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Insecticidal and Repellent Activities of *Mimosa pudica* L. (Fabaceae) against *Cryptolestes pusillus* (Schon) (Coleoptera: Cucujidae)

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A B S T R A C T

The insecticidal and repellent activities were observed by residual film method and surface film application methods respectively. Leaf, stem and root of *Mimosa pudica* L. were screened through Petroleum ether, chloroform, ethyl acetate and methanol extracts against flat grain beetle, *Cryptolestes pusillus* (Schon.). The plant extracts showed less to high mortality by using 0.25, 0.50, 1.0 and 2.0 mg/cm^2 doses and the root extract showed most potency. In 72 h of exposure, chloroform extract of root showed the lowest LD_{50} value (2.024 mg/cm^2) and 95% confidence limit was 1.730-2.292. In case of leaf, stem and root the highest mortality was recorded in chloroform extracts were 73.3, 70.0, 80.0%; 80.0, 76.7, 90.0% and 90.0, 86.7, 96.7% respectively in 24, 48 and 72 h. The repellency response among the three parts of the tested plant was different ($p<0.05$) and dose effect was more effective than exposure effect. The root extracts showed strong repellent activity than leaf and stem. The highest F-value was found 189.6542 and 83.48169 for dose and time effect respectively for petroleum ether extract of root. The order of dose-mortality effect of *M. pudica* extracts was root > leaf>stem and the susceptibility of contact toxicity in intensity of solvents was chloroform> petroleum ether > methanol > ethyl acetate. The order of intensity of repellent activity was root (petroleum ether) > leaf (petroleum ether) > stem (ethyl acetate).

Introduction

Mimosa pudica L. is one of the important medicinal plants. It was first formally described by Carl Linnaeus in *Species Plantarum* in 1753 (*'Mimosa pudica*'. Australian Plant Name Index (APNI), IBIS database).The generic name *Mimosa* is derived from the Greek 'mimos' (meaning mimic) alluding to the fact that the leaves move in response to something moving against them. The specific epithet is taken from the Latin word '*pudica*' meaning bashful or shrinking to contact (Barneby 1991). *Mimosa* is a genus of about 400 species of herbs and shrubs, in the sub-family Mimosoideae of the Legume family Fabaceae. *M. pudica* (Amador-Vegas & Dominguez 2014) (from Latin: *pudica* 'shy, bashful or shrinking'; also called sensitive plant, humble plant, sleepy plant, action plant, chuimui, ant-plant ('*Mimosa pudica*'. Royal Horticultural Society 2018) Dormilones, touch-me-not, shame plant or shy plant) is a

creeping annual or perennial flowering plant of the pea/legume family Fabaceae.

M. pudica has been used as a traditional medicine for the treatment of neurasthenia, insomnia, traumatic injury, pulmonary tuberculosis and others. It mainly grows on the hillside, jungle, glade and roadside of Asia. Many previous studies on *M. pudica* revealed the presence of flavonoids, phenolics and others (Yuan *et al.*, 2007; Yuan *et al.*, 2007; Yuan *et al.*, 2006; Yuan *et al.*, 2006 and Yuan *et al.*, 2006). In addition, many bioactivities of *M. pudica* were also studied, such as antioxidant, antibacterial, antihepatotoxic activities and so on (Samuel *et al.*, 2008, Nazeema and Brindha 2009, Adhikarimayum *et al.*, 2010, Sadia *et al.*, 2008).

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 (Berenbaum, 1995). 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 (Sreelatha and Geetha, 2011).

The protection of stored-grains against insect attack is essential, especially for countries that have inadequate storage facilities and climatic conditions that favor deterioration of grains (Pascual-Villalobos and Robledo 1999). Currently, the control of insect pests is largely dependent on the use of synthetic chemicals in most parts of the world. However, the continuous use of synthetic insecticides is leading to problems such as pest resurgence, resistance and environmental hazards, including human poisoning and toxicity to other non-target organisms (Subramanyam Hagstrum, 1995; Banwo and Adamu, 2003; Bughio and Wilkins, 2004).

