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Insecticidal and Repellent Activity of *Clerodendrum viscosum* Vent. (Verbenaceae) Against *Tribolium castaneum* (Herbst) (Coleoptera:tenebrionoidea)

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Abstract: In continuation of our study for plant origin substances with insecticidal effects, we have screened out root, leaf and stem of *C. viscosum* against red flour beetle *T. castaneum,* extracted into ethyl alcohol, ethyl acetate and chloroform. A correlation was found between the insecticidal and repellent activity observed by residual film assay technique and repellency test. It is interesting to note that these plant extracts showed higher to less mortality activity by using 3.93, 1.97, 0.98, 0.49 and 0.25 mgcm^{-2} of doses. The highest degree of toxicity 88% mortality was found using root and stem extracts. The LC_{50} values 0.252208 along with 95% confidence limit 0.095135 for stem and LC_{50} values 0.2863626 along with 95% confidence limit 0.125406 for root were showed at 72h of observation. The repellent activity was done using ethyl alcohol fraction of *C. viscosum.* All the extracts showed 100% repellency (Class V category repellency) in dose no 1 to dose no 5 except stem. The degree of repellency response among the three parts of test insect was significantly different ($P < 0.05$). Class V category repellency was observed for root up to dose 0.06 mgcm^{-2} (Dose no 7). Whereas, the lowest dose at which the leaf exhibited class V category repellency was at 0.12 mgcm^{-2} (Dose no 6) and stem showed class V category repellency at the lowest 0.25 mgcm^{-2} (Dose no 5). The root extract showed strong repellent activity than the leaf and stem. The susceptibility of contact toxicity in intensity of solvents were found ethyl alcohol > ethyl acetate > chloroform fraction. The dose-mortality order of the *C. viscosum* extracts is root > stem > leaf in comparison with insect to *T. Castaneum.*

Key words: Residual film assay · Ethyl alcohol · Contact toxicity · Probit mortality · Log dose

INTRODUCTION

Today, humans are waging an undeclared war against insects in the competitive struggle for existence and almost no crop in the World is free from attack by insects, at least to some degree [1]. Farmers have been using plant extracts in pest control for centuries. Botanical insecticides are one of the best alternatives for the hazardous chemical insecticides. Phytochemicals are able to induce different types of abnormalities in insects that could safely be used for insect pest control [2]. Plants powder can be used as one of the component for IPM programmers, which could further reduce the application of the synthetic chemicals [3]. Different solvents are utilized, the most commonly used as acetone, chloroform, ethanol, hexane, petroleum ether, ethyl acetate or methanol. Extracts have been applied in various forms of insect bioassay; residual film assay technique or topical application as insecticidal test, repellency trials etc.

Published information on the toxic effects of plant products as well as extracts especially of *C. viscosum* against stored product insect pests are poorly presented and this lead to the present experiment is carried out on root, leaf and stem of *C. viscosum* Vent. (Verbenaceae) using three solvents (ethanol, ethyl acetate and chloroform) against red flour beetle *T. castaneum*

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(Herbst). *Tribolium castaneum* is an important stored product insect in grain storage [4]. *Tribolium castameum* live on cracked grain on breakfast food or meal, rice, dried fruit, bleached and unbleached wheat flour, cornmeal, barley flour and at meal [5]. In Bangladesh, *T. castaneum* is abundantly found in stored grain of different cereals. The genus *Clerodendrum* exhibits a wide spectrum of folk and indigenous medicinal uses [6-9]. It is logical to expect biologically active compounds to be produced by plants as a chemical defense measure against their enemies [10].

MATERIALS AND METHODS

Plant Collection and Identification: The plant specimen was collected from PRajshahi University campus, Rajshahi-6205, Bangladesh. Identification of voucher specimen was confirmed at the taxonomical section, Department of Botany, Rajshahi University, Bangladesh and dried in the shade. The Plant materials root, leaf and stem were powdered in a grinding machine. Before grinding the plants were well dried in an oven at 37±0.5°C for overnight.

