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Insecticidal activity of proteinous venom from tentacle of jellyfish *Rhopilema esculentum* Kishinouye

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Abstract—Insecticidal activity of proteinous venom from tentacle of jellyfish *Rhopilema esculentum* Kishinouye was determined against three pest species, *Stephanitis pyri* Fabriciusa, *Aphis medicaginis* Koch, and *Myzus persicae* Sulzer. *R. esculentum* full proteinous venom had different insecticidal activity against *S. pyri* Fabriciusa, *A. medicaginis* Koch, and *M. persicae* Sulzer. The 48 h LC_{50} values were 123.1, 581.6, and 716.3 μg/mL, respectively. Of the three pests, *R. esculentum* full proteinous venom had the most potent toxicity against *S. pyri* Fabriciusa, and the corrected mortality recorded at 48 h was 97.86%. So, *S. pyri* Fabriciusa could be a potential target pest of *R. esculentum* full proteinous venom.

Rhopilema esculentum Kishinouye (*R. esculentum*), a cnidarian of the class Scyphozoa, the order Rhizostomeae, and the family Rhopilema, distributes widely from the South China Sea, the Yellow Sea to the Bohai Sea and is abundant in late summer to early autumn.[1] *R. esculentum* sting on swimmers and fishermen is very painful, with erythematous eruptions, itching, and burning sensations. Syndrome includes fever, fatigue, muscle ache, tight breath, dropsy, blood pressure depression, and even death.[2] Jellyfish proteinous venom from tentacle with unique structure has many bioactivities such as enzymatic activities, hemolysis, hepatocyte toxicity, cardiac toxicity, and antioxidant activity.[3–6] However, no report on the insecticidal activity has yet been made.

Stephanitis pyri Fabriciusa, *Aphis medicaginis* Koch, and *Myzus persicae* Sulzer are the main and common pests in China. They parasitize on crops, fruits, and vegetables such as corn, cabbage, cucumber, and peach, and they sting plants and suck their sap, resulting in malnutrition, retardation, and poor productivity. Additionally, they transmit most of the viruses causing disease problems.[7,8] In order to protect the plants, the organophosphates, carbamates, pyrethroids, and other classes of insecticides have been used to control pests. However, significant insect resistance has emerged. In addition, residual agrochemicals in the environment have been causing a serious impact. So, in the recent years, there has been increasing interest in the development of novel, powerful, target-selective, and environment-friendly pesticide. A multitude of insecticidal substances have already been isolated from plant, animal, and bacteria materials,[9–12] they constitute a broad range of compounds including polyphenolic compounds, halogenated diterpenes, sesquiterpenes, and piperoctadecalidine.[13–15] However, the study on the insecticidal activity of protein was comparatively deficient. Moreover, insecticidal activity of protein from marine invertebrates was never studied before to our knowledge. In this study, insecticidal activity of proteinous venom from tentacle of jellyfish *R. esculentum* against three pests mentioned above is assessed.

Proteinous venom preparation. Jellyfish *Rhopilema esculentum* were collected in the Aoshan Bay in Qingdao, Shandong Province, China, in August 2004. Tentacles were manually excised in vivo, packed in polythene bags, and frozen immediately at −20 °C. The frozen tentacles were then sonicated in cold (4 °C) phosphate

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buffer solution (0.01 M, pH 6) eight times for 30 s each time at 100 mV. The resultant fluids were clarified by centrifugation (13,000 rpm) for 20 min at 4 °C and used as *R. esculentum* full proteinous venom (RFV). The concentration was determined by the method of Bradford,[16] using bovine serum albumin (BSA) as a standard.

Collection of pests. *Stephanitis pyri* Fabriciusa (*S. pyri*), *Aphis medicaginis* Koch (*A. medicaginis*), and *Myzus persicae* Sulzer (*M. persicae*) were collected on pears, tobaccos, and kidney beans in experimental fields of Laiyang Agricultural College, respectively. Prior to the experiment, pests were maintained at 25 ± 1 °C, 50–60% relative humidity, and a photoperiod of 16:8 (L:D) h for 3 days.

Insecticidal activity bioassays. A glass platelet dipping method recommended by FAO was applied for determining insecticidal activity of RFV against *S. pyri* (imagoes), *A. medicaginis* (second instars), and *M. persicae* (second instars).[17] Three pests with the same size and age were picked up with a camel hair brush and their backs were stuck tightly on double-sided adhesive tapes (1 cm × 2 cm) on a glass platelet, with 20 on each platelet. One hour later, the pests were observed with a magnifier. Dead and improperly positioned ones were removed and then refilled the empty. The loaded glass platelets were dipped in RFV solutions, shaken gently for 5 s, and then taken out. The excess solution was absorbed with a filter paper immediately. Then the dipped platelets were held at 25 ± 1 °C, 50–60% relative humidity, and a photoperiod of 16:8 (L:D) h. There were three replicates for each of the five RFV dosages (569, 285, 143, 71.5, and 35.6 μg/mL) and phosphate buffer solution (0.01 M, pH 6) was used as control. The test pests were considered dead if appendages did not respond to a touch with a camel hair brush and the mortality was recorded at 24 and 48 h after the treatment. The mortality was corrected by Abbot's formula.[18]

