Scholars Academic Journal of Biosciences (SAJB)
Abbreviated Key Title: Sch. Acad. J. Biosci.

A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

ISSN 2347-9515 (Print)
ISSN 2321-6883 (Online)

Zoology

Bio-efficacy of *Tabernaemontana divaricata* (L.) Leaf and Stem Bark Extract against *Callosobruchus chinensis* L.

Md. Samiul Islam, Nurul Islam, Md. Kamrul Ahsan, Shah H. A. Mahdi*
Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh

Original Research Article

***Corresponding author**
Shah H. A. Mahdi

Article History
Received: 08.02.2018
Accepted: 21.02.2018
Published:30.03.2018

DOI:
10.36347/sajb.2018.v06i03.002

Abstract: A thorough screening of the indigenous plant *Tabernaemontana divaricata* (L.) leaf and stem bark were extracted separately in petroleum ether (Pet-ether), chloroform ($CHCl_3$) and methanol (CH_3OH) has been done for the detection of bioactive compounds. The extracts of leaf and stem bark were subjected to repellency and dose mortality assay against the stored product pest *Callosobruchus chinensis* L. adults. The Pet-ether extract of leaf showed moderate repellency at 1% level of significance ($p < 0.01$), while the CH_3OH extract of leaf and the Pet-ether, $CHCl_3$ and CH_3OH extract of stem bark gave mild repellency at 5% level of significance ($p < 0.05$), but the $CHCl_3$ extract of leaf did not show any repellency. According to the intensity of activity the results could be arranged in a descending order: Pet-ether > CH_3OH > $CHCl_3$ for the leaf extracts and CH_3OH > $CHCl_3$ > Pet-ether for the stem bark extracts respectively. The Pet-ether extract of both the leaf and stem bark were tested against the adult beetles of *C. chinensis* L. through dose mortality assay, and LD_{50} values were established as 3.289, 2.796, 2.512, 2.053, 1.846 and 1.572 mg/cm^2 and 4.219, 3.604, 3.579, 3.041, 2.419 and 1.953 mg/cm^2 for leaf and stem bark respectively at 6h, 12h, 18h, 24h, 36h and 48h of exposure. However, the CH_3OH extract gave the LD_{50} values 6.689, 9.136E-05, 6.336, 4.591, 2.569 and 1.813 mg/cm^2 and 4.854, 5.272, 4.066, 3.17, 2.667 and 2.431 mg/cm^2 for both the leaf and stem bark extracts respectively at the same exposure time. The $CHCl_3$ extracts of both the leaf and stem bark did not show any mortality to the test organism.
Keywords: *Tabernaemontana divaricata* (L.), *Callosobruchus chinensis* L., bio-efficacy, extracts, mortality, repellency.

INTRODUCTION

Despite the successful achievement in food production, ensuring food security for all is one of the major challenges till now. The major concern for this is the pest infestation during food storage that causes substantial economic losses by reducing the quantity and quality of grain. This problem definitively impacts the worldwide economy, as the damage related cost from the presence of insect pests in stored foods may reach up to 475 million dollars per year [1]. About 600 species of insects belonging to different families have been identified from stored products in various parts of the world. *Callosobruchus chinensis* L. is one of the major stored grain pests that attack several crops including wheat, rice, maize, pulse, etc. *C. chinensis* are polyphagous in nature and one of the most damaging crop pests to the stored legume industry due to their generalized legume diets and wide distribution [2]. The species most preferred to habitat in the tropics, on green gram or chickpeas and many countries of the world [3, 4]. They reach the height of egg production and legume infestation in July–August [5].

Chemical pesticides and fumigants are often used by the farmers to control such pest, but these pesticides may remain in food as residue and may harmful to human health as well as environment. Besides, genetic resistance to pest, environmental pollution and hazards from handling is common effects from the indiscriminate use of pesticides [6]. In this aspect plants may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals. Recent studies on plant extract *viz*, *Argemone mexicana* L., *Abutilon hirtum* (Lam.) and *Saraca indica* L. have been shown the control potentials against *Culex quinquefasciatus*, *Tribolium castaneum* (Hbst.), *Callosobruchus chinensis* (L.), *Artemia salina* L. and *Sitophilus oryzae* L. [7- 9]. It is also revealed that the repellent activity of some plant extract can be the possible way to protect stored grain insects [8, 10].