Insects: Adults of *T. castaneum* were collected from the Crop Protection Lab of the Department of Agriculture and Environmental Science, University of Newcastle upon Tyne, UK and successfully reared for more than a decade in the Crop Protection and Toxicology Lab of the Institute of Biological Science, Rajshahi University. Mass cultures were maintained in Jars (1000 ml) containing food medium and kept in an incubator at 30±0.5°C and 70-80% RH. A standard mixture of sterilized (at 60°C for 24 hours) whole-wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium in the experiments [11- 13].

Output of Extracts: The root, leaf and stem were extracted separately with ethyl alcohol, chloroform and ethyl acetate. The extracts condensed using rotary evaporator and weighed. The yield of the extracts according to solvents and plant parts are presented in Table 1.

Pilot Experiment of Application of *C. Viscosum:* Same ages of 25 adult pests were used for making different doses of this experiment with five replications. The pilot experiments were carried out on the basis of indications made by the produces for the users, to obtain doses in which mortality rate was in between 12-88% (Table 3). Similar findings were reported on another plant against *T. castaneum* [14]. The actual doses were calculated from the amount of extract present in 1 ml of the solution and then the amount of active ingredient was also worked out. The calculated amount of the active ingredient of the extract was expressed in mg cm^{-2}.

Table 1: Extracts from different parts of *C. viscosum* with different solvents

Plants part	Solvents	Wt. of dust. (g)	Wt. of ext. (g)
Root	Ethyl alcohol	100	10.0
	Ethyl acetate	100	6.25
	Chloroform	100	6.25
Leaf	Ethyl alcohol	100	12.5
	Ethyl acetate	100	6.25
	Chloroform	100	6.25
Stem	Ethyl alcohol	100	6.25
	Ethyl acetate	100	3.12
	Chloroform	100	6.25

Dose preparation and Bioassay: From the result of pilot experiment a dose was selected. That was considering as the stock solution and then the final doses were made by serial dilution technique with the same solvent at the proportion of 1: 2 in treatments. Five different condensed extracts *viz.* 12.5, 10.0, 6.25 and 3.125 gram were dissolved in requisite amount of respective solvents and transferred uniquely into 3.93, 1.97, 0.98, 0.49 and 0.25 mg cm^{-2} concentrations by measuring the dry-weight of extracted materials and divided by the surface area of petri dish (90 mm) according to the residual film method [15] The extract was dispersed into each petri dish for each dose. After evaporating the solvents from the petri dish 25 same-aged (3-5 day old) adult *T. castaneum* was released separately by an aspirator sub sequentially control batch was maintained with respective solvent only. Then the petri dish kept within an incubator at 30±0.5°C. The mortality was recorded after 24, 48 and 72 h of exposure. The dose was calculated by measuring the dry-weigh of the crude extracts applied in petri dish divided by surface area of respective petri dish.

Statistical Analysis: Mortality percentage was corrected using Abbott's formula [16]. Then this percentage of mortality was subjected to statistical analysis according to Finney [17].

Repellency Test: To study the repellent activity of plant extracts on the basis of McDonald *et al*. [18] with some modification, filter paper strips (Whatman No. 40) 9 cm in diameter cutting in half. To make stock solution 10 mg-dried samples was dissolved into 1 ml ethyl alcohol and considered as the highest dose. The eight doses of 3.93, 1.97, 0.98, 0.49, 0.25, 0.12, 0.06 and 0.03 (mg cm^{-2}) were made by serial dilution technique at the proportion 1:2 from the stock solution and were used in repellency

Table 2: Average value of repellency rate of *T. castaneum*

Class	Repellency rate (%)	Class	Repellency rate (%)
0	>0.01 to <0.1	III	40.1 to 60
I	0.1 to 20	IV	60.1 to 80
II	20.1 to 40	V	80.1 to 100

treatments of the subjected insects. one ml solution of plant extract from the respective dose was applied uniformly on the half of filter papers with a micropipette. Then the treated half circles were air dried to evaporate the solvent completely. Attaching a treated half to an untreated half circle of the same dimension by transparent non-toxic adhesive tape full circle then remake one. Ten insects were released on the centre of each filter paper circles and petri dishes were covered immediately. There were three replications for each treatment. Counts of the insects present on each strip were made at one-hour interval up to five hours observation. The averages of counts were converted into percentage repulsion (PR) using the formula of Talukder and Howse [19, 20]:

$$PR = (N_c - 5) \times 20$$

Where, N_c is the number of insects present in the control half.

