Repellent activities of *Syzygium cumini* L. (Myrtaceae) extracts against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

Md. Hasanuzzaman, W. Islam^{*} and S. Parween¹

Institute of Biological Sciences, University of Rajshahi, Bangladesh ¹Department of Zoology, University of Rajshahi, Bangladesh

> **Abstract** The root, leaf, stem bark and seed were extracted into n-Hexane, acetone, chloroform and methanol from *Syzgium cumini* L and were used against *Callosobruchus chinensis* L. for the repellent activities. All the tested extracts showed repellent activities against adult beetles of *C. chinensis* at concentrations of 251.50, 125.70, 62.86, 31.43 and 15.72 μ g/cm² on filter paper. The chloroform extracts of all plant parts showed the best repellency at 0.01% level of significance, except the leaf (chloroform) extract which was found active at 0.1% level of significance. According to the intensity of repellency the result could be arranged in a descending order: leaf (chloroform extract) >stem bark (chloroform extract) >root (methanol extract) > seed (methanol extract) and in all the cases significant differences were observed.

Key words: Syzygium cumini extracts, Callosobruchus chinensis, repellency

Introduction

Pulses are important and rich sources of several amino acids; containing 20-30% protein which is almost three times higher than the protein content of cereals (Doharey *et al.*, 1990). These grains also contain minerals and certain vitamins, and provide high energy. Pulse legumes are infested by several bruchid species (Arora, 1977) of which *Callosobruchus* species are notorious and cosmopolitan during both pre-and post-harvest periods. The major pests of pulses are *Callosobruchus chinensis* L. and *C. maculatus* (F.). The infested pulses loss the seed quality and viability, and the market value is decreased accordingly (Caswell, 1980).

Synthetic chemical pesticides have been used for a long time to control stored legumes (Bhalla *et al.*, 2008). However, over use of chemical insecticides led a number of problems including hazards to human health and destruction of the ecosystem balance (Shaheen & Khaliq, 2005). Plant based pesticides were found to be potent candidates for insect pest control in stored legumes (Rajapakse & Ratnasekera, 2008).

In Bangladesh presence of both species of *Callosobruchus* have been recorded from infested pulses at both field and storage systems, of which *C. chinensis* is the commonest.

Syzygium cumini L. is a common, large evergreen fruit tree of Bangladesh, commonly known as

'Jamun' or 'Jam' which belongs to the family Myrtaceae. The original home of *S. cumini* is India or the East India and have been successfully introduced into many other tropical countries (Kirtikar & Basu, 1975). The plant extract has been frequently used by a number of researchers and they reported that the plant possesses insecticidal activity.

However, there is a lack of information on the insect management potentiality of *S. cumini* against the stored-product insects, especially against *C. chinensis*. Therefore, the present research was designed to determine the repellent effect of the extracts of root, leaf, stem bark and seed of *S. cumini* against *C. chinensis* under laboratory condition.

Materials and Methods

Plant collection: The plant *S. cumini* was collected from the University of Rajshahi campus.

Test insect: Adults of *C. chinensis* were collected from the lentil infested stock cultures maintained for five years at the Control Temperature room (CT Room: $30 \pm 0.5^{\circ}$ C and 70-80% RH) with photoperiod (12h L: 12h D) of the Entomology and Insect Biotechnology Laboratory, Institute of Biological Sciences, University of Rajshahi. The fresh lentil seeds was used as food medium for the sub-cultures of *C. chinensis*.

^{*}Corresponding author: mwislam2001@yahoo.com

Preparation of extracts: Root, leaf, stem bark and seeds were dried in shade and stored separately in cotton bags. Prior to experimental set up the dried plant parts were powdered individually with the help of a grinder. Each ground material (500 g) was soaked in 500 ml n-Hexane, acetone, chloroform and methanol separately for 24-72 h and filtered through Whatman no 1. The filtrate was then allowed to vaporize in rotary evaporator until completely dried up which was kept in a refrigerator at 4°C with proper labelling. Hundred dried extract were dissolved in 10 ml of each of the solvents. The concentration of the final extract thus was 100 μ g/10 μ l for each solvent of each plant part.