The fascinating results produced by synthetic insecticides against pest, have been overshadowed by recent debates surrounding their hazards to human health and effects on non-target organisms amongst other issues (Adedire *et al.*, 2011). Furthermore, their frequent usages sometimes result in the development of insecticide resistance in target species. The challenge of finding sustainable alternatives to these synthetic insecticides has led to the bio-prospecting of plants with repellent and toxicological properties (Osipitan *et al.*, 2012). Natural products of plant origin have been reported to be useful and desirable tools in pest management because they are effective (Chaubey 2017, Uyi and Obi 2017). Several experiments using plant extracts and powders in human and animal health protection, agriculture and in household pest management have been particularly promising. Although a number of studies have empirically evaluated the use of extracts or powders from several indigenous plant species against insect pests (Habiba *et al.*, 2010, Addisu *et al.*, 2014, Uyi and Osarieme 2016), but little is known about the use of extracts from invasive alien plants to control these noxious species (Uyi and Obi, 2017; Uyi and Osarieme, 2016).

The plant-derived chemicals have been used as potential seed protectant (insecticides and antifeedants) often begins with the screening of plant extracts (Pavela 2007). It is logical to expect biologically active compounds to be produced by plants as a chemical defense measure against their enemies (Oly *et al.*, 2011). The application of plant materials with insecticidal or repellent properties to stored grains is a common traditional method in rural areas around the world (Regnault-Roger *et al.*, 2012, Kedia *et al.*, 2013).

Cryptolestes pusillus (Coleoptera: Cucujidae) is one of the most harmful insect of stored grains. Stored wheat, wheat flour, rice bran, corn, maize, beans, cassava, cocoa, cowpeas, groundnuts, nere seed, shorghum etc. are attacked by a large number of insect pests and causes a major amount of damage. *C. pusillus* is one of the common and major insect pests of stored grains. It is cosmopolitan in distribution and occurs throughout the whole world especially tropical and subtropical region of the world including Bangladesh. *C. pusillus* completes the life cycle within 30 to 34 days at 30°C temperatures. It multiplies rapidly in worm conditions and causes a large amount of damage about 100% after six months of storage. The damage is caused both by the larval and adult stages of *C. pusillus.*

Natural products from locally available plants with insecticide activity represent a low-cost and sustainable alternative to protect agricultural production. Furthermore, botanical insecticides supposedly pose little threat to the environment or human health compared with synthetic insecticides, and they represent a suitable alternative to controlling mites and insect pests worldwide (Isman 2006, Regnaut-Roger *et al.,* 2012, Kedia *et al.,* 2013).

Materials and Methods

Plant collection and identification

The sample plant touch-me-not, *Mimosa pudica* L. was collected from the Botanical Garden of Rajshahi University Campus. The voucher specimen was then confirmed from the Taxonomical section of the Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extracts

Sample plants with roots were collected and taken in the laboratory. The soil attached with roots was cleaned by a brush. The leaf, stem and root were separated and the stem and root were chopped in small pieces with the help of scissor and chopper. The leaf, stem and root were taken in wooden trays and kept open in the laboratory for drying in room temperature and tried to keep contamination-free by any cost.

After drying, the samples were taken in a grinding machine and grinded the leaf, stem and root samples separately and preserved in air-tight bags. 100 g dust of each part (leaf, stem and root) was taken in baspata envelope, sealed and kept them in air-tight transparent polythine bags. The bags were marked with date and preserved for extraction.

Chemical extraction of the plant parts

To yield the extracts of leaf, 500 g dust of leaf was taken in five conical flasks of 500 ml (100 g in each conical flask) and poured 400 ml petroleum ether in each of the five flasks. All the flasks were covered by aluminum foil and shaked for 72 h in an electric shaker. After shaking the extracts of leaf of petroleum ether was filtered by Whatman filter paper No.1 and collected the extracts in a beaker. After filtering again the flasks were poured by 300 ml petroleum ether, shaked for 48 hours and collected the extract in the same beaker. Repeated the work for third time the beaker was kept at room temperature for evaporation of the solvent. After evaporation, the extract was collected in a glass vial and preserved at 4°C. The same procedures were followed to yield the extracts of stem and root respectively.

When the extracts of leaf, stem and root of petroleum ether were collected, the dusts in the conical flasks were dried in room temperature to evaporate the solvent. Again the dusts were taken in conical flasks and used chloroform as solvent and followed the same procedures as taken for the petroleum

ether extracts of leaf, stem and root; taken in glass vials and preserved at 4°C. The same procedures were followed to yield the ethyl acetate and methanol extracts of leaf, stem and root and preserved at 4°C for the future experiments.