Tabernaemontana divaricata (commonly called Crape Jasmine, Bengali name- Togor; Family- Apocynaceae) is a general garden plant in Southeast Asia and other tropical countries. It is also used as traditional medicine in many countries. It is a beautiful evergreen shrub, with large shiny leaves, may appear sporadically throughout the year. The plant generally grows to a height of 5–6 feet and is dichotomously-branched. The large shiny leaves are deep green and about 6 inches (15 cm) in length and 2 inches (5.1 cm) in width. The waxy blossoms are found in small clusters on the stem tips. This medicinal plant is a rich source of alkaloids with various pharmacological properties and has been used in the folk medicine for anti-infection, anti-inflammation, analgesic, anti-tumor, anti-oxidative effect and the effect in neuronal activity [11, 12]. It is also used as anthelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, purgative, remedy against poisons and tonic to the brain, liver and spleen [13, 14]. The insecticidal properties of this plant are still unknown. The present study was carried out to investigate the insecticidal and repellent activity against stored grain pest the *C. chinensis* L.

MATERIALS AND METHODS

Preparation of plant materials and extraction

After collection of leaf and stem bark of the plant, the materials sliced into pieces and spread on a tray. They were allowed to dry under shade to avoid direct sunlight. After drying, the plant parts were powdered using a grinder. Three solvents as Petroleum ether (Pet-ether), Chloroform ($CHCl_3$) and Methanol (CH_3OH) were used to prepare the extract. The solvent were mixed at a ratio of 3:1 (solvent: dust) in a conical flask and shaked for 48 hours before filtration. The output extract then transferred to a glass vials and preserved.

Culture of test insects

The culture of *C. chinensis* L. was maintained in earthen pots with common lentil as food medium for it. About 300-400 beetles were placed in the medium and allowed them to oviposit in it. The grain contained eggs then transferred to other containers and kept in room temperature for hatching.

Repellent activity test

The repellency test was adopted from of McDonald *et al*. [15] with some modification by Talukder and Howse [16]. Each of the Petri dishes was divided into three parts and marked with two narrow sticks by using adhesive tape. Then both the sides were filled with food where in one side was with treated food. Ten insects were released in the middle part of the Petri dish. Observation was made for one hour interval and up to five successive hours.

Insecticidal activity test

The application of doses carried out by residual film method [17]. Each of the doses was prepared by dissolving it in 1ml of solvent and poured into the Petri dish (50mm) that contained lentils and allowed to dry. After drying 10 test organisms were released in each of the Petri dishes and the whole experiment was done in three replicates. The mortality first counted after 30 min and then 6 hour interval up to 48 hour.

Statistical analysis

The repellency values in the recorded data were calculated for percent repulsion and again developed by arcsine transformation for the calculation of ANOVA. But the data recorded of the dose mortality effects were corrected by the formula [17, 18]:

$$Pr = \frac{(Po - Pc)}{(100 - Pc)} \times 100$$

The dose mortality relationship was expressed as a median lethal dose (LD_{50}).

RESULTS

Repellent activity

The Pet-ether and CH_3OH extracts of *T. divaricata* (L.) leaf and stem bark showed repellency to adult *C. chinensis* L. for a concentration of 0.157, 0.079, 0.039, 0.02 and 0.01 mg/cm^2, while the $CHCl_3$ extract of leaf exhibited no repellent activity against this pest (Table 1). The Pet-ether extracts of leaf offered moderate repellency at 1% level of significance ($p < 0.01$); the $CHCl_3$ extract of leaf and the Pet-ether, $CHCl_3$ and CH_3OH extract of stem bark gave mild repellency at 5% level of significance ($p < 0.05$). However, the $CHCl_3$ extract of leaf against *C. chinensis* L. did not show any repellency. According to the intensity of activity the results could be arranged in a descending order: Pet-ether > CH_3OH > $CHCl_3$ for leaf extracts, and CH_3OH > $CHCl_3$ > Pet-ether for stem bark extracts.

Table-1: Result of repellent activity test with leaf and stem bark extracts of *T. divaricata* (L.) against *C. chinensis* L.