Preparation of concentrations: The concentration $100 \mu/10 \mu$ was considered as the stock solution. and then the final concentrations were made by serial dilution of the same solvent at the proportion of 1:2. From this solution of each extract 12.5, 10.0, 6.25 and 3.125 were taken into separate vials and dissolved in requisite amount of the respective solvent and spread equally on the filter paper placed at the bottom of separate petridish (90 mm). Now the concentrations were attained as 251.5, 125.7, 62.86, 31.43 and 15.72 mg of extract per 0.01 cm of the petri dish. The concentration controlling light and humidity was calculated by measuring the dry-weigh of the crude extracts applied in petri dish divided by surface area of respective petri dish. The petri dishes were then dried at room temperature. Such five concentrations were prepared for each four extracts of each part (root, seed, leaf and stem) of S. cumini.

Repellency test: Half filter paper discs (Whatman No. 40, 9 cm diam) were prepared and selected concentrations of all the n-Hexane, acetone, chloroform and methanol extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 10-15 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc (without any extract) with adhesive tape and placed in a Petri dish (9 cm diam), the inner wall of which was smeared with glu on to prevent insects escaping. Ten adult insects (3-5d old) were released in the midline of each filter-paper. Insects that settled on each half of the filter paper discs were counted after 1 h and then at hourly intervals for 5 h. No significant difference was detected between the repellency of only solvent impregnated and untreated filter papers in tests designed to check for any possible influence of solvents. Each test was repeated five times. The average of the counts was converted to percentage repellency (PR) using the formula of Talukder & Howse (1995):

$$PR = (N_c - 5) X 20$$

Where, N_c is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values for attractant activity.

Statistical analysis

The PR data was further transformed into arcsin values for the calculation of Analysis of Variance (ANOVA).

Results and Discussion

All the test extracts of leaf, seed, stem bark and root of *S. cumini* collected in n-Hexane, acetone, chloroform and methanol showed repellency by contact action against the adult *C. chinensis* at concentration levels 251.50, 125.70, 62.86, 31.43 and 15.72 μ g/cm² on filter paper.

All the extracts (n-Hexane, Acetone, Chloroform and Methanol) at all concentrations, except the lowest ones, showed repellent action against the adult *C. chinensis* (Tables 1-4). The 3-67% repellency were recorded in acetone, chloroform and methanol extracts of root in higher concentrations. The highest 93.32% repellency was recorded in chloroform and methanol extracts of leaf and seed in higher concentrations (Tables 2 and 4). The lowest concentrations showed the lowest repellency.

Adults showed significantly different levels of repulsion at different concentrations of each extract of all parts of S. cumini (Table 5). However, the repellent effect did not vary with exposure time. The acetone and chloroform extracts of leaf were found to be comparatively less repellent than the other parts (Table 5). Among the tested chloroform extracts all the rest offered repellency at 0.01% level of significance (P<0.001) except the leaf (chloroform) extract which was found active at 0.1% level of significance (P<0.01) (Table 5). According to the intensity of repellency the result could be arranged in a descending order: leaf (chloroform extract)>stem bark (chloroform extract)>root (methanol extract)>seed (methanol extract) and in

Repellent activity of S. cumini L.