Selection of *C. pusillus*

To carry on the tests for insecticidal activities and also for the repellent potentials of the extracts of *M. pudica*, *Cryptolestes pusillus* was selected, because it is an easy cultivable and noble laboratory insect. The life history made this insect as popular choice as test insect for biological studies.

Collection and culture of *Cryptolestes pusillus*

Adult beetles of *C. pusillus* were collected from the stock culture of Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi, Bangladesh and reared as mass-cultures and sub-cultures to be used in the experiments. A standard mixture of sterilized (at 60°C for 24 h) cracked wheat and wheat flour with powdered dry yeast in a ratio of 19:1 was used as a food medium in the experiments (Khalequzzaman *et al.*, 1994).

Insecticidal activity test

The test was carried out by residual film method. 254.34 mg extracts were mixed in 2 ml of the respective solvents to make stock solutions. From these stock solutions other successive doses were prepared by serial dilution method. Thus four doses were prepared as 0.25, 0.5, 1.0 and 2.0 mg/cm^2. To conduct the surface film activity test 9.0 cm Petri dishes were taken for all the doses and their replications. 1ml of each dose was poured into the lower part of Petri dishes and allowed them to dry out. Then 10 insects were released in each of the Petri dishes. A control experiment by applying the only solvents into the Petri dishes was also set at the same time and three replicates were taken. The whole experiment was observed from time to time and the mortality was counted after every 24, 48 and 72 h of exposure. A simple microscope was used to check each beetle by tracing natural movement. In some cases hot needle was taken closer to the bodies (without movement) to confirm death.

Statistical analysis

The mortality recorded was corrected by the Abbott's (1925) formula in the following manner:

$$P_r = \{(P_o - P_c) / (100-P_c)\} \times 100$$

Where, P_r = Corrected mortality (%)
P_o = Observed mortality (%)
P_c = Control mortality (%), sometimes called natural mortality (%).

The mortality percentages were subjected to statistical analysis according to Finney (1947) and Busvine (1971) by using software developed in the Department of Agricultural Environmental Science, University of Newcastle upon Tyne, UK. The dose-mortality relationship was expressed as median lethal dose (LD_{50}).

Repellent activity test

The repellent activity test was performed by following the surface film application method. A stock solution for each of the petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and root was prepared. From this stock solution five doses *i.e.* 0.625, 1.25, 2.5, 5.0 and 10.0 mg/ml were made by serial dilution. One control was taken using respective solvent. Half-disc filter papers (Whatman No. 1) were prepared and

applied doses of all the petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and root onto half-discs and allowed to dry out in the air. Each treated half-disc then attached lengthwise, edge-to-edge to a control half-disc with a scotch-tape and placed in a Petri dish (9cm diam.). Then 10 adult insects were released in the middle of each filter paper circle. Insects were counted on the untreated half of the filter paper disc at one hour interval up to five consecutive hours. Three replications were taken and the averages of the counts were converted to percentage repulsion (PR) using the formula of Talukder and Howse (1993, 1995) which was again developed by arcsine transformation for the calculation of ANOVA.

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

Where, N_c is the number of insects on the untreated half-disc.

Positive values (+) expressed repellency and negative values (-) for attractant activity.

Preparation of doses for repellent activity of *C. pusillus*

The repellent activity test used was adopted from the method of McDonald *et al.*, (1970) with some modifications by Talukder and Howse (1993). Half filter paper discs (Whatman No.1, 9.0 cm diam.) were prepared and 60 mg extract of each sample of different solvents were taken in vials. If the extracts were insoluble in the definite solvent, pure DMSO were added to dissolve the extracts and then 6 ml definite solvent was added with the extracts separately. So the concentration became 60 mg/6 ml *i.e*. 10 mg/ml. From this 6 ml solution, 3 ml were taken to three half-disc filter papers as R_1, R_2 & R_3 each contained 1 ml solution whose concentration was 10 mg/ml. Then 3 ml same solvent were added to the rest 3 ml solution to give the concentration 30 mg/6ml, *i.e*. 5 mg/ml. From this concentration 3 ml were taken to another three half-disc filter papers as R_1, R_2 & R_3 each contained 1 ml solution of 5 mg/ml. In the same way 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml doses were prepared and took three replications for each of the doses. The half-disc filter papers with doses were left for sometimes to dry up the solvent in the air. Three half-disc filter papers were soaked with only solvent as control (C_1, C_2 & C_3) and all the Petri dishes were marked.

