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Bioactive potentials of *Melia azedarach* L. with special reference to insecticidal, larvicidal and insect repellent activities

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Abstract
A lab experiment was conducted during June 2015 - Feb 2016 at the Crop protection & Toxicology Lab., University of Rajshahi, Bangladesh to evaluate the insecticidal, repellent and larvicidal efficacy of Petroleum ether (Pet. ether), $CHCl_3$ and CH_3OH extracts of the fruits and leaves of *Melia azedarach* against adults of *Tribolium castaneum* and larvae of. *Tribolium* adults exhibited mortality when exposed to all the doses of Pet. ether extracts of fruits and leaves whereas the $CHCl_3$ and CH_3OH extracts of the same didn't show mortality at all. All extracts showed larvicidal activity by yielding different LC_{50} values in different time exposure. The Pet. ether extracts of fruit did not show repellent activity, while the Pet. ether extract of leaf showed repellent activity at 5% level of significance ($P<0.05$) and the $CHCl_3$ and CH_3OH extracts of both fruit and leaf offered repellent activity at 5% level of significance ($P<0.05$).

Keywords: *Melia azedarach, Tribolium castaneum, Culex quinquefasciatus,* Insecticidal, Larvicidal and Insect repellent activity

1. Introduction

Increasing concern about the risks from synthetic insecticides to the environment and human health has led to a major trend in current pest management strategy, which involves searching for less hazardous chemicals or biologically based products [1]. Botanical products fit within this strategy, being biodegradable and effective against pests without harming beneficial insects [2]. *M. azedarach* L. (locally known Ghoda Neem) belongs to the family Meliaceae, is a highly significant medicinal plant found almost everywhere in Bangladesh [3]. Each and every part of the *M. azedarch* have traditional medicinal uses like stem is prescribed internally against asthma, bark is used in case of fever to relieve thirst, nausea, vomiting and general debility, loss of appetite and skin diseases. Leaves also relieve from headache and cure the eruption on the scalp. Leaf juice is anthelmintic, diuretic, vermifuge and their decoction is astringent and stomachic. Fruits are used for the preparation of tonic which is purgative, emmolient and anthelmintic. Seeds are bitter, expectorant, anthelmintic and aphrodisiac and are useful in helminthiasis, typhoid fever, pain in the pelvic region and scrofula. Roots are also bitter, astringent, anodyne, depurative, vulnerary, antiseptic, anthelmintic, constipating, expectorant, febrifuge, antiperiodic, and bitter tonic in low doses [4]. The present work aimed at evaluation of the insecticidal and repellent activities of *M. azedarach* leaf and fruit extracts against the severe stored product pest, *T. castaneum* (Hbst.) (Coleoptera: Tenebrionidae) adults and larvicidal activity of same against the filarial vector, *C. quinquefasciatus* Say (Diptera: Culicidae). *T. castaneum* is a primary pest of flour and other milled products of cereals and a secondary pest of stored wheat [5-8] causing severe damages to quantity and quality of these food grains [9]. Adults are reddish-brown in color, flattish curved sided body, head is visible from above, antennae capitate type and thorax has slightly curved sides [10]. On the other hand *C. quinquefasciatus* is a widely distributed species of mosquito throughout the world. This mosquito has been found breeding in shallow ponds within streams [11] and artificial habitats such as drains and drain sumps [12], septic tanks, rain water containers, tyres, etc. [13-14]. Adult *C. quinquefasciatus* vary from 3.96 to 4.25mm in length [15]. The eggs are not desiccation resistant and are laid as rafts on the water surface [16]. Most eggs hatch into larvae (Wrigglers) within 2-3 days and they swim actively in water by wriggling movements [17].
The present work aimed to evaluate the repellent and insecticidal effects of Pet. ether, $CHCl_3$ and CH_3OH extracts of *M. azedarach* fruits and leaves. For the evaluation of insecticidal activities and for the repellency test the red flour beetle, *T. castaneum* (Hbst.) adults were used

in this investigation. This study also aimed at investigation of the larvicidal potential of the extracts to control the filarial vector, *C. quinquefasciatus* Say (Diptera: Culicidae).

2. Materials and Methods

2.1 Collection and preparation of test materials: The fruits and leaves of *M. azedarach* were collected from the village, Ranihati under Chapai Nawabganj District, Bangladesh. The collected plant parts were cut into small pieces and spread out to dry (under shade at room temperature) in separate trays. Well dried plant materials ground to coarse powder with an electric blender, weighted, kept in separate conical flasks and then extracted with sufficient amount of solvents (Pet. ether, $CHCl_3$ and CH_3OH) (200g × 600ml × 2 times) for 48h. Filtration was done by Whatman No. 40 filter paper and after evaporation residues (extracts) left were collected in glass vials and kept in a cool place with appropriate labeling.