SDS–PAGE. SDS–polyacrylamide gel electrophoresis was carried out according to the procedure of Laemmli.[19] Running gels containing 7% acrylamide and stacking gels of 5% acrylamide were used. RFV was diluted 1:1 with sample buffer 0.05 M Tris (pH 6.8), 2% SDS, 20% glycerol, 2% of 2-mercaptoethanol, and 0.04% bromophenol bule and then was boiled for 5 min. Gels were silver stained according to Wray et al.[20] Myosin heavy chain (200 kDa), Calmodulin-binding protein (130 kDa), rabbit phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43 kDa) were used as standards for molecular mass determination.

Statistical analysis. The results were presented as means ± SD of three parallel measurements. Data were analyzed by Student's *t* test and all tests were considered statistically significant at $P < 0.05$. The LC_{50} (median lethal dosage), LC_{95} (95% lethal dosage), and CI_{95} (95% confidence intervals) were analyzed using probit analysis performed with the statistical software SAS.

Figure 1. SDS–PAGE of RFV. Lane 1: Marker; lane 2: RFV.

SDS–PAGE. Figure 1 shows the bands of RFV. RFV was composed of several proteins and the five bands appeared after staining of the SDS–PAGE gel. The molecular mass of these bands was 64, 84, 98, 154, and 187 kDa, respectively. The components of jellyfish venom were complex. Differentia of species resulted in differentia of protein in jellyfish venom; furthermore, components of venom from the same species jellyfish were different sometimes. Radwan reported that the SDS–PAGE of CxTX showed protein bands with molecular masses ranging from 200 to below 10 kDa and Bloom reported that the SDS-CE of *Chironex fleckeri* established molecular masses of 201, 174, 112, 71, 39, and 31 kDa.[21,22] The component which was essential for the insecticidal activity to occur needs to be further investigated.

Insecticidal activity of RFV against three pests. Tables 1–3 show the insecticidal activity of RFV against *S. pyri*, *M. persicae*, and *A. medicaginis*. RFV had the strongest insecticidal activity against *S. pyri* of the three pests, and the corrected mortality at a concentration of 569 μg/mL recorded at 24 and 48 h was 70.86% and 97.86%, respectively. RFV exhibited insecticidal activity against *A. medicaginis*, and the maximal corrected mortality recorded at 24 and 48 h was 36.43% and 41.45%, respectively. RFV had low insecticidal activity against *M. persicae* and the mortality was below 31%. The insecticidal activity of RFV against three pests depended on the concentration. Table 4 shows the results of regress analysis of corrected morality recorded at 48 h. In the three pests, *S. pyri* was the most sensitive to the RFV and its 48 h LC_{50} value was 123.1 μg/mL. *A. medicaginis* and *M. persicae* had better endurance against RFV than *S. pyri*, and the 48 h LC_{50} values were 581.6 and 716.3 μg/mL, respectively. The 48 h LC_{95} value of *S. pyri* was 673.6 μg/mL, and the 48 h LC_{95} values of *A. medicaginis* and *M. persicae* were much bigger being 53.3 times and 29.4 times of that of *S. pyri*, respectively. The figures above clearly indicate some very tolerant individuals against RFV in the groups of *A. medicaginis* and *M. persicae*. In the regress equation, *b* is the slope and denotes

Table 1. The insecticidal activity of RFV against *S. pyri*

Concentration (μg/mL)	24 h		48 h	
	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)
Control	8.33		8.33	
569	73.28 ± 20.32	70.86 ± 19.65	98.04 ± 3.40***	97.86 ± 3.39
285	40.40 ± 29.11	34.99 ± 25.21	72.12 ± 23.73*	69.59 ± 22.90
143	30.20 ± 3.04**	23.86 ± 2.40	57.07 ± 8.62**	53.17 ± 8.03
71.5	23.61 ± 10.49	16.67 ± 7.41	40.28 ± 8.67*	34.85 ± 7.50
35.6	15.29 ± 6.64	7.59 ± 3.30	21.19 ± 7.76*	14.02 ± 5.13

Data are presented as means ± SD of three parallel measurements.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $n = 3$.