Solvents	Conc.	Dist		n of adu rea/hou		ated	Percent repulsion (PR)				
	(µg/cm²)	1	2	3	4	5	1	2	3	4	5
	251.5	6.67	7.00	7.33	7.00	6.33	33.32	40.0	46.66	40.0	26.66
	125.7	6.0	5.67	6.33	5.33	5.67	20.00	13.32	26.66	6.6	13.56
n-Hexane	62.86	5.33	5.67	5.67	5.67	5.33	6.66	13.32	13.32	13.32	6.66
	31.63	4.67	4.67	4.33	4.33	3.67	-6.68	-6.68	-13.34	-13.34	-26.68
	15.72	3.0	2.33	3.33	3.33	2.33	-40.0	-53.34	-33.34	-33.34	-53.34
	251.5	8.33	8.0	7.67	7.33	7.67	66.66	60.0	53.32	46.66	53.32
	125.7	7.67	7.0	6.33	6.33	6.67	53.32	40.0	26.66	26.66	33.32
Acetone	62.86	6.33	6.0	5.67	5.33	5.67	26.66	20.0	13.32	6.66	13.32
	31.63	5.67	5.33	5.33	4.67	5.33	13.32	6.66	6.66	6.68	6.66
	15.72	5.33	4.0	4.0	3.33	4.67	6.66	-20.0	-20.0	-33.34	-6.68
	251.5	8.33	8.0	7.67	7.33	8.0	66.66	60.0	53.32	46.66	60.0
	125.7	7.33	7.33	6.33	6.67	6.33	46.66	46.66	26.66	33.32	26.66
Chloroform	62.86	6.33	6.0	5.67	6.0	5.67	26.66	20.0	13.32	20.0	13.32
	31.63	5.33	5.33	5.33	5.0	4.0	6.66	6.66	6.66	0.0	-20.0
	15.72	4.33	4.0	4.0	3.67	3.0	-13.34	-20.0	-20.0	-26.56	-40.0
Methanol	251.5	5.67	5.33	5.67	5.67	4.33	13.32	6.66	13.32	13.32	-13.32
	125.7	8.0	7.67	8.0	7.67	7.0	60.0	53.32	60.0	53.32	40.0
	62.86	7.68	6.67	6.33	6.67	6.33	53.32	33.32	26.66	33.32	26.66
	31.63	6.33	5.67	6.33	5.67	5.33	26.66	13.32	26.66	13.32	6.66
	15.72	5.67	5.33	5.67	5.67	4.33	13.32	6.6	13.32	13.32	-13.32

all the cases significant differences were observed. **Table 1.** Repellency effect of root extracts of different solvents of *S. cumini* against *C. chinensis* adults (N=10).

Table 2. Repellency effect of leaf extracts of different solvents of S. cumini against C. chinensis adults (N=10).

Solvents	Conc. (µg/cm²)	Dist	ributior a	n of adu irea/hou		ated	Percent repulsion (PR)				
	(µg/cm)	1	2	3	4	5	1	2	3	4	5
n-Hexane	251.5	8.67	9.0	8.33	8.67	8.67	73.32	80.0	66.66	73.32	73.32
	125.7	7.67	7.33	7.67	7.67	7.67	53.32	46.66	53.32	53.32	53.32
	62.86	6.33	6.67	6.33	6.67	6.67	26.66	33.32	26.66	33.32	33.32
	31.63	5.67	6.33	5.33	5.67	5.67	13.32	26.66	6.66	13.32	13.32
	15.72	5.0	5.67	4.33	4.33	4.33	0.0	13.32	-13.34	6.66	-13.34
	251.5	8.67	8.33	8.33	8.0	8.33	73.33	66.66	66.66	60.0	66.66
	125.7	7.67	7.33	7.67	7.33	7.67	53.32	46.66	53.32	46.66	53.32
Acetone	62.86	6.33	6.33	6.67	6.33	7.0	26.66	26.66	33.32	26.66	40.0
	31.63	6.0	5.33	6.33	5.67	6.0	20.0	6.66	26.66	13.32	20.0
	15.72	6.0	5.33	5.67	5.33	4.67	20.0	6.66	13.32	6.66	-6.68
	251.5	9.67	9.33	9.0	9.0	9.67	93.32	86.66	80.0	80.0	93.32
	125.7	8.67	8.33	8.0	7.67	8.0	73.32	66.66	60.0	53.32	60.0
Chloroform	62.86	7.33	6.67	7.0	6.67	6.67	46.89	33.32	40.0	33.32	33.32
	31.63	6.0	5.67	6.33	5.67	5.33	20.0	13.32	26.66	13.32	6.66
	15.72	5.67	5.33	5.67	5.33	4.67	13.32	6.66	13.32	6.66	-6.68
Methanol	251.5	8.67	7.33	8.0	8.0	9.67	92.32	83.33	80.0	80.0	93.32
	125.7	8.67	7.33	8.0	7.67	8.0	73.32	66.66	60.0	53.32	60.0
	62.86	7.33	6.67	7.0	7.67	6.67	46.89	33.32	40.0	33.32	33.32
	31.63	5.0	5.67	6.33	5.67	5.33	20.0	13.32	26.66	13.32	6.66
	15.72	5.67	5.33	6.67	5.33	4.67	13.32	6.66	13.32	6.66	-6.68