2.2 Collection and culture of the test insect: The test insects *T. castaneum* of 3 to 5 days old used in this investigation were received from the sub cultures of the Crop Protection Laboratory, Department of Zoology, University of Rajshahi, Bangladesh and the culture of *C. quinquefasciatus* were maintained in the same laboratory throughout the experimental period for continuous supply of larvae.

2.3 Dose-mortality tests on *T. castaneum*: For dose-mortality response of the beetles through surface film method doses for the fruit extract were 2.037, 1.783, 1.528 1.273 and 1.019mg cm^{-2} and for the leaf extract were 1.783, 1.528, 1.273, 1.019 and 0.764mg cm^{-2} selected by pilot experiments. Each of the doses were diluted in 1ml of solvent, poured into a Petri dish and allowed to dry. Ten adult beetles were released in each Petri dish. The whole experiment was set using all the doses for each of the extracts in 3 replicates; and was started observation to record mortality data from 0.5h and then 6h, 12h and by every 12h interval up to 72h.

2.4 Larvicidal activity test: The extracts of fruit and leaf were tested against the one day aged *C. quinquefasciatus* larvae throughout the lethality test. The doses were 600, 500, 400, 300 and 200ppm for the fruit ($CHCl_3$) and leaf (Pet. ether and CH_3OH) extracts; 500, 400, 300, 250, 200, 150 and 100ppm for the fruit (Pet. ether and CH_3OH) and 170, 150, 140, 130 and 110ppm for the leaf ($CHCl_3$) extracts were set through pilot experiments. Ten freshly emerged larvae were added to each of the test tubes with different concentrations mentioned above and observation of mortality was made after 6h of application with 6h interval up to 36h.

2.5 Statistical Analysis: The mortality records of the residual film experiments on *T. castaneum* adults and the lethality records on larvae were corrected by the Abbott's formula [18]

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

Where, P_r = Corrected mortality (%), P_o= Observed mortality (%), P_c= Control mortality (%). The data were then subjected to probit analysis according to Finney and Busvine [19-20]. The dose-mortality relationship was expressed as a median lethal dose (LD_{50}) for *T. castaneum* whereas lethality relationship as a median lethal concentration (LC_{50}) for the mosquito larvae.

2.6 Repellent activity test against *T. castaneum* adults: The methodology for repellency test used in the experiment was adopted from the method (No. 3) of McDonald *et al*. [21] with some modifications by Talukder and Howse [22-23]. Half filter paper discs (Whatman No. 40, 9cm diam.) were treated with the selected doses of 0.314, 0.157, 0.079, 0.039 and 0.019mg cm^{-2} of all extracts and were then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in the Petri dishes. Ten adult insects were released in the middle of each of the filter paper circles.

2.7 Observation and analysis of repellency data: Each concentration was tested for five times. Insects that settled on each of the non-treated half of the filter paper discs were counted after 1h and then observed repeatedly at hourly intervals for five hours. The average of the counts was converted to percent repellency (*PR*) using the formula of Talukder and Howse [22, 24]: *PR* = (Nc – 5) 20, where, 'c' is the percentage of insects on the untreated half of the disc.

3. Results

3.1 Dose mortality effects: The Pet. ether, $CHCl_3$ and CH_3OH extracts of *M. azedarach* fruit and leaf were tested against the adult beetles of *T. castaneum* through residual film assay and only the Pet. ether extract was found active. The results of the dose-mortality assay for Pet. ether extracts of fruit and leaf are represented in Table 1. The highest and the lowest mortality have been observed from the Pet. ehter extract of *M. azedarach* leaf (LD_{50} 0.312mg cm^{-2}) and the Pet. ether extract of *M. azedarach* fruit (LD_{50} 1.359mg cm^{-2}) both after 72h of exposure. According to the intensity Pet. ehter extracts of leaves were dominating over the Pet. ether extracts of fruits.

Table 1: LD_{50} values of Pet. ether extracts of *M. azedarach* fruit and leaf against *T. Castaneum*

Extract	**solvent**	**LD_{50} (mg cm^{-2}) at different exposures (in hours)**							
		0.5h	**6h**	**12h**	**24h**	**36h**	**48h**	**60h**	**72h**
Fruit	Pet. ether	-	2.129	1.889	1.663	1.559	1.504	1.455	1.359
Leaf	Pet. ether	-	1.413	1.152	0.965	0.885	0.601	0.372	0.312

3.2 Larvicidal effects: The lethality records of Pet. ether, $CHCl_3$ and CH_3OH extracts of fruit and leaf of *M. azedarach* represented in Table 2. The highest and lowest lethality has been observed for the $CHCl_3$ extracts of fruits (LC_{50} 3.184ppm) and CH_3OH extracts of leaves (LC_{50} 828.367ppm) after 18h of exposure against *C. quinquefasciatus* larvae. According to the intensity the extracts of *M. azedarach* could be arranged in the following descending order: fruit ($CHCl_3$) > fruit (Pet.ether) > fruit (CH_3OH) > leaf (Pet. ehter) > leaf (CH_3OH) extract.

