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Zaidur Rahman Sabuj
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Sadequl Islam
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Mohammad Abdullah
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Dewan Mohammad Badruddoza
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Nurul Islam
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Correspondence
Nurul Islam
Department of Zoology,
University of Rajshahi,
Rajshahi, Bangladesh

Repellent potentials of *Morus alba* L. extractives against three stored product pests *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.)

Zaidur Rahman Sabuj, Sadequl Islam, Mohammad Abdullah, Dewan Mohammad Badruddoza and Nurul Islam

Abstract

Crude extracts of white mulberry by Petroleum Ether (Pet. ether), Chloroform ($CHCl_3$) and Methanol (CH_3OH) were subjected to repellent activity tests against the adults of three stored product pests *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.). The CH_3OH extract of leaves against *C. chinensis* was found highly repellent at 0.1% level of significance ($P<0.001$); the Pet. ether extract of leaves against *C. chinensis* and stem bark extract against *T. castaneum*; the $CHCl_3$ extract of stem bark against *S. oryzae* and *T. castaneum* showed moderate repellency at 1% level of significance ($P<0.01$). The Pet. ether extracts of root and stem bark, the $CHCl_3$ extracts of leaves and stem bark, and the CH_3OH extracts of stem bark against *C. chinensis*; the CH_3OH extract of leaves and stem bark, the Pet. ether and $CHCl_3$ extracts of roots against *T. castaneum*; the $CHCl_3$ extracts of stem bark against *S. oryzae* showed mild repellent action at 5% level of significance ($P<0.05$). However, the CH_3OH extracts of leaves and root didn't show repellent activity at all.

Keywords: Repellency, *Morus alba*, *Callosobruchus chinensis*, *Sitophilus oryzae*