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MORTALITY AND REPELLENT ACTIVITY OF *CLERODENDRUM VISCOSUM* VENT. (VERBENACEAE) AGAINST *SITOPHILUS ORYZAE* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT: In continuation of our study we have screened out root, leaf and stem of *C. viscosum* against rice weevil, *S. oryzae* extracted into ethanol, 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 doses. From the overall assessment suggested that the root extracts showed most potency. Ethanol fraction of root extract exposed 44-84% mortality with 0.39 mg/ml of LC₅₀ value at 72h of exposure and 95% confidence limit was 0.19 - 0.80. Repellency test carried out ethanol fraction. All the extracts showed 100% repellency (Class V category repellency) in dose no 1 to dose no 5. 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 and leaf up to dose 0.06 mgcm⁻² (Dose no 7). Whereas, the lowest dose at which the stem exhibited class V category repellency at 0.12 mgcm⁻². 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.

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 degree¹. 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 control². This method of pest control provides an ideal source of low cost, safe and effective pesticides. Extracts of plant products rely on the solubility of the active compounds. 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 mortality test, repellency trials etc.

The plant bhant tree, *Clerodendron viscosum* Vent. (Verbenaceae), exhibits a wide spectrum of folk and indigenous medicinal uses^{3, 4, 5, 6} but little

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information about its bio-efficacy against stored product insect pest rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae). This scarcity was addressed in the present experiment using three solvents (ethanol, ethyl acetate and chloroform) carrying on root, leaf and stem extracts. *S. oryzae* is the most widespread and destructive major insect pest of stored cereals throughout the world. Female rice weevil oviposits directly into the seeds and completes larval development inside the seeds and emerges as adults. *S. oryzae* has been reported to developed resistance to synthetic insecticides⁷. The plant-derived chemicals have been used as potential seed protectant (insecticides and antifeedants) often begins with the screening of plant extracts⁸. It is logical to expect biologically active compounds to be produced by plants as a chemical defense measure against their enemies⁹.

MATERIALS AND METHODS:

Plant collection and identification-The plant specimen was collected from Rajshahi 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 $37\pm 0.5^{\circ}\text{C}$ for overnight.

Insects- Adults of *S. oryzae* were collected from the wheat infested rearing cultures maintained in the Control Temperature (CT Room $30\pm 0.5^{\circ}\text{C}$) and 70-80% RH with photoperiod 12h L: 12h D), Integrated Pest Management (IPM) laboratory, Institute of Biological Sciences, University of Rajshahi for five years. A standard mixture of sterilized (at 60°C for 24h) whole-wheat flour with powdered dry yeast in a ratio of 19:1 was used as food medium in the experiments^{10, 11, 12}.

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**.

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

Pilot experiment-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 12-84% (Table 3). 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 mgcm^{-2} .

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 mgcm^{-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 method¹³. 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 *S. oryzae* 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 $30\pm 0.5^\circ\text{C}$. The mortality was recorded after 24, 48 and 72h of exposure. The dose was calculated by measuring the dry-weight 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 formula¹⁴. Then this percentage of mortality was subjected to statistical analysis according to Finney¹⁵.

Repellency test- To study the repellent activity of plant extract on the basis of McDonald¹⁶ 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 (mgcm^{-2}) were made by serial dilution technique at the proportion 1:2 from the stock solution and were used in repellency 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 center 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^{17, 18};

$$\text{PR} = (\text{N}_c - 5) \times 20$$

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**.