Table 2: LC_{50} values of extracts of *M. azedarach* leaf and fruit against *C.quinquefasciatus* larvae

Extracts	solvent	LC_{50} ppm at different exposures (in hours)					
		6h	12h	18h	24h	30h	36h
Fruit	Pet.E.	-	502.687	387.485	339.011	258.781	195.131
	$CHCl_3$	759.492	40.168	3.184	All dead		
	CH_3OH	-	615.988	539.090	326.536	266.487	154.743
Leaf	Pet.E.	1889.108	763.843	415.125	283.498	138.948	128.148
	$CHCl_3$	-	-	-	1118.701	462.446	388.434
	CH_3OH	-	996.866	828.367	657.194	478.637	331.359

3.3 Repellent effect on *T. castaneum*

The Pet. ether extract of leaf showed repellency at 5% level of significance (P<0.05) while the Pet. ether extract of fruit did not show repellency. The $CHCl_3$ and CH_3OH extracts of leaf and fruit showed the significant repellent activity at 5% level of significance (P<0.05) (Table 3). According to the intensity of repellency the results could be arranged in a descending order of: Leaf (Pet. ether) > Fruit (CH_3OH) > Leaf (CH_3OH) > Leaf ($CHCl_3$) > Fruit ($CHCl_3$).

Table 3: ANOVA results of repellency of the Pet. ether, $CHCl_3$ and CH_3OH extracts of fruit and leaf of *M. azedarach* against *T. castaneum* adults

Types of extract		Source of variation			F- ratio with level of significance		P- value	
Plant parts	Solvents	Between doses	Between time intervals	Error	Between doses	Between time intervals	Between doses	Between time intervals
Fruit	Pet. ether	4	4	16	3.097	0.549	0.046	0.702
	$CHCl_3$	4	4	16	12.916*	1.043	6.95E-05	0.416
	CH_3OH	4	4	16	16. 930*	1.471	1.33E-05	0.257
Leaf	Pet. ether	4	4	16	17.454*	0.309	1.10E-05	0.868
	$CHCl_3$	4	4	16	13.992*	0.905	4.31E-05	0.484
	CH_3OH	4	4	16	15.541*	1.045	2.27E-05	0.415
* = P<0.05								

4. Discussion

The findings of this work receive supports from previous researchers' achievements. The ethanol extracts of different parts of *Melia azedarach*, *Mentha longifolia*, *Myrtus communis*, *Cymbopogon citratus* and *Datura stramonium* possess toxic principles with significant insecticidal effects against *Oryzaephilus surinamensis*, *T. castaneum* (Herbst) and *Callosobruchus chinensis* L. [25]. Ahmed *et al.* [26] reported that *M. azedarach, Aegle marmelos, C. splendens* and *C. inerme* afforded a significant degree of feeding deterrent to different instars of *Spodoptera littoralis*. Khan and Siddiqui [27] recorded good repellency of *M. azedarach* (Bakain) seeds and leaves against *Tribolium castenteum*. Another report described the repellent properties of *M. azedarach* against the red pumpkin beetle (*Aulacophora foveicollis* Lucas) attacking musk melon (*Cucumis melo* L.) crop. [28]. Methanol extracts of fruits and seeds of *M. azedarach* showed strong antifeedant activity against larvae of *S. nonagrioides* (Lepidoptera: Noctuidae) [29-30]. Fruit extract of *M. azedarach* caused growth inhibition and larval mortality above 80% against *Spodoptera frugiperda* [31]. Extracts obtained from seed kernels of *M. azedarach* with petroleum ether showed antifeedant effect against 2^{nd} and 3^{rd} instar nymphs of *Nilapavarta lugens* Stal. (Hemiptera: Delphacidae) [32]. Methanol extracts of the leaves and seeds of the *M. azedarach* were tested against mature and immature stages of the mosquito vector *A. stephensi*. Different fractions of the ethanol extract of leaves and unripe fruit of *M. azedarach* proved as an effective repellent against the nymphs of *Triatoma infestans*. The ethyl acetate extract of *M. azedarach* leaf showed excellent larvicidal potential in controlling *C. quinquefasciatus* and *A. aegypti* [33].

5. Conclusion

The findings depicted in the present study approved the repellency, mortality and larvicidal activity of the extractives of *M. azedarach* leaf and fruit on *T. castaneum* adults and *C. quinquefasciatus* larvae. Pet. ether extracts of both the test parts exposed good activity against *T. castaneum* and on *C. quinquefasciatus* larvae. In case of the repellent activity test, except Pet. ether extract of fruits all the extracts showed significant repellency. So, no doubt this plant is a potential source of promising biologically active compounds, and thus further investigation should be attempted on this natural resource to ascertain the active ingredient(s) of their potentials until ensuring their use in the respective fields.

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