{b}	7.00 ± 0.577^{b}
0.75 µl	2.66 ± 0.57^{b}	7.66 ± 0.88^{b}
1 µl	3.33 ± 0.88^{b}	9.66 ± 0.33^{c}

The means with different letters in columns are significantly differed at P<0.05.

(df = 3,8; F = 25.22 ; P < 0.001 at 6 hours and df = 3,8; F = 140 ; P < 0.001 at 12 hours respectively) (Table 1). The calculated LD_{50} and LD_{95} values for *B. zonata* were 6.53 µl (0.61-10.17) and 18.84 µl (14.32-31.22) respectively. Dose killed 95% of flies was about three fold higher than the dose which killed 50% of *B. zonata.* The LT_{50} values were 9.60h, 9.10h and 7.16h for 0.5µl, 0.75µl and 1µl respectively. The highest LT_{50} value was recorded for 0.5µl concentration. Similarly, the calculated LT_{95} values were 15.17h, 13.91h and 10.70h for 0.5µl, 0.75µl and 1µl respectively.

DISCUSSION

Present study clearly demonstrates that mixture of low molecular weight peptide fractions of *O. odontrous* is effective against *B. zonata,* which is a serious pest of fruits and vegetables. The isolation of two new toxins from the South American scorpion *Tityus bahiensis,* Toxin TbIT-I and Tb2-II was done and Toxin TbIT-I had almost no activity or pharmacological effects in vertebrate tissues whereas it was lethal to house flies. In contrast,

Tb2-II was active against both mammals and insects [10]. The venom components from Israeli yellow scorpion were employed and expressed them in bacteria genetically. These genetically engineered toxins percolate into insect's brain and damage their nervous system, resulting in death of pests so quickly. These toxins contain poisonous effects on some insect pests and safer to mammals [11]. The venom of the scorpion *Leiurus quinquestriatus hebraeus* contains a depressant insect specific toxin counterpart [12]. This venom when injected in to blowfly larvae cause fast contraction paralysis [13]. The LqhIT2 a depressant insect toxin derived from the venom of Israeli yellow scorpion *Leiurus quinquestriatus hebraeus* (Ehrenberg), a polypeptide of 61 residues [14]. Administration of LqhIT2 toxin into blowfly larvae induced symptoms of excitatory effect, prolonged flaccidity, immobilization and ultimate death [15-17]. These investigations are supporting the effectiveness of *O. odontrous* venom against *B. zonata*. These low molecular weight peptide fractions of *O. odonturus* can be used as eco-friendly bio-pesticides.

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