Plant part	Name of extracts	Source 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
Leaf	Pet-ether	4	4	16	40.636**	1.368	3.44E-08	0.289
	$CHCl_3$	4	4	16	6.594^{ns}	1.698	0.002	0.199
	CH_3OH	4	4	16	15.918*	1.242	1.96E-05	0.333
Stem Bark	Pet-ether	4	4	16	3.990*	0.735	0.020	0.581
	$CHCl_3$	4	4	16	9.100*	6.972	0.001	0.002
	CH_3OH	4	4	16	10.790*	0.709	0.001	0.597

*** $p < 0.001$, * $p < 0.01$, * $p < 0.05$, and ns= Non-significant.

Insecticidal activity

The results of the dose mortality assays, Pet-ether extracts of *T. divaricata* (L.) leaf and stem bark against beetles of *C. chinensis* L. are represented in Table 2. The LD_{50} values for leaf extract were 3.289, 2.796, 2.512, 2.053, 1.846 and 1.572 mg/cm^2 after 6h, 12h, 18h, 24h, 36h and 48h of exposure hours respectively. For the stem bark extract the LD_{50} values were 4.219, 3.604, 3.579, 3.041, 2.419 and 1.953 mg/cm^2 after 6h, 12h, 18h, 24h, 36h and 48h of exposures respectively. Consistent with insecticidal activity of the extracts of *T. divaricata* (L.) could be arranged in the following descending order: leaf > stem bark.

Table-2: Dose mortality results of the Pet-ether extracts of *T. divaricata* (L.) against *C. chinensis* L.

Plant part	Exposure (h)	LD_{50} value (mg/cm^2)	95% confidence level		Regression Equation	χ^2 value
			Lower	Upper		
Leaf	6	3.289	2.353	4.599	Y = 2.399 + 5.028 X	4.304
	12	2.796	2.299	3.398	Y = 2.599 + 5.376 X	1.011
	18	2.512	2.082	3.031	Y = 3.404 + 3.989 X	1.144
	24	2.053	1.873	2.249	Y = 3.369 + 5.222 X	0.799
	36	1.846	1.677	2.033	Y = 3.553 + 5.435 X	0.717
	48	1.572	1.331	1.856	Y = 4.072 + 4.726 X	9.494E-02
Stem Bark	6	4.219	2.429	7.331	Y = 2.088 + 4.657 X	3.349
	12	3.604	2.507	5.181	Y = 2.408 + 4.655 X	0.608
	18	3.579	2.165	5.918	Y = 3.34 + 2.998 X	0.502
	24	3.041	2.074	4.461	Y = 3.76 + 2.568 X	0.54
	36	2.419	1.963	2.982	Y = 4.08 + 2.398 X	0.116
	48	1.953	1.686	2.262	Y = 3.75 + 4.299 X	0.183

The lethal activity of CH_3OH extracts of *T. divaricata* (L.) leaf and stem bark against *C. chinensis* L. are represented in Table 3. The LD_{50} values for leaf extract were 6.689, 9.136E-05, 6.336, 4.591, 2.569 and 1.813 mg/cm^2 after 6h, 12h, 18h, 24h, 36h and 48h of exposures respectively. For the stem bark extract the LD_{50} values were 4.854, 5.272, 4.066, 3.17, 2.667 and 2.431 mg/cm^2 after 6h, 12h, 18h, 24h, 36h and 48h of exposures respectively. According to insecticidal activity of the extracts of *T. divaricata* (L.) could be arranged in the following downward order: leaf > stem bark.

Table-3: Dose mortality results of the methanol (CH_3OH) extracts of *T. divaricata* (L.) against *C. chinensis* L.

Plant part	Exposure (h)	LD_{50} value (mg/cm^2)	95% confidence level		Regression equation	χ^2 value
			Lower	Upper		
Leaf	6	6.689	1.155	38.756	Y = 2.139 + 3.465 X	1.74
	12	9.136E-05	1.331	48.856	Y = 3.87 + 0.279 X	0.308
	18	6.336	0.72	55.789	Y = 3.753 + 1.555 X	0.612
	24	4.591	1.067	19.751	Y = 3.952 + 1.582X	0.218
	36	2.569	1.694	3.895	Y = 4.223 + 1.897 X	0.521
	48	1.813	1.511	2.175	Y = 4.242 + 2.933X	0.106
Stem Bark	6	4.854	2.533	9.299	Y = 1.409 + 5.234 X	0 .468
	12	5.272	2.089	13.304	Y =2.586 + 3.343 X	0.323
	18	4.066	2.486	6.65	Y = 2.78 + 3.644 X	0.999
	24	3.17	2.562	3.922	Y = 2.888 + 4.215 X	1.463
	36	2.667	2.405	2.958	Y = 2.937 + 4.841 X	0.297
	48	2.431	2.261	2.613	Y = 2.377 + 6.8 X	0.378