TABLE 2. AVERAGE VALUE OF REPELLENCY RATE OF *S. ORYZAE*

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

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 Torrie¹⁹ 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 *S. oryzae* adults treated with root, leaf and stem of *C. viscosum* extracts within three solvents. Ethyl alcohol root and stem extract

of *C. viscosum* showed highest mortality activity 44-84% at 72h of exposure and 40-80% mortality for root extract; 36-76% mortality for stem extract at 24h of observation period within 0.25 and 3.93 mgcm^{-2} doses. LC_{50} value was 0.39 and 0.51 mg/ml for root extract; 0.43 and 0.88 mg/ml for stem extract at 72h and 24h of exposure. Leaf extract exposed moderate activity 32-72% mortality at 72h of exposure and 28-60% mortality at 24h of exposure with 3.93 and 0.25 mgcm^{-2} doses. LC_{50} value was 0.93 and 1.81 mg/ml at 72h and 24h of exposure (**Table 3**). Regression equations are presented in **Figure 1**.

TABLE 3. MORTALITY (%) OF *S. ORYZAE*

Exp. (h)	Dose mgcm^{-2}	Root		Leaf		Stem	
		Kill	%Kill	Kill	%Kill	Kill	%Kill
Ethyl alcohol extract							
24	3.93	20	80	15	60	19	76
	0.25	10	40	7	28	9	36
72	3.93	21	84	18	72	21	84
	0.25	11	44	8	32	11	44
Ethyl acetate extract							

24	3.93	17	68	14	56	15	60
	0.25	7	28	7	28	8	32
72	3.93	20	80	20	80	19	76
	0.25	10	40	10	40	12	48
Chloroform extract							
24	3.93	12	48	7	28	10	40
	0.25	4	16	3	12	4	16
72	3.93	14	56	11	44	13	52
	0.25	6	24	5	20	5	20

No of insects=25

S. oryzae

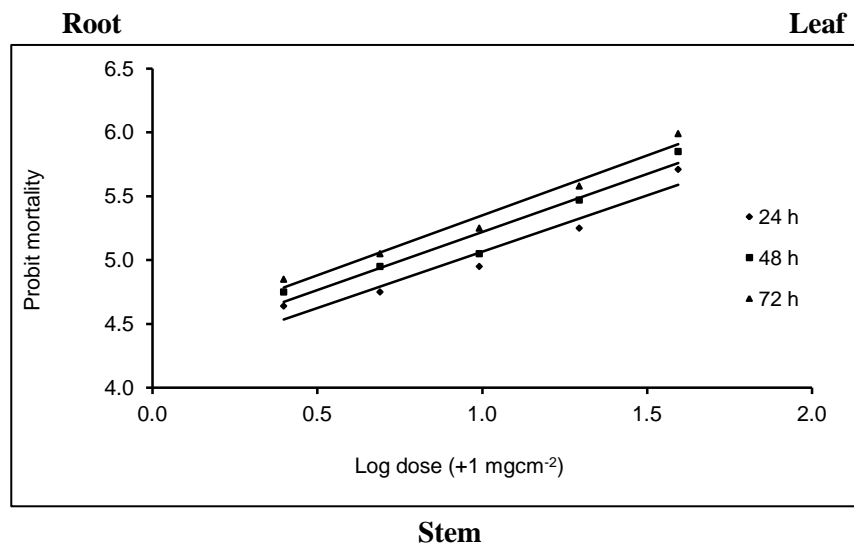
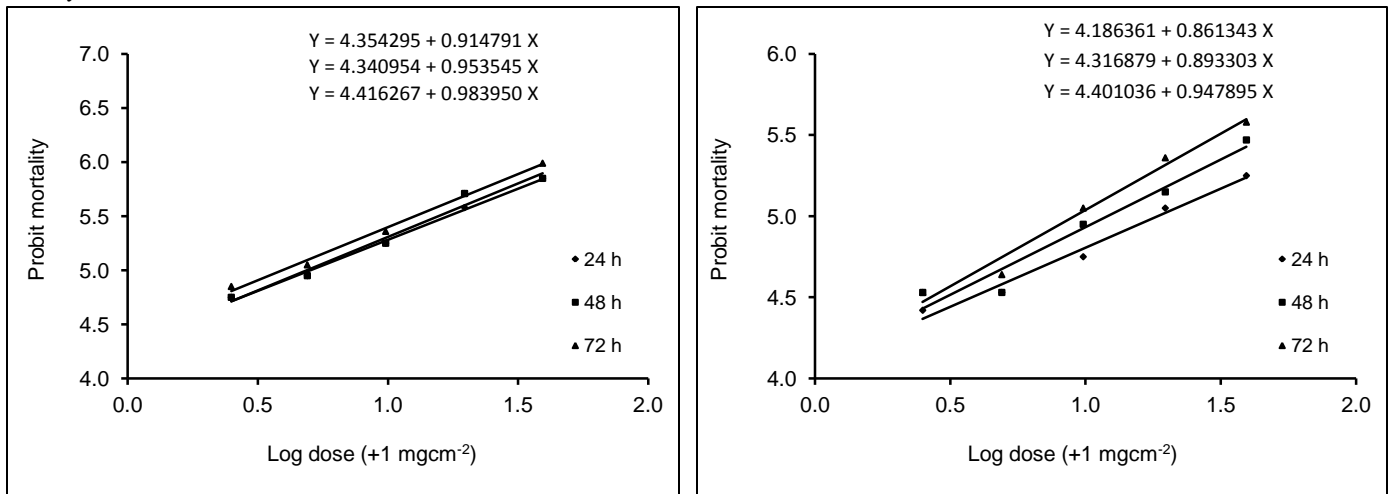