Statistical analysis

Repellency activity test was conducted according to complete randomized experimental design with three replications for each treatment. Data of percentage repulsion (PR) were subjected to arcsine transformation of the proportion and then calculate the ANOVA.

Results and Discussion

Insecticidal activity

The test was carried out by residual film method. The effect of different doses of petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf of *M. pudica* on the mortality of *C. pusillus* was shown in Table 1. In case of leaf, the highest activity was found in chloroform extract and the LD_{50} values were 2.891 mg/cm^2, 2.472 mg/cm^2 and 2.190 mg/cm^2 in 24, 48 and 72 h respectively. The LD_{50} values of 72 hours of exposure showed significant along with 95% confidence than 48 and 24 h of application. The exposure wise dose mortality was 72 > 48 > 24 h.

The effect of similar doses of petroleum ether, chloroform, ethyl acetate and methanol extracts of stem of *M. pudica* on the mortality of *C. pusillus* was shown in Table 2. Stem

extracts were somewhat less toxic than the leaf extracts except the petroleum ether extract. In case of stem, the highest activity was also found in chloroform extract where the LD_{50} values were 3.144 mg/cm^2, 2.691 mg/cm^2 and 2.354 mg/cm^2 in 24, 48 and 72 hours respectively. Also the LD_{50} values of 72 hours of exposure showed significant along with 95% confidence than 48 and 24 hours of application.

Table.1 LD_{50}, 95% confidence limits and regression equations of different extracts of leaf of *M. pudica* against adult *C. pusillus*

Test extracts	Time exposed (h)	LD_{50} value (mg/cm^2)	95% confidence limits		Regression equation	X^2 value (df)
			Lower limit	Upper limit		
Petroleum ether	24	5.560	4.296	10.250	y= -1.37+1.64x	2.300 (3)
	48	4.535	3.674	6.711	y= -1.13+1.59x	3.298 (3)
	72	3.870	3.206	5.134	y= -0.92+1.53x	4.493 (3)
Chloroform	24	2.891	2.442	3.409	y= -0.75+1.91x	5.549 (3)
	48	2.472	2.074	2.866	y= -0.48+1.87x	7.475 (3)
	72	2.190	1.854	2.503	y= -0.55+2.57x	6.779 (3)
Ethyl acetate	24	4.314	3.540	6.067	y= -1.15+1.72x	3.166 (3)
	48	3.577	2.997	4.516	y= -0.93+1.69x	4.356 (3)
	72	2.936	2.470	3.489	y= -0.67+1.71x	6.026 (3)
Methanol	24	3.456	2.898	4.313	y= -0.86+1.65x	4.892(3)
	48	2.980	2.510	3.549	y= -0.72+1.75x	5.782 (3)
	72	2.506	2.106	2.907	y= -0.52+1.91x	7.226 (3)

Table.2 LD_{50}, 95% confidence limits and regression equations of different extracts of stem of *M. pudica* against adult *C. pusillus*

Test extracts	Time exposed (h)	LD_{50} value (mg/cm^2)	95% confidence limits		Regression equation	X^2 value (df)
			Lower limit	Upper limit		
Petroleum ether	24	3.809	3.196	4.891	y= -1.13+1.93x	3.299 (3)
	48	3.025	2.560	3.591	y= -0.85+1.96x	4.822 (3)
	72	2.648	2.226	3.091	y= -0.58+1.84x	6.790 (3)
Chloroform	24	3.144	2.651	3.691	y= -1.02+2.22x	3.826 (3)
	48	2.691	2.274	3.134	y= -0.71+2.04x	5.745 (3)
	72	2.354	1.987	2.706	y= -0.59+2.3x	7.021 (3)
Ethyl acetate	24	5.085	4.013	8.459	y= -1.23+1.57x	2.873 (3)
	48	4.435	3.573	6.614	y= -0.94+1.3x	4.322 (3)
	72	3.576	2.946	4.668	y= -0.58+1.08x	6.790 (3)
Methanol	24	4.210	3.477	5.784	y= -1.17+1.79x	3.014 (3)
	48	3.554	2.996	4.430	y= -1.04+1.91x	3.826 (3)
	72	2.980	2.510	3.549	y= -0.72+1.75x	5.782 (3)

Table.3 LD_{50}, 95% confidence limits and regression equations of different extracts of root of *M. pudica* against adult *C. pusillus*