DISCUSSION

Most of the test extracts used in this investigation showed repellent activity against adult beetles of *C. chinensis* L. The present results agreed with the previous findings of Murugan [19], who reported that the repellent activity of extracts of neem seed kernel and *Anisomeles malabarica* leaf against *C. maculatus* at 1h interval was 81% and 73% respectively at 2% concentration when tested through olfactometer. The finding also revealed that along with increasing exposures time, the repellent activity was decreased. Ogendo *et al*. [20] performed repellency test of *Ocimum gratissimum* L. and its major compound eugenol against *C. chinensis* through choice bioassay in Petri plates. After 24 h, 78–93% repellency was observed at the concentration of 0.05–0.2% v/w. It is reported that the *Saraca indica* L. extracts were found repellent to the stored pest. However, CH_3OH extracts of leaves, $CHCl_3$ and CH_3OH extracts of stem bark didn't show any repellent activity [9]. A recent study has been reported that the $CHCl_3$ extract of *Abutilon hirtum* (Lam.) showed significant result at 1% level of significance (*p*

< 0.01), while the CH_3OH extract showed moderate repellency at 5% level of significance ($p < 0.05$), but the Pet-ether extract didn't show any significant repellent activity against adult *T. castaneum* (Hbst.) [8].

The result of the present study demonstrated that both Pet-ether and CH_3OH extract of *T. divaricata* (L.) leaf and stem bark showed insecticidal potential against *C. chinensis* L. But the chloroform extracts of leaf and stem bark showed no insecticidal activity against test insect. These findings receive supports from the works done by Nowshin *et al*.[21] studied the cytotoxic potential of methanol extract of *T. divaricata* (L.) leaves on brine shrimp through lethality bioassay and reported the LC_{50} value 3.12±0.11 (µg/ml). Previous study has also reported that Pet-ether, $CHCl_3$ and CH_3OH extract of *Argemone mexicana* L. were found active against adult beetles of *T. castaneum* and the LD_{50} values were 0.608, 0.428, 0.293, 0.192 mg cm^{-2}; 1.261, 0.394, 0.241, 0.198, 0.194 mg cm^{-2}; and 1.481, 1.212, 1.171, 1.099 mg cm^{-2} after 1h, 6h, 12h, 18h; 1h, 6h, 12h, 18h, 24h and 12h, 18h, 24h, 30h of exposures respectively [7].

Plant-based pesticides have been in use as synthetic form for over 150 years, and the synthetic insecticides occupied good position in the marketplace as the prominent agrochemicals for controlling all forms of agricultural pests [22]. It was indicated the presence of potent bioactive components in this crude extract of which might be very useful as anti-proliferative, antitumor, pesticidal and other bioactive agents [23]. The overall assessment of toxicity of *T. divaricata* (L.), the Pet-ether and methanol extract of leaf are very much promising and their efficacy on stored grain pest might have future to be used as a control agent or tool.

CONCLUSION

The selected test plant *T. divaricata* (L.) is a common garden plant which traditionally used in different medicinal purposes. Its Pet-ether and methanol extract of leaf and stem bark showed repellent and good insecticidal activity against *C. chinensis* L. To prevent the problem of resistance and to conserve a healthy environment, *T. divaricata* (L.) would be a good source of such control agent if further attempts could be taken and the active potentials accomplished and furnished so far.

ACKNOWLEDGEMENT

The author (Md. Samiul Islam) is grateful to the Ministry of Science and Technology (MST) of Bangladesh for his scholarship. The authors would like to extend thanks to the Chairman, Department of Zoology, University of Rajshahi, for providing laboratory facilities.

