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Insecticidal, insect repellent, cytotoxic and larvicidal activities of *Cnesmone javanica* Blume extracts

Samama Begum Chhabi[1], Dipali Sarkar[1], Shahriar Zaman[1], Sadequl Islam[2], Zaidur Rahman Sabuj[2] and Nurul Islam[2*]

[1] Department of Genetic Engineering and Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh.

[2] Department of Zoology, University of Rajshahi, Rajshahi-6205, Bangladesh.

Abstract: Leaf, flower, stem wood, stem bark and roots of *Cnesmone javanica* Blume extracted into petroleum ether, chloroform and methanol have thoroughly been screened for their dose mortality, insect repellency against *Tribolium castaneum* adults, cytotoxicity against *Artemia salina* nauplii and larvicidal activity against *Culex quinquefasciatus* under laboratory conditions. The CH_3OH extract of *C. javanica* root showed the highest mortality against *T. castaneum* with LD_{50} value of 0.24 mg cm^{-2} after 48 hours of exposure. The Pet. ether, $CHCl_3$ and CH_3OH extracts of different parts of *C. javanica* showed repellent activity against *T. castaneum* adults at $P<0.01$ and $P<0.05$ levels of significance. Methanol extract of flower showed activity against *A. salina* nauplii and *C. quinquefasciatus* larvae and gave LC_{50} values of 1.4E-07ppm and 837.49 ppm after 30 hours of exposure respectively. The susceptibility of the test agents to the toxicity of *C. javanica* extracts could be arranged in a descending order of chloroform> Pet. ether > methanol.

Key words: *Cnesmone javanica*, Dose-mortality, Repellency, Cytotoxicity, Larvicidal activity, *Tribolium castaneum*, *Artemia salina, Culex quinquefasciatus*.

Introduction

Cnesmone javanica Blume (Euphorbiaceae) is found in Assam, Myanmar, Indo-China, Thailand, Malay Peninsula, Sumatra, Java, Bali and Borneo. In Bangladesh the common name of this plant is 'Rakhal khoskhoshi' or 'Agnishwar' and is found mainly in North Bengal, Chittagong Hill Tracts, Cox's Bazar and Sylhet. The plant is strongly hirsute, covered with stinging hairs. It exhibits a wide spectrum of indigenous medicinal uses (Prain, 1857). The seeds are used as coriander in curry in Patna. The juice of the root is used in long-standing fevers in North Lakhimpur. In Kelantan (Malaya) it is used as a poison by criminals while the flowers and leaves are mixed in cakes that may cause death. The chemical composition of this plant is not yet investigated so much, however the information are available only on the cellulose properties of *C. javanica* which are highly biocidal in nature while tested on mustard and radish plants (Ghayal *et al.,* 2008).

The present investigation was carried out to find out the insecticidal, insect repellency against *Tribolium castaneum*, cytotoxicity or lethality against *Artemia salina* nauplii and larvicidal activity against *Culex quinquefasciatus. T. castaneum* is a worldwide pest of stored products, particularly food grains, and a model organism for food safety research (Grunwald, 2013). The plant derived chemicals have been used potential seed protectant (insecticides and antifeedants) often begins with screening of plant extracts (Pavela, 2007). Therefore, it is important to follow proper pest management practices to reduce potential insect vectors in feeds and in the feed manufacturing environments (Lakshmikantha *et al.,* 2009). The *A. salina* belong to a genus of very primordial crustacean (crawfish-crayfish) the *Anostraca*, and it is very nice to grow. It is a possible vector for white spot syndrome virus (WSSV) transmission to *Penaeus indicus* (Indian prawn) and *Macrobrachium rosenbergii* nodavirus (MrNV) and its associated extra small virus (XSV) transmission to *M.*

Corresponding author: n_islamm@yahoo.com

rosenbergii post-larvae (Sudhakaran *et al*., 2006; Hameed *et al*., 2002).