FIGURE 1. PROBIT REGRESSION LINES BETWEEN PROBIT MORTALITY OF *S. ORYZAE* AND LOG DOSE (+1 MGCM⁻²) OF ETHYL ALCOHOL EXTRACTS OF *C. VISCOSUM* AFTER 24, 48 AND 72H EXPOSURE.

Ethyl acetate fraction of root also exposed significant mortality 40-80% and 28-68% with the recorded LC₅₀ value were found 0.66 and 1.30 mg/ml respectively at 72h and 24h of exposure within 0.25 and 3.93 mgcm⁻² doses. Leaf and stem extracts of ethyl acetate fraction were also showed promising activity, 40-80% and 28-56% mortality

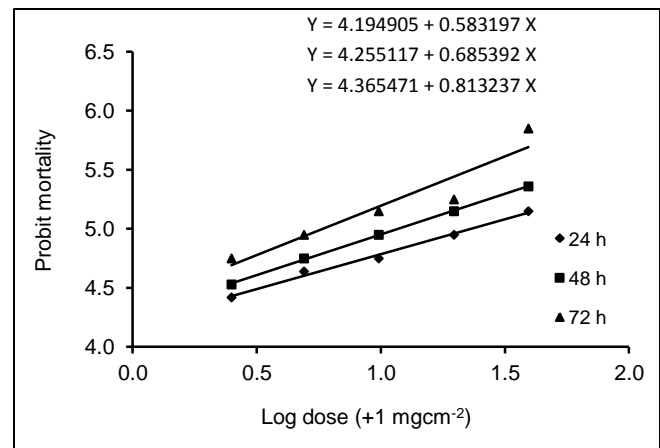
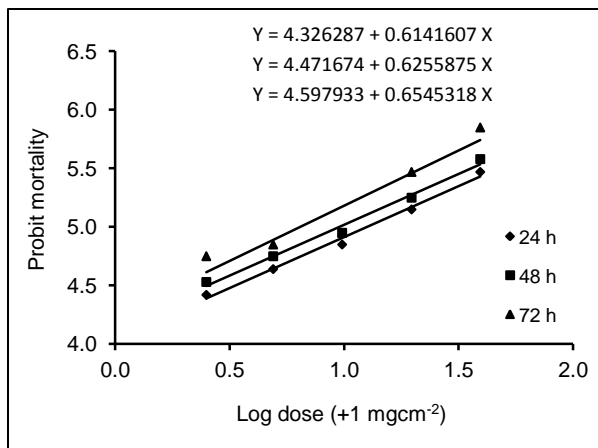
with 0.60 and 2.40 mg/ml of LC₅₀ value for leaf;

48-76% and 32-60% mortality with 0.41 and 1.25mg/ml of LC₅₀ value for stem respectively at 72h and 24h of exposure period within 0.25 and 3.93 mgcm⁻² doses (**Table 4**). Regression equations are shown in **Figure 2**.