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Solvents	Conc. (µg/cm ²)	Dist	tributior a	n of adu rea/hou		ated	Percent repulsion (PR)				
	(µg/cm)	1	2	3	4	5	1	2	3	4	5
	251.5	9.67	9.0	9.33	9.67	9.0	93.32	80.0	86.66	93.32	80.0
	125.7	8.33	7.67	8.0	8.33	7.0	66.66	53.32	60.0	66.66	40.0
n-Hexane	62.86	7.0	6.33	6.33	7.33	5.67	40.0	26.66	26.66	46.66	13.32
	31.63	6.0	6.0	5.33	6.33	5.33	20.0	20.0	6.66	26.66	6.66
	15.72	5.33	5.67	5.33	5.0	4.0	6.66	13.32	6.6	0	-20.0
	251.5	8.67	9.0	8.67	8.67	9.0	73.32	80.0	73.32	73.32	80.0
Acetone	125.7	7.67	8.0	8.33	8.0	8.0	53.32	60.0	66.66	60.0	60.0
	62.86	6.67	6.67	7.0	6.67	6.67	33.32	33.32	40.0	33.32	33.32
	31.63	5.67	6.0	5.67	5.33	5.33	13.32	20.0	13.32	6.66	6.66
	15.72	5.33	4.67	4.33	4.33	3.67	6.66	-6.68	-13.35	-13.34	-26.68
	251.5	8.33	8.0	7.67	8.0	7.67	66.66	60.0	53.32	60.0	53.32
	125.7	7.67	7.0	7.0	7.0	6.33	53.32	40.0	40.0	40.0	26.66
Chloroform	62.86	6.33	5.33	6.0	6.33	5.33	26.66	-6.66	20.0	26.66	6.66
	31.63	5.67	4.33	5.33	5.67	4.67	13.32	-13.34	6.66	13.32	-6.68
	15.72										
Methanol	251.5	8.33	7.67	8.33	7.33	8.0	66.66	53.32	66.66	46.66	60.0
	125.7	6.67	7.33	7.0	6.67	7.0	33.32	46.66	40.0	33.32	40.0
	62.86	6.0	6.33	6.67	5.67	6.0	20.0	26.66	33.32	13.32	20.0
	31.63	5.67	5.33	6.33	5.33	5.67	13.32	6.66	26.66	6.66	13.32
	15.72	5.33	4.33	5.33	4.33	4.67	6.66	-13.34	-13.34	6.66	-6.68

Table 3. Repellency effect of stem bark extracts of different solvents of S. cumini against C. chinensis adults (N=10).

Table 4. Repellency effect of seed extracts of different solvents of S. cumini against C. chinensis adults (N=10).