Test extracts	Time exposed (h)	LD_{50} value (mg/cm^2)	95% confidence limits		Regression equation	X^2 value (df)
			Lower limit	Upper limit		
Petroleum ether	24	3.119	2.638	3.732	y= -0.87+1.92x	4.873 (3)
	48	2.722	2.289	3.193	y= -0.6+1.79x	7.009 (3)
	72	2.241	1.895	2.566	y= -0.57+2.5x	7.256 (3)
Chloroform	24	2.548	2.151	2.948	y= -0.65+2.12x	6.142 (3)
	48	2.218	1.879	2.534	y= -0.6+2.61x	6.782 (3)
	72	2.024	1.730	2.292	y= -0.74+3.49x	6.574 (3)
Ethyl acetate	24	3.805	3.154	5.011	y= -0.88+1.48x	4.632 (3)
	48	3.081	2.579	3.729	y= -0.62+1.48x	6.470 (3)
	72	2.611	2.192	3.046	y= -0.54+1.8x	7.039 (3)
Methanol	24	4.649	3.705	7.274	y= -0.96+1.27x	4.508 (3)
	48	3.765	3.103	5.000	y= -0.7+1.26x	5.638 (3)
	72	3.184	2.662	3.897	y= -0.64+1.44x	6.569 (3)

Table.4 LD_{50} values of different solvent extracts of leaf, stem and root of *M. pudica* in 24h, 48h and 72h of exposure

Test extracts	24 h			48 h			72 h		
	Leaf LD_{50} value (mg/cm^2)	Stem LD_{50} value (mg/cm^2)	Root LD_{50} value (mg/cm^2)	Leaf LD_{50} value (mg/cm^2)	Stem LD_{50} value (mg/cm^2)	Root LD_{50} value (mg/cm^2)	Leaf LD_{50} value (mg/cm^2)	Stem LD_{50} value (mg/cm^2)	Root LD_{50} value (mg/cm^2)
Petroleum ether	5.560	3.809	3.119	4.535	3.025	2.722	3.870	2.648	2.241
Chloroform	2.891	3.144	2.548	2.472	2.691	2.218	2.190	2.354	2.024
Ethyl acetate	4.314	5.085	3.805	3.577	4.435	3.081	2.936	3.576	2.611
Methanol	3.456	4.210	4.649	2.980	3.554	3.765	2.506	2.980	3.184

Table.5 % Mortality of *Cryptolestes pusillus* in different extracts

Exposure (h)	Dose (mg/cm^2)	% Kill											
		Petroleum ether			Chloroform			Ethyl acetate			Mehtanol		
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
24	0.25	20.0	30.0	40.0	43.3	36.7	50.0	26.7	23.3	33.3	36.7	26.7	30.0
	0.50	26.7	40.0	50.0	56.7	50.0	63.3	36.7	30.0	43.3	46.7	36.7	33.3
	1.00	33.3	50.0	60.0	63.3	63.3	73.3	43.3	36.7	50.0	53.3	46.7	40.0
	2.00	43.3	60.0	70.0	73.3	70.0	80.0	53.3	46.7	56.7	63.3	53.3	50.0
48	0.25	26.7	40.0	50.0	53.3	46.7	60.0	33.3	30.0	43.3	43.3	33.3	36.7
	0.50	33.3	53.3	56.7	66.7	60.0	70.0	46.7	36.7	53.3	53.3	43.3	43.3
	1.00	43.3	63.3	66.7	73.3	70.0	83.3	53.3	43.3	60.0	63.3	53.3	50.0
	2.00	50.0	70.0	76.7	80.0	76.7	90.0	60.0	50.0	66.7	70.0	63.3	56.7
72	0.25	33.3	50.0	60.0	60.0	56.7	66.7	43.3	40.0	50.0	53.3	43.3	43.3
	0.50	40.0	60.0	70.0	73.3	66.7	76.7	56.7	46.7	63.3	63.3	53.3	50.0
	1.00	50.0	70.0	80.0	83.3	76.7	90.0	63.3	53.3	70.0	73.3	63.3	56.7
	2.00	56.7	76.7	90.0	90.0	86.7	96.7	70.0	56.7	76.7	80.0	70.0	66.7

Table.6 ANOVA results for repellent activity of leaf extracts of *M. pudica* against adult *C. pusillus*