TABLE 4. 95% CONFIDENCE OF MORTALITY RANGED OF *S. ORYZAE*

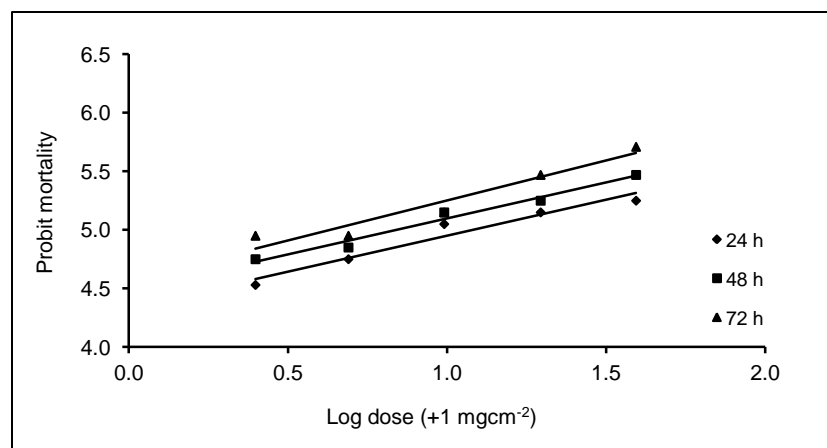
Fraction	95% Confidence		
	Lower	Leaf	Upper
	Root	24h of Exposure	Stem
		72h of Exposure	
Ethyl alcohol	0.26 - 1.00	0.81 - 4.00	0.48 - 1.61
Ethyl acetate	0.19 - 0.80	0.53 - 1.64	0.21 - 0.87
	0.67 - 2.42	0.73 - 7.81	0.53 - 2.95
Chloroform	0.35 - 1.21	0.30 - 1.22	0.15 - 1.17
	1.31 - 25.24	65.53 - 3058.8	1.15 - 65.31
	0.90 - 6.44	0.96 - 63.40	1.07 - 9.45

S. oryzae



Root

Leaf



Stem

FIGURE 2. PROBIT REGRESSION LINES BETWEEN PROBIT MORTALITY OF *S. ORYZAE* AND LOG DOSE (+1 MGCM⁻²) OF ETHYL ACETATE EXTRACTS OF *C. VISCOSUM* AFTER 24, 48 AND 72H EXPOSURE.

Contact toxicity of chloroform fraction showed lesser to moderate activity. 24-56% mortality rate with 2.41 mg/ml of LC₅₀ value at 72h of observation; 16-48% mortality with 5.76 mg/ml of LC₅₀ value at 24h of observation for root extract. The stem extract of chloroform fraction showed 20-52% mortality rate with 3.17 mg/ml of LC₅₀ value at 72h of observation; 16-40% mortality with 8.65

mg/ml of LC₅₀ value at 24h of observation and the toxicity result of leaf extract was 20-44% mortality with 7.79 mg/ml of LC₅₀ value at 72h of exposure; 12-28% mortality with 44.77 mg/ml of LC₅₀ value at 24h of experiment period respectively within 0.25 and 3.93 mgcm⁻²doses (**Table 4**). Regression equations are shown in **Figure 3**.