Solvents	Conc. (µg/cm ²)	Dist	ributior a	n of adu rea/hou		ated	Percent repulsion (PR)				
	(µg/cm)	1	2	3	4	5	1	2	3	4	5
	251.5	7.67	7.33	7.0	7.67	7.33	53.32	46.66	40.0	53.32	46.66
	125.7	6.33	6.67	6.0	6.33	6.67	26.66	33.32	20.0	26.66	33.32
n-Hexane	62.86	6.33	5.67	5.33	5.67	5.67	26.66	13.32	6.66	13.32	13.32
	31.63	6.33	5.67	5.33	5.67	5.67	26.66	13.32	6.66	13.32	13.32
	15.72	4.33	4.33	4.0	3.33	4.0	-13.34	-13.34	-20.0	-33.34	-20.0
	251.5	8.67	8.67	8.33	8.0	8.33	73.32	73.32	66.66	60.0	66.66
	125.7	8.0	7.67	7.33	7.0	6.67	60.0	53.32	46.66	40.0	33.32
Acetone	62.86	6.67	6.33	6.0	5.67	5.67	33.32	26.66	20.0	13.32	13.32
	31.63	6.0	5.67	5.33	4.33	5.33	20.0	13.32	6.6	13.34	6.66
	15.72	4.67	5.0	3.67	3.0	4.33	-6.68	0.0	-26.68	-40.0	-13.34
	251.5	6.67	7.33	6.67	7.0	7.33	33.32	46.66	33.32	40.0	46.66
	125.7	6.33	6.0	6.33	6.0	6.33	26.66	20.0	26.66	20.0	26.66
Chloroform	62.86	5.67	5.33	5.67	5.33	5.67	13.32	6.66	13.32	6.66	13.32
	31.63	4.67	4.33	5.33	4.33	4.0	-6.68	-13.34	-6.66	-13.34	-20.0
	15.72	3.33	3.67	4.0	3.33	3.0	-33.34	-26.68	20.0	33.34	40.0
Methanol	251.5	9.67	9.33	9.0	8.67	9.0	93.32	86.66	80.0	73.32	80.0
	125.7	8.67	8.0	8.0	7.33	8.0	73.32	60.0	60.0	46.66	60.0
	62.86	7.33	6.67	7.0	6.33	6.67	46.66	33.32	40.0	26.66	33.32
	31.63	6.0	5.67	6.33	5.33	5.67	20.0	13.32	26.66	6.66	13.32
	15.72	5.33	4.67	5.67	4.33	4.0	6.66	-6.68	13.32	-13.34	-20.0

Plant parts	Extracts	Factors	F-value	P-value (df=4)
	n-Hexane	Concentrations	253.296	3.03
	II-Hexalle	Exposure Time	2.95	0.0526
	Acetone	Concentrations	51.036	6.55
Deet	Acelone	Exposure Time	6.778	0.002
Root	Chlarafarm	Concentrations	54.435	4.07
	Chloroform	Exposure Time	3.901	0.021
	Mathanal	Concentrations	43.044	2.27
	Methanol	Exposure Time	5.447	0.0057
	n Havana	Concentrations	83.589	1.63
	n-Hexane	Exposure Time	3.835	0.023
	A set set s	Concentrations	64.509	1.15
0	Acetone	Exposure Time	5.669	0.005
Seed	Chlarafarm	Concentrations	75.793	3.43
	Chloroform	Exposure Time	1.4005	0.278
		Concentrations	50.394***	7.19
	Methanol	Exposure Time	4.244	0.016
	n Hevene	Concentrations	37.389	6.26
	n-Hexane	Exposure Time	9.653	0.0003
	A set set s	Concentrations	28.478	4.24
Last	Acetone	Exposure Time	5.453	0.006
Leaf	Chlarafarm	Concentrations	17.687**	1.01
	Chloroform	Exposure Time	0.722	0.589
	Mathemal	Concentrations	63.545	1.29
	Methanol	Exposure Time	7.798	0.001
	n Hevene	Concentrations	121.978	9.03
	n-Hexane	Exposure Time	2.953	0.0527
Stem bark	Apotono	Concentrations	70.1196	6.16
	Acetone	Exposure Time	1.604	0.222
	Chloraform	Concentrations	41.638	2.89
	Chloroform	Exposure Time	7.299	0.0015
	Mathemal	Concentrations	67.354	8.34
	Methanol	Exposure Time	1.86	0.167

Table 5. ANOVA on repellent action of S. cumini extracts against C. chinensis by contact.