Human lymphatic filariasis, alternatively known as infectious tropical disease (Hati, 2002), caused by the worms *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* transmitted by female *C. quinquefasciatus* mosquito from man to man (Bockarie, 2009). *W. bancrofti* individuals exploit human body as their definitive host for survival efficiently transmitted by the vector *C. quinquefasciatus*. Stored product insect pests have been reported to develop resistance to synthetic insecticides (Benhalima *et al.*, 2004). There is no sufficient information available on the biological activities of *C. javanica*, and this led to the present investigation.

Materials and Methods

Plant collection and preparation: The plant of *C. javanica* was collected from the district of Gybandha, Bangladesh, and identified by a plant taxonomist in the Department of Botany, University of Rajshahi, Bangladesh. The plant part materials viz. leaf, flower, stem wood, stem bark and roots were chopped into small pieces, dried under shade and powdered by using a grinder, weighed and placed in separate conical flasks to add Pet. ether, $CHCl_3$ and CH_3OH (Merck, Germany) (100gm × 300ml × 2times) for 48h. Filtration was done by Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract were left. The extracts were then removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

Collection and culture of insects: Adult *T. castaneum* were reared in glass beakers (500ml) in a standard mixture of whole-wheat flour (Park, 1962; Park and Frank, 1948) with powdered dry yeast (19:1) in an incubator at 30 ±0.5°C without light and humidity control for continuous supply of adults during experimentation.

Brine shrimp eggs were purchased from Kalabagan, Dhaka and kept in aerated seawater at (25-30°C) room temperature and took 30-48h to give nauplii. Egg strips of *C. quinquefasciatus* were assembled with acute precision from some drains contiguous to the campus of the University of Rajshahi and reared in the Insect and Microbiology Research laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, and placed in a 500ml beaker containing pond water. The pond water was filtrated and the rafts were placed carefully on a rack in the laboratory at room temperature. After 24 hours the larvae were hatched out and collected for their use in the experiments.

Dose-mortality test: The doses were prepared through serial dilution technique and the dose-mortality responses of different parts of *C. javanica* were observed by surface film method. The concentrations were used for leaf in Pet. ether: 1.783, 1.529, 1.274, 1.019, 0.764 and 0.510 mg cm^{-2}; in CH_3OH: 0.510, 0.408,0.306, 0.204, 0.102, 0.005,0.003, 0.001 and 0.0006 mg cm^{-2}; in $CHCl_3$: 1.273, 1.146, 1.019, 0.891 and 0.764 mg cm^{-2}; for flower in Pet. ether: 1.273, 1.146, 1.019, 0.891 and 0.764 mg cm^{-2}; for roots in CH_3OH: 0.510, 0.408, 0.306, 0.204, 0.102 mg cm^{-2}; for stem bark in CH_3OH: 0.510, 0.408, 0.306, 0.204, 0.102 mg cm^{-2}; for stem wood in CH_3OH: 0.510, 0.408, 0.306, 0.204, 0.102 mg cm^{-2}. Each of the doses were diluted in 1ml of solvent, poured into each of the Petri dishes and allowed them to dry. Ten adult *T. castaneum* were released separately in each of the Petri dishes, and the experiment of the extracts were replicated thrice. The mortality was assessed after 12, 24, 36 and 48h of exposures.

Statistical analysis: Mortality (%) was corrected using Abbott's formula (Abbott, 1925). The data were then subjected to Probit analysis according to Finney (1947) and Busvine (1971).

Repellent activity: The repellency test was adopted from the method (No. 3) of McDonald *et al.* (1970) with some modifications. Half filter paper discs (Whatman No. 40, diameter 9 cm) were treated with the selected doses of 0.629, 0.315, 0.157, 0.079, 0.039 and 0.011 mg cm^{-2} concentrations for Pet. ether extract and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. The orientation was changed in the two remaining replicates to avoid the effects of any external directional stimulus affecting the distribution of the test insects. Ten adult insects were released in the middle of each of the filter paper circles. The similar process was done for the $CHCl_3$ and CH_3OH extracts as well.

The concentration of extracts in each of the solvents was tested for five times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was

converted to percent repellency (*PR*) using the formula of Talukder and Howse (1993, 1995): *PR* = (Nc – 5) × 20, where, Nc is the percentage of insects on the untreated half of the disc. The data was subjected to ANOVA.