REFERENCES

1. Dominguez J, Marrero L. Catalogo de la entomofaunaasciada an almacenes de alimentos en la provincia de Matanzas. Fitosanidad. 2010; 14: 75-82.
2. Yanagi S, Saeki Y, Tuda M. Adaptive egg size plasticity for larval competition and its limits in the seed beetles *Callosobruchus chinensis*. Entomologia Experiment et Applicata. 2013; 148(2): 182-187.
3. Valsala KK, Gokuldas M. Repellent and oviposition deterrent effects of *Clerodendrum infortunatum* on the pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae). Journal of Entomology and Zoology Studies. 2015; 3(4): 250-255.
4. Mahdi SHA. Ovicidal and repellent effects of some spice powders against the *Callosobruchus chinensis L.* and *C. maculatus* (F.). Bangladesh Journal of Zoology. 2016; 44(1): 51-59.
5. Varma S, Anadi P. Biology of pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) and their management through botanicals on stored mung grains in Allahabad region. Legume Research-An International Journal. 2010; 33(1): 38-41.
6. Champ BR, Dyte CE. Report of the FAO global survey of pesticides susceptibility of stored grain pests. In: FAO Plant Production and Protection series, UN, Rome. 1976; 5: 257.
7. Ali H, Islam S, Sabiha S, Rekha SB, Nesa M, Islam N. Lethal action of *Argemone mexicana* L. extracts against *Culex quinquefasciatus* Say larvae and *Tribolium castaneum* (Hbst.) adults. Journal of Pharmacognosy and Phytochemistry. 2017; 6(1): 438-441.
8. Hossain S, Rimi SA, Ali H, Shawon RA, Abdullah M. Islam N. Dose-mortality, Cytotoxicity and Repellent Activity of *Abutilon hirtum* (Lam.) Sweet against *Callosobruchus chinensis* (L.), *Artemia salina* L. and *Tribolium castaneum* (Hbst.). Journal of Scientific Research. 2017; 9(4), 367-373.
9. Sabuj ZR, Islam S, Haque S, Rekha SB, Islam N. Control potentials of *Saraca indica* L. extracts against the adults of stored product pests *Callosobruchus chinensis* L., *Sitophilus oryzae* L. and *Tribolium castaneum* (Hbst.) Journal of Entomology and Zoology Studies. 2017; 5(4): 11-15.
10. Sabuj ZR, Islam S, Abdullah M, Badruddoza DM, Islam N. Repellent potentials of *Morus alba* L. extractives against three stored product pests *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.) Journal of Pharmacognosy and Phytochemistry. 2017; 6(3): 825-828.
11. Ghani A. Medicinal plants of Bangladesh: Chemical constituents and uses. Dhaka: Asiatic Society of Bangladesh. 2003; 381: 1-16.

12. Pratchayasakul W, Pongchaidecha A, Chattipakorn N, Chattipakorn S. Ethnobotany & ethnopharmacology of *Tabernaemontana divaricata*. Indian Journal of Medical Research. 2008; 127: 317-335.
13. Hoernle AF. The Bower manuscript (Archaeological Survey of India, New Imperial Series, Vol. 22). Superintendent of Government Printing, Culcutta, India. 1912:18-20.
14. Ali KM. Effect of *Tabernaemontana divaricata* (crepe jasmine) flower methanolic extract on pylorus ligated rats. Malays J Pharm Sci. 2010;1:104-5.
15. McDonald LL, Guy RH, Speirs RD. Preliminary evaluation of new candidate materials as toxicants, repellents, and attractants against stored-product insects-1. Preliminary evaluation of new candidate materials as toxicants, repellents, and attractants against stored-product insects-1.. 1970(882).
16. Talukder FA, Howse PE. Laboratory evaluation of toxic and repellent properties of pithraj tree, *Aphanamixis polstachya* Wall & Parker, against *Sitophilus oryzae* L. International Journal of Pest Management. 1995; 40: 274-279.
17. Busvine JR. A critical review of the techniques for testing insecticides. Commonwealth Agricultural Bureau, Lodon. 1971; pp. 345.
18. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18: 265-267.
19. Murugan K. Bioefficacy of plant derivatives on the repellency, damage assessment and progeny production of the cowpea weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). 10th International Working Conference on Stored Product Protection, Julius- Kühn-Archiv 2010; 425: 874–880.
20. Ogendo J, Kostjukovski M, Ravid U, Matasyoh J, Deng A, Omolo E, Kariuki S, Shaaya E. Bioactivity of *Ocimumgratissimum* L. oil and two of its constituents against five insect pests attacking stored food products. Journal of Stored Products Research. 2008; 44:328–334.
21. Nowshin NR, Rahman MM, Kazal MK. Antioxidant and cytotoxic potential of methanol extract of *Tabernaemontana divaricata* leaves. International Current Pharmacological Journal. 2012; 1(2): 27-31.
22. Du J, He ZD, Jiang RW, Ye WC, Xu HX, But PPH. Antiviral flavonoids from the root bark of *Morus alba* L. Phytochemistry. 2003; 62: 1235-1238.
23. Meyer BN, Ferringni NR, Puam JE, Lacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active constituents. Planta Medica. 1982; 45: 31-32.