Positive values (+) indicated repellency and negative values (-) attraction.

The average values were then categorized according to the following scale presented in Table 2.

Statistical Analysis: Repellency activity test was conducted according to complete randomized experimental design with three replications for each treatment. Using Duncan's Multiple Range Test (DMRT) as described by Steel and Torrie [21] did repellent significance. Data on percentage were subjected to arcsine transformation of the proportion before analysis and were transformed back to percentage of presentation.

RESULTS AND DISCUSSION

The mortality (%) of *T. castaneum* adults treated with root, leaf and stem of *C. viscosum* extracts within three solvents. Ethyl alcohol stem extract of *C. viscosum* showed highest mortality activity 88% and 48% (Table 3), LC_{50} value was 0.25 and 0.42 mg/ml at 72 h and 24 h of exposure. Though root extract also exposed significant activity 88% and 44% (Table 3) with LC_{50} value 0.29 and 0.52 mg/ml at 3.93 and 0.25 mg cm^{-2} doses but the leaf extract was found less toxic (56% and 28% mortality with 1.65 and 2.94 of LC_{50} value) than the stem and root extracts respectively at 72 h and 24 h of exposure. Regression equations are presented in Fig. 1. The ethyl acetate fraction of root exposed a significant dose mortality 84% and 72% with the recorded LC_{50} value were found 0.44 and 0.95 mg/ml. Stem and leaf extracts of ethyl acetate fraction were also showed promising activity. 76% mortality (Table 3) with 0.67 and 1.50 mg/ml of LC_{50} value for stem; 0.78 and 1.60 mg/ml of LC_{50} value for leaf respectively 72 h and 24 h of exposure period. Regression equations are shown in Fig. 2. Contact poisoning of chloroform fraction showed the moderate to lesser activity 60% and 24% mortality rate with 1.46 and 5.75 mg/ml of LC_{50} value for root extract; 10.60 and 15.85 mg/ml of LC_{50} value for stem extract; 15.89 and 26.70 mg/ml LC_{50} value for leaf extract but the mortality rate were observed 36% and 32% for both stem and leaf extracts. Regression equations are presented in Fig. 3. The 95% Confidence of the mortality of beetles is presented in Table 4.

The result of the current investigation described above that the contact insecticidal properties of the ethyl alcohol fraction of stem extract showed although the highest degree of toxicity 88% mortality and the LC_{50} values 0.25 mg/ml along with 95% confidence limit 0.095-0.67 at 72 h of observation. But the overall assessment suggested that the root extracts showed most potency using three kind of fraction. Basically, ethyl alcohol extract of root exposed the equal toxicity 88% mortality and LC_{50} values 0.29 mg/ml along with 95% confidence limit 0.13-0.65 at 72 h of observation. The susceptibility of contact toxicity in intensity of solvents were found ethyl alcohol > ethyl acetate > chloroform fraction. The dose-mortality order of the *C. viscosum* extracts is root > stem > leaf in comparison with insect to *T. castaneum*.

On the basis of the susceptibility of contact toxicity in intensity of solvent was ethyl alcohol > ethyl acetate > chloroform fraction. So, the repellent activity was done using ethyl alcohol fraction of root, leaf and stem of *C. viscosum.* All the extracts showed 100% repellency (Class V category repellency) in dose no 1 to dose no 5 (Table 5) except stem. The degree of repellency response among the three parts of test insect was significantly different ($P < 0.05$). Class V category repellency was observed for root up to dose 0.06 $mgcm^{-2}$ (Dose no 7). Whereas, the lowest dose at which the leaf exhibited class V category repellency was at 0.12 $mgcm^{-2}$ (Dose no 6) and stem showed class V category repellency