S. oryzae

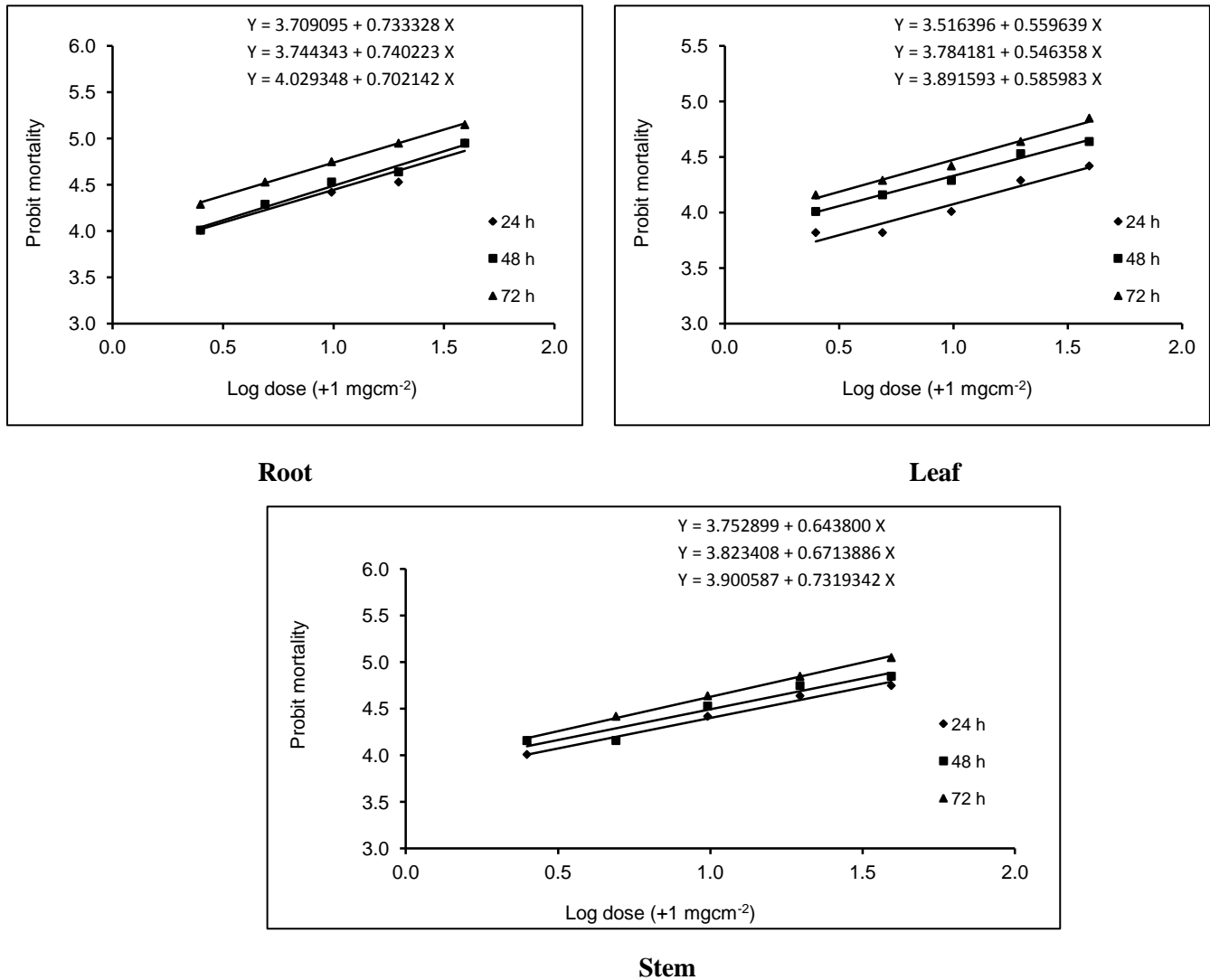


FIGURE3. PROBIT REGRESSION LINES BETWEEN PROBIT MORTALITY OF *S. ORYZAE* AND LOG DOSE (+1 MGCM⁻²) OF CHLOROFORM EXTRACTS OF *C. VISCOSUM* AFTER 24, 48 AND 72H EXPOSURE.