Table 2. The insecticidal activity of RFV against *A. medicaginis*

Concentration (μg/mL)	24 h		48 h	
	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)
Control	11.36		27.42	
569	43.65 ± 5.99**	36.43 ± 4.99	57.50 ± 17.77*	41.45 ± 12.81
285	26.35 ± 8.06*	16.91 ± 5.17	44.56 ± 1.54***	23.62 ± 0.82
143	18.13 ± 4.27*	7.64 ± 1.79	44.21 ± 11.28*	23.13 ± 5.90
71.5	10.89 ± 9.82	−0.53 ± 0.47	36.00 ± 3.63**	11.82 ± 1.19
35.6	20.34 ± 8.57	8.97 ± 3.78	33.93 ± 3.11**	10.13 ± 0.93

Data are presented as means ± SD of three parallel measurements.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; $n = 3$

Table 3. The insecticidal activity of RFV against *M. persicae*

Concentration (μg/mL)	24 h		48 h	
	Mortality (%)	Corrected mortality (%)	Mortality (%)	Corrected mortality (%)
Control	5.10		13.70	
569	11.92 ± 7.47	7.20 ± 4.51	34.39 ± 7.17	23.97 ± 4.99
285	15.82 ± 0.83*	11.30 ± 0.59	40.14 ± 12.43	30.63 ± 9.48
143	13.80 ± 8.43	9.17 ± 5.60	25.84 ± 14.74	14.06 ± 8.02
71.5	8.27 ± 7.41	3.35 ± 3.00	17.24 ± 6.64	4.10 ± 1.58
35.6	3.33 ± 2.89	−1.86 ± 1.61	16.67 ± 5.77	3.43 ± 1.19

Data are presented as means ± SD of three parallel measurements.
*$P < 0.001$; $n = 3$.

the differentia of sensitive degree against pesticides. Bigger *b* value presents more consistency of pests against pesticides. *b* value of *S. pyri* was biggest (2.23) among the three species, compared to those of *M. persicae* (1.14) and *A. medicaginis* (0.92) (Table 4). *b* values showed that the sensitivity of *S. pyri* against RFV was quite consistent; however, for *A. medicaginis* and *M. persicae*, the behavior against the RFV was quite different individually.

To *A. medicaginis*, RFV had more potency than the extraction of *Sophora alopecuroides* L. but less potency that the extraction of *Aconitum szechenyianum* Gay, two plants distributed in China widely, and the LC_{50} values of *S. alopecuroides* L. and *A. szechenyianum* Gay were 159.1 and 107.6 μg/mL, respectively.[17] To *M. persicae*, RFV had more potent toxicity than those pesticides such as pirimicarb and dimethoate, and the LC_{50} values of pirimicarb and dimethoate were 1583 and 4543 μg/mL, respectively, but was less potent than pesticides such as imidacloprid and emulsion of Metam-Sodium, and the LC_{50} values of imidacloprid and emulsion of Metam-Sodium were 198.6 and 45.3 μg/mL, respectively.[23] Possible reasons for low insecticidal activity of RFV against *A. medicaginis* and *M. persicae* were interpreted from three factors: (1) *A. medicaginis* and *M. persicae* were not target pests of RFV. (2) The concentration of RFV was too low to kill most of

Table 4. Results of regression analysis of corrected morality recorded at 48 h

Pests	Regress equation	Correlative coefficient (*r*)	LC_{50} (CI_{95}) μg/mL	LC_{95} (CI_{95}) μg/mL
S. pyri	$Y = 7.03 + 2.23X$	0.9653	123.1 (96.6–156.9)	673.6 (528.7–858.3)
A. medicaginis	$Y = 5.22 + 0.92X$	0.9696	581.6 (240.6–1406)	3.59×10^4 (1.49×10^4–8.69×10^4)
M. persicae	$Y = 5.17 + 1.14X$	0.9222	716.3 (296.5–1731)	1.97×10^4 (0.82×10^4–4.79×10^4)

Y: Probit of corrected mortality recorded at 48 h; *X*: Log (Dos).

A. medicaginis and *M. persicae*. (3) External conditions such as temperature, sunlight, and microorganism might deactivate the RFV.

The study on the insecticidal activity of protein was comparatively deficient and mainly focused on the bacteria *Bacillus thuringiensis*.[24–26] Recently, Castro reported the identification and molecular cloning of insecticidal toxins from the venom of the brown spider *Loxosceles intermedia*;[27] also, Zhang reported a novel insecticidal peptide toxin from the spider *Selenocosmia huwena*.[28] In the present study, insecticidal activity of proteinous venom from tentacle of *R. esculentum* was first investigated providing a useful beginning for the development of bioinsecticides from marine biology. Moreover, proteinous venom of jellyfish is mainly in tentacles but the tentacles are discarded as waster because of their high content of water and venomous sting. Thus, assaying the insecticidal activity of proteinous venom from tentacle of *R. esculentum* will lead to a clean and economic application of *R. esculentum*.

Of the three pests, *S. pyri* was the most sensitive to RFV, so *S. pyri* could be the potential target pest of RFV. For *A. medicaginis* and *M. persicae*, the morality may be improved by enhancing stabilization and concentration of RFV. The purification of insecticidal protein from RFV and the insecticidal mechanism are under investigation.

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