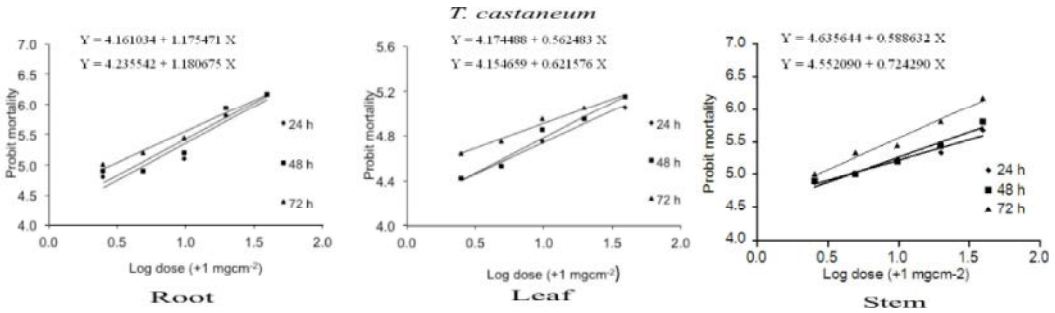

Fig. 1: Probit regression lines between probit mortality of *T. castaneum* and log dose (+1 mg cm^{-2}) of ethyl alcohol extracts of *C. viscosum* after 24, 48 and 72h exposure.

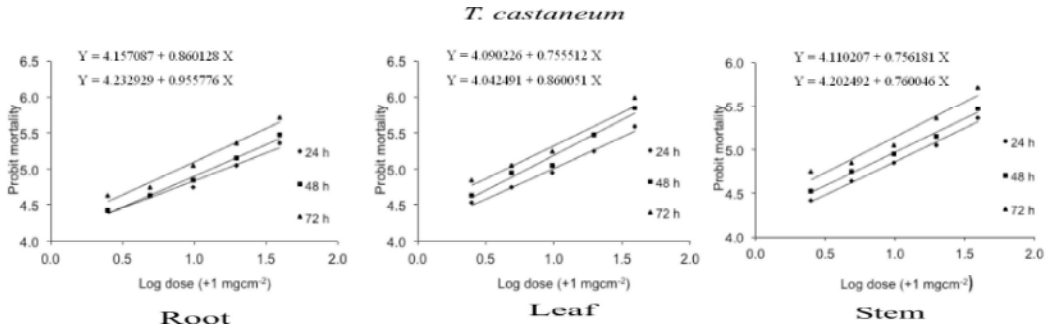

Fig. 2: Probit regression lines between probit mortality of *T. castaneum* and log dose (+1 $mgcm^{-2}$) of ethyl acetate extracts of *C. viscosum* after 24, 48 and 72 h exposure.

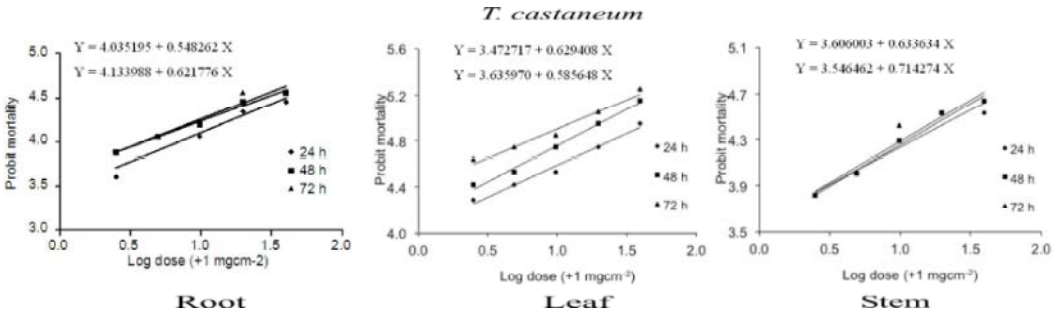

Fig. 3: Probit regression lines between probit mortality of *T. castaneum* and log dose (+1 $mgcm^{-2}$) of chloroform extracts of *C. viscosum* after 24, 48 and 72 h exposure.