The 95% Confidence of the mortality of common stored product coleopteran insect pest *S. oryzae* is presented in **Table 5**.

TABLE 5. REPELLENCY EFFECT OF ETHYL ALCOHOL EXTRACT ON *S. ORYZAE*

Sl. Nos.	Mean repellency values			Mean repellency %			
	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	10.0	100a	100a	100a
Dose 6	0.12	9.66	9.53	8.53	96.6a	95.3a	85.3b
Dose 7	0.06	9.33	9.26	8.00	93.3a	92.6a	80.0b
Dose 8	0.03	6.80	6.13	5.73	68.0b	61.3b	57.3c

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

From the result of the current investigation the overall assessment suggested that the root extracts showed most potency using three kind of fraction.

Though ethyl alcohol root and stem extracts exposed the equal toxicity 44-84% mortality at 72h of exposure but the LC₅₀ value(0.39 mg/ml) of root

extract is more effective than the LC_{50} value (0.43 mg/ml) of stem extract along with 95% confidence limit 0.19 - 0.80 at 72h 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 against *S. oryzae*.

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 carried out using ethyl alcohol fraction

of root, leaf and stems of *C. viscosum*. All the extracts showed 100% repellency (Class V category repellency) in dose no 1 to dose no 5. 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 and leaf up to dose 0.06 mgcm^{-2} (Dose no 7). Whereas, the lowest dose at which the stem exhibited class V category repellency at 0.12 mgcm^{-2} (Dose no 6) presented in Table 5. The root extract showed strong repellent activity than the leaf and stem (**Figure 4**).

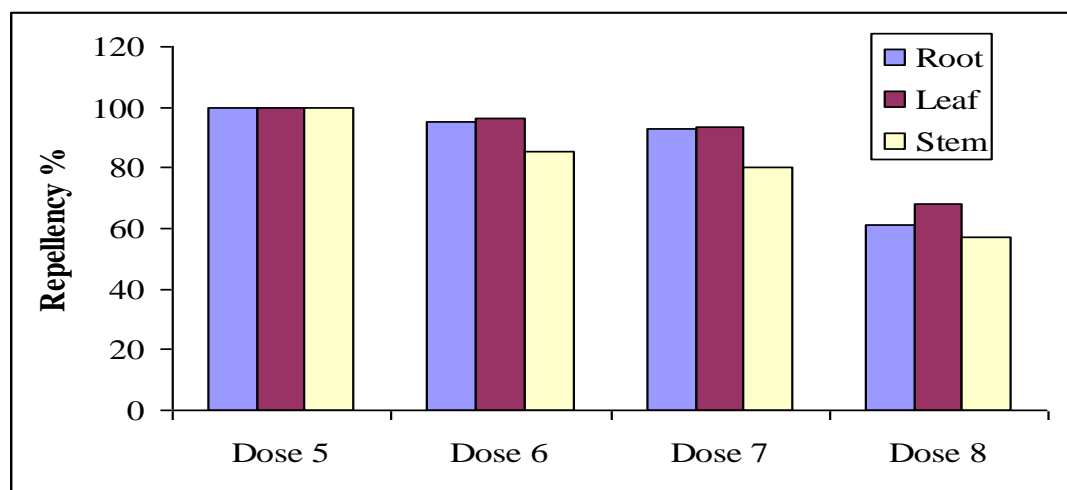


FIGURE 4. REPELLENCY EFFECT OF ETHYL ALCOHOL EXTRACT OF ROOT ON *S. ORYZAE*

The above findings of the present study are in general agreement with the findings of Yankanchi and Gadache²⁰. Our study supports the observation of Husain and Rahman²¹; Husain and Hasan²² who conducted repellent and toxicity experiment on the larvae and adults of *T. confusum*.

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 pathogens²³. In conclusion, the present research revealed that the *C. viscosum* extracts has strong insecticidal and repellent effect against *S. oryzae*.

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