Note: *=** Highly significant, ****** = Significant

Repellency by the n-Hexane, acetone, chloroform, and methanol extracts of *S. cumini* against *C. chinensis* adults was very much promising, while all the extracts found to repel at 0.01% level of significance (P<0.001) except the leaf (chloroform) extract which was found active at 0.1% level of significance (P<0.01). The repellency record triggers a hope for the use of *S. cumini* extracts as repellent to manage *C. chinensis* in stored pulses.

Nattudurai *et al.* (2015) investigated insecticidal and repellent activity of *Toddalia asiatica* (L.) Lam. (Rutaceae) leaf and fruit extracts (hexane, diethyl ether and methanol) against *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.) adults and found that all the extracts of both leaf and fruits had the mortality and repellency against the tested insects in a concentration dependent manner. They found *C. maculatus* was the most susceptible. Lethal concentrations for 50 percent mortality (LC₅₀) of

C. maculatus, S. oryzae and T. castaneum were recorded as 39.19, 44.13 and 61.10 µl/L respectively. They concluded diethyl ether fruit extract exhibited 100% repellent activity against C. maculatus and S. oryzae and 92% against *T. castaneum* adults at 20 µl/L concentration. They concluded that diethyl fruit extract of T. asiatica could be used as ecofriendly and repellent against C. maculatus, S. oryzae and T. castaneum. Badshah et al. (2015) studied repellency evaluation of some selected indigenous plant materials against Rhyzopertha dominica and found the tested insect was exposed for ten days to five plant extracts (distilled water) viz. Mentha longifolia, Momordica charantia, Luffa aegyptica, Carum copticum and Curcuma longa at the concentrations of 25%, 50% and 75%. The results depicted that dose rate and exposure period had significant effects on the repellency. They reported that the repellency increased with increasing dose and

exposure periods. M. longifolia was found to be the most effective repellent followed by M. charantia, L. aegyptica, C. copticum and C. longa. They concluded that complete repellency was achieved with M. longifolia while minimum repellency was recorded with C. longa at 75% and 25% dose rates respectively. Repellency and feeding deterrency of S. oryzae of three distributed plants extracted in ethanol, chloroform and hexane were performed by Viglianco et al. (2008) in Argentina. The studied plant species were Aloysia polystachia (Griseb) Moldenke, Solanum argentinum Bitter et Lillo and Tillandsia recurvata (L.) L. Percentual repellency (PR) was determined for each extract. A moderate repellent effect of S. argentinum and A. polystachia on S. oryzae was observed; however, the hexane extract of S. argentinum was the one with strongest repellent (Class 4), Abdullah et al. (2011) assessed the mortality and repellency of the chloroform extracts of different parts of Urena sinuate on T. castaneum adults. The root and stem extracts showed significant repellent effect on the beetles but the fruit and leaf extracts produced no repellency at all. Talukder & Howse (1995) tested MeOH extract of pitraj (Aphanamixis polystachya (Wall.) against T. castaneum and reported 100% repellency. Mondal et al. (2011) assessed the repellent activity of Derris indica extracts against T. castaneum adults. The fruit shell, leaves, seed and stem bark extracts also showed repellent activity.

The result of the present study showed that all the tested concentrations of the extracts have considerable repellent activity against adult *C. chinensis*. An unpleasant pungent smell was present in the extracts due to the presence of certain highly volatile chemical components, which caused the repellency to the test insect.

It may be concluded that *S. cumini* possess chemicals, which are repellent to insects and therefore are potential against *C. chinensis*. The results thus provide important tools for further development of Integrated Pest Management (IPM) Program.

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Manuscript accepted on 23.06.2015