Test material	Extracts	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Leaf	Petroleum ether	Dose effect	1685.274	4	421.3184	49.74491	7.91E-09	3.006917
		Time effect	578.5058	4	144.6265	17.07599	1.26E-05	3.006917
		Error	135.5133	16	8.469579			
		Total	2399.293	24				
	Chloroform	Dose effect	1821.602	4	455.4006	13.11165	6.36E-05	3.006917
		Time effect	332.332	4	83.083	2.392082	0.093914	3.006917
		Error	555.7202	16	34.73251			
		Total	2709.655	24				
	Ethyl acetate	Dose effect	1651.455	4	412.8639	19.99361	4.57E-06	3.006917
		Time effect	643.0277	4	160.7569	7.784918	0.001107	3.006917
		Error	330.3966	16	20.64979			
		Total	2624.88	24				
	Methanol	Dose effect	1280.406	4	320.1015	46.44371	1.31E-08	3.006917
		Time effect	451.473	4	112.8683	16.37612	1.64E-05	3.006917
		Error	110.2759	16	6.892247			
		Total	1842.155	24				

Table.7 ANOVA results for repellent activity of stem extracts of *M. pudica* against adult *C. pusillus*

Test material	Extracts	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Stem	Petroleum ether	Dose effect	1472.515	4	368.1288	13.4846	5.38E-05	3.006917
		Time effect	1052.202	4	263.0505	9.635567	0.000365	3.006917
		Error	436.7991	16	27.29995			
		Total	2961.516	24				
	Chloroform	Dose effect	1800.978	4	450.2444	9.968747	0.000303	3.006917
		Time effect	685.2142	4	171.3035	3.792788	0.023581	3.006917
		Error	722.6496	16	45.1656			
		Total	3208.841	24				
	Ethyl acetate	Dose effect	1855.095	4	463.7739	36.56298	7.34E-08	3.006917
		Time effect	925.2233	4	231.3058	18.23568	8.29E-06	3.006917
		Error	202.9479	16	12.68425			
		Total	2983.267	24				
	Methanol	Dose effect	1675.989	4	418.9973	20.1373	4.37E-06	3.006917
		Time effect	1093.728	4	273.4319	13.14133	6.27E-05	3.006917
		Error	332.9124	16	20.80702			
		Total	3102.629	24				

Table.8 ANOVA results for repellent activity of root extracts of *M. pudica* against adult *C. pusillus*

Test material	Extracts	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Root	Petroleum ether	Dose effect	1547.642	4	386.9106	189.6542	2.93E-13	3.006917
		Time effect	681.239	4	170.3098	83.48169	1.65E-10	3.006917
		Error	32.64136	16	2.040085			
		Total	2261.523	24				
	Chloroform	Dose effect	1126.007	4	281.5017	66.00051	9.71E-10	3.006917
		Time effect	530.9797	4	132.7449	31.1232	2.29E-07	3.006917
		Error	68.2423	16	4.265144			
		Total	1725.229	24				
	Ethyl acetate	Dose effect	1278.837	4	319.7093	102.7792	3.37E-11	3.006917
		Time effect	581.417	4	145.3543	46.72803	1.25E-08	3.006917
		Error	49.7703	16	3.110644			
		Total	1910.025	24				
	Methanol	Dose effect	1000.317	4	250.0793	127.6997	6.34E-12	3.006917
		Time effect	526.9535	4	131.7384	67.27046	8.42E-10	3.006917
		Error	31.33342	16	1.958339			
		Total	1558.604	24				

The effect of contact poisoning of root extracts of *M. pudica* against *C. pusillus* showed the highest activity to all the three exposures of application except methanol extract which was shown in Table 3. Among the root extracts, the highest activity was also found in chloroform extract where the LD_{50} values were 2.548 mg/cm^2, 2.218 mg/cm^2 and 2.024 mg/cm^2 in 24, 48 and 72 hours respectively. The recorded LD_{50} values of 72 hours of exposure observed higher mortality along with 95% confidence than 48 and 24 hours of application.

Among the extracts of leaf, stem and root, the chloroform extracts of root showed the highest activity. In 24 hours the lowest LD_{50} value was 2.548 mg/cm^2 for chloroform extract of root and the highest was 5.560 mg/cm^2 for petroleum ether extract of leaf. In case of 48 hours the lowest LD_{50} value was 2.218 mg/cm^2 for chloroform extract of root and the highest was 4.535 mg/cm^2 also for petroleum ether extract of leaf. And that of 72 hours the lowest LD_{50} value was 2.024 mg/cm^2 also for the chloroform extract of root and the highest was 3.870 mg/cm^2 for petroleum ether extract of leaf (Table 4).