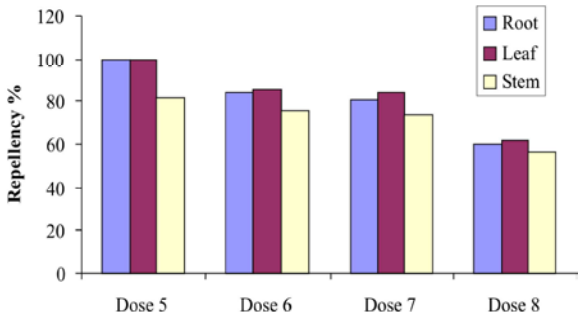

Fig. 4: Repellency effect of ethyl alcohol extract on *T. castaneum*

Table 3: Mortality (%) of *T. castaneum*

		Root		Leaf		Stem	
Exp. (h)	Dose mgcm^{-2}	Kill	%kill	Kill	%kill	Kill	%kill
		Ethyl alcohol extract					
24	3.93	22	88	13	52	19	76
	0.25	11	44	7	28	12	48
72	3.93	22	88	14	56	22	88
	0.25	13	52	9	36	13	52
		Ethyl acetate extract					
24	3.93	18	72	16	64	16	64
	0.25	8	32	7	28	7	28
72	3.93	21	84	19	76	19	76
	0.25	11	44	9	36	10	40
Chloroform extract							
24	3.93	12	48	8	32	8	32
	0.25	6	24	3	12	3	12
72	3.93	15	60	9	36	9	36
	0.25	9	36	4	16	3	12

No of insects=25

Table 4: 95% Confidence of mortality ranged of *T. castaneum*

	95% Confidence		
	Lower	Lower	
	Root	Leaf	Stem
	72h of Exposure		
Fraction	24h of Exposure		
Ethyl alcohol	0.12 - 0.65	0.45 - 6.06	0.09 - 0.66
	0.30 - 0.87	0.75 - 11.37	0.13 - 1.31
Ethyl acetate	0.19 - 0.95	0.43 - 1.39	0.33 - 1.33
	0.52 - 1.74	0.75 - 3.41	0.71 - 3.14
Chloroform	0.52 - 4.06	1.10 - 229.55	1.36 - 82.43
	0.82 - 40.24	0.97 - 731.68	1.13 - 221.48

Table 5. Repellency effect of ethyl alcohol extract on *T. castaneum*

Mean repellency values					Mean repellency %		
Sl. Nos.	Dose (mgcm^{-2})	Root	Leaf	Stem	Root	Leaf	Stem
Dose 1	3.93	10.0	10.0	10.0	100a	100a	100a
Dose 2	1.97	10.0	10.0	10.0	100a	100a	100a
Dose 3	0.98	10.0	10.0	10.0	100a	100a	100a
Dose 4	0.49	10.0	10.0	10.0	100a	100a	100a
Dose 5	0.25	10.0	10.0	8.20	100a	100a	82.0b
Dose 6	0.12	8.53	8.40	7.53	85.3b	84.0b	75.3c
Dose 7	0.06	8.40	8.06	7.40	84.0b	80.6b	74.0c
Dose 8	0.03	6.17	6.00	5.66	61.7c	60.0c	56.6c

Means within a column having the same letter are not statistically significant ($P < 0.05$) according to Duncan's Multiple Range Test.

at the lowest 0.25 mgcm^{-2} (Dose no 5). The root extract showed strong repellent activity than the leaf and stem (Figure 4).

Leaf extract of *C. viscosum* possesses repellent properties to adult and larvae of *T. castaneum* [22]. Further, Husain and Hasan [23] were observed that both the larvae and adults of flour beetle, *T. confusum* were repelled by contact with different food media when mixed with leaf dusts of the plant. The above findings of the present study are in general agreement with the findings of [22-24]. Alam *et al.* [25] conducted an investigation on other plant showed that residual film toxicity and repellent toxicity against *T. castaneum.*

In continuation of our search for substances of plant origin with insecticidal effects, we have screened out root, leaf and stem of *C. viscosum,* extracted into ethyl alcohol, ethyl acetate and chloroform. A correlation was found between the insecticidal and repellent activity observed by residual film assay technique and repellency test. It is interesting to note that these plant extracts showed higher to less mortality activity. Our previous study indicates promising antibacterial and antifungal properties of *C. infortunatum* against life threatening pathogens [26]. In conclusion, the present research reveled that the *C. viscosum* extracts has strong insecticidal and repellent effect against *T. castaneum.*

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