Brine shrimp nauplii lethality test: Different concentrations of *C. javanica* extracts collected in all the three solvents were 600, 500, 400, 300 and 200 ppm. Ten freshly hatched nauplii were added to each of the test tubes with the concentrations and observed mortality after 12, 18, 24 and 30 h of exposure. The data was subjected to Probit analysis.

Larvicidal activity test: A series of concentrations of *C. javanica* were 700, 600, 500, 400 and 300 ppm for extracts collected in all the three solvents. Ten newly hatched larvae were added to each of the test tubes containing the concentrations mentioned above. The mortality was observed after 6, 12, 18, 24 and 30 h of exposure. The data was subjected to Probit analysis.

Results

Dose mortality effects: The root extract of CH_3OH offered highest mortality giving LD_{50} values ranged between 31.13 to 0.24 mg cm^{-2} followed by the leaf extract of CH_3OH ranged between 2.14 to 0.33 mg cm^{-2}; and the lowest mortality for Pet. ether and $CHCl_3$ extracts of leaves ranged between 2.12 to 0.68 mg cm^{-2} and 3.56 to 0.68 mg cm^{-2} respectively (Table 1).

Repellent activity: The Pet. ether extract of flower, stem bark, stem wood and roots; the $CHCl_3$ extract of flower, leaf and stem wood; and the CH_3OH extract of stem wood of *C. javanica* offered repellent effects against *T. castaneum* adults. The Pet. ether extract of flower and CH_3OH extract of stem wood showed repellency at 1% level of significance ($P<0.01$) and the $CHCl_3$ extracts of flower, leaf, stem wood and Pet. ether extracts of stem bark, stem wood and roots offered repellency at 5% level of significance ($P<0.05$), while the other extract of the plant parts did not show any repellency (Table 2).

Brine shrimp lethality: The CH_3OH extract of flower offered the highest activity with LC_{50} value 1.4E-08 ppm, followed by the CH_3OH extract of stem bark with LC_{50} value 2.93E-9 ppm and Pet. ether extract of roots with LC_{50} value 4.86 ppm after 30 h of exposure (Table 3).

Larvicidal effect: All the extracts showed activity against the 1-day old larvae of *C. quinquefaciatus,* where the highest lethality offered by CH_3OH extract of stem bark with LC_{50} value 83.17 ppm followed by the $CHCl_3$ extract of stem wood with LC_{50} value 221.24 ppm; and the lowest lethality observed for the $CHCl_3$ extract of flower with LC_{50} value 837.49ppm after 30 h of exposure (Table 4).

Table 1: LD_{50} values of *C. javanica* extracts against *T. castaneum* adults.

Plant part	Solvent of extraction	LD_{50} at different exposures (in hours)			
		12	24	36	48
Flower	Pet. ether	0.72	0.64	0.511	0.56
Leaves	Pet. ether	1.09	0.85	0.76	0.68
	$CHCl_3$	1.05	0.79	0.710	0.68
	CH_3OH	2.14	0.47	0.41	0.33
Stem wood	CH_3OH	-	11.94*	1.18*	0.65*
Stem bark	CH_3OH	4.59	3.82	0.46*	0.34*
Roots	CH_3OH	31.13	0.31	0.29	0.24

* Variance has been adjusted for heterogeneity

Table 2: ANOVA results of repellency by the *C. javanica* extracts on *T. castaneum*.

Plant part	Solvent of extraction	Sources of Variation			F-ratio with level of significance		P- value	
		Between doses	Between time interval	Error	Between doses	Between time interval	Between doses	Between time interval
Flower	Pet. ether	4	4	16	21.91**	5.48	2.51E-06	0.005
	$CHCl_3$	4	4	16	13.51*	0.20	5.33E-05	0.933
	CH_3OH	4	4	16	7.67	0.45	0.001192	0.772
Leaves	Pet. ether	4	4	16	1.04	1.02	0.417639	0.429
	$CHCl_3$	4	4	16	11.07*	1.36	0.00017	0.291
	CH_3OH	4	4	16	5.48	1.14	0.005646	0.375
Stem Bark	Pet. ether	4	4	16	19.52*	2.55	5.35E-06	0.080
	$CHCl_3$	4	4	16	3.96	1.27	0.020324	0.321
	CH_3OH	4	4	16	4.89	1.72	0.00909	0.196
Stem wood	Pet. ether	4	4	16	11.45*	0.38	0.00014	0.821
	$CHCl_3$	4	4	16	8.66*	2.39	0.000645	0.094
	CH_3OH	4	4	16	27.28**	0.406	5.7E-07	0.802
Roots	Pet. ether	4	4	16	17.79*	0.38	9.72E-06	0.820
	$CHCl_3$	4	4	16	2.55	0.36	0.079413	0.833
	CH_3OH	4	4	16	4.24	3.13	0.015811	0.044