Among the petroleum ether, chloroform, ethyl acetate and methanol extracts of leaf, stem and root the highest percentages of mortality was found in chloroform extract *i.e.* 73.3%, 70.0% and 80.0% respectively in 24 hours and that was for 48 and 72 hours the highest percentages were also for chloroform extracts *i.e.* 80.0%, 76.7%, 90.0% and 90.0%, 86.7%, 96.7% respectively for leaf, stem and root (Table 5).

Mortality of *Macrotermes* species caused by the root extract of *Mimosa diplotricha* was high and observed to be concentration and exposure time dependent. The highest concentration [10% (w/v)] of *M. diplotricha* root extract accounted for 100% mortality against *Macrotermes* species after a 36 hour exposure period. Following a 36 hour exposure period, the median lethal concentration (LC_{50}) of *M. diplotricha* against the termites was 4.12% (w/v) (Uyi *et al.*, 2018). In our study we found the related results *i.e.* root extracts were most effective and 96.7% mortality was observed by root extract in72 hour exposure period.

The leaf and stem powders of *M. diplotricha* plant exhibited some degrees of mortality against *Sitophilus zeamais* Motschulsky which was both concentration and exposure time dependent. At the highest concentration (3.5g), the leaf and stem powders of *M. diplotricha* accounted for only 28% mortality of *S.zeamais* after a five hour exposure period (Uyi and Samugana 2018). In our study the chloroform extracts showed the highest percent mortality among the four solvent extracts of leaf, stem and root and the root extracts were the most potential in all the three exposure time *i.e.* 24, 48 and 72 hours.

Repellent activity test of *C. pusillus*

The repellent activity test was performed by following the surface film application method with doses of 314.56, 157.28, 78.64, 39.32 and 19.66 µg/cm^2 on half-disc filter paper. The data was recorded with one hour interval for up to five consecutive hours of exposure and the percentage repulsion data was then subjected to ANOVA after transforming into arcsine percentage values. The tables showed that the F-values were 49.74491, 13.11165, 19.99361 and 46.44371 for the analysis between doses and 17.07599, 2.392082, 7.784918 and 16.37612for the analysis between time interval for leaf extracts of petroleum ether, chloroform, ethyl acetate and methanol (Table 6). In case of stem the F-values were 13.4846, 9.968747, 36.56298 and 20.1373 for the analysis between doses and 9.635567, 3.792788, 18.23568 and 13.14133

for the analysis between time interval for petroleum ether, chloroform, ethyl acetate and methanol extracts (Table 7) and that of the root were 189.6542, 66.00051, 102.7792 and 127.6997 for the analysis between doses and 83.48169, 31.1232, 46.72803 and 67.27046 for the analysis between time interval (Table 8). Among the extracts tested the petroleum ether extract of root offered the highest repellent activity at 5% ($P<0.05$) level of significance. According to the intensity of repellent activity the result could be arranged in a descending order: Root (petroleum ether) > Leaf (petroleum ether) > Stem (ethyl acetate).

The following results were found during repellent activity test of *C. pusillus*with extracts of different parts of *M. pudica* using different solvents.

The leaf and stem powders of *M. diplotricha* exhibited some degrees of repellent activity against *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), although, the repellent activity was a function of both concentration and exposure time. After a 3 hour exposure period, the 3.5g of the leaf (53%) and stem (58%) powders exhibited the highest repellent activity against *S. zeamais* (Uyi and Samugana, 2018). In our study the root extracts showed strong repellent activity than leaf or stem extracts.

The highest concentration [10% (w/v)] of the root extract of *Mimosa diplotricha* significantly repelled 100% of *Macrotermes* species following a 30 min. exposure period (Uyi *et al.*, 2018). In our investigation we observed that the root extracts possessed the higher potentiality (highest 90% repellency for petroleum ether extract of root).

In conclusion this work was a basic approach to investigate the insecticidal and repellent activities of the medicinal plant *M. pudica* against *C. pusillus* and the study revealed that the plant extract tested had a strong insecticidal and repellent effect against the pest. Leaf, stem and root each part of the plant had some insecticidal and repellent activities, but root extracts possessed a strong bioactive potential. The findings suggested that the certain bioactive components of *M. pudica* had potential that act as a grain protectant and might be exploited for the control of *C. pusillus* in stored grains in an environment-friendly way.

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