** = Significant at 1% level (P<0.01) * = Significant at 5% level (P<0.05)

Table 3: LC_{50} values of Pet. ether, $CHCl_3$ and CH_3OH extracts of *C. javanica* flower, leaf, stem bark, stem wood and root extracts against *A. salina* nauplii.

Plant part	Solvent of extraction	LC_{50} (ppm) at different exposures (in hours)				
		6	12	18	24	30
Flower	Pet. ether	*	1063.72	568.16	543.79	513.38
	$CHCl_3$	*	*	*	*	*
	CH_3OH	185.67	83.23	43.37	1.04E-07	1.4E-08
Leaves	Pet. ether	*	740.89	375.93	311.22	219.35
	$CHCl_3$	334.58	205.97	144.82	60.05	28.37
	CH_3OH	*	*	*	*	*
Stem bark	Pet. ether	*	*	148.76	95.40	5.76
	$CHCl_3$	*	587.35	316.82	292.40	229.97
	CH_3OH	558.61	78.73	2.28E-03	1.14E-7	2.93E-9
Stem wood	Pet. ether	503.34	514.65	498.21	278.38	137.73
	$CHCl_3$	*	*	338.55	87.77	51.62
	CH_3OH	*	999.60	54.44	40.95	33.08
Roots	Pet. ether	50.83	19.59	13.31	8.19	4.86
	$CHCl_3$	*	503.27	212.19	86.77	68.57
	CH_3OH	341.20	173.46	24.45	26.45	15.314

*Poorly active, where high throughput was required to ensure mortality.

Table 4: LC_{50} values of Pet. ether, $CHCl_3$ and CH_3OH extracts of *C. javanica* flower, leaf, stem wood, stem bark and roots against *C. quinquefasciatus* larvae.

Plant part	Solvent of extraction	LC_{50} (ppm) at different exposures (in hours)				
		6	12	18	24	30
Flower	Pet. ether	*	567.90	379.96	325.75	323.33
	$CHCl_3$	*	*	*	997.60	837.49
	CH_3OH	782.25	666.77	513.94	420.17	312.25
Leaf	Pet. ether	*	829.84	441.01	347.17	239.62
	$CHCl_3$	*	666.98	513.43	377.00	320.75
	CH_3OH	*	*	*	771.16	464.03
Stem wood	Pet. ether	*	799.83	719.74	582.23	506.42
	$CHCl_3$	*	568.70	408.18	273.36	221.24
	CH_3OH	-	-	-	-	-
Stem bark	Pet. ether	*	800.24	640.51	406.55	334.32
	$CHCl_3$	208.16	134.17	108.83	87.61	83.17
	CH_3OH	-	-	-	-	-
Roots	Pet. ether	*	897.97	783.32	577.06	424.19

*Poorly active, - no activity at all.

Discussion

It is interesting to note that these plant extracts showed higher to moderate mortality activity. Hence, the present result reports that this toxic as well as medicinal plant bears killing and repealing potentials in its organs. These findings receive supports from previous researchers' achievements (Khan *et al.,* 2013); and the test plant *C. javanica* is also used in the treatment of snake bite and blood cancer by Chakma people of Bangladesh (Roy *et al.,* 2008). It's root cause diarrhea if eaten or drunk as water decoction. In conclusion, the present research revealed that the *C. javanica* extracts has strong insecticidal and repellent effects against *T. castaneum* adults, lethal effect against *A. salina* nauplii and *C. quinquefasciatus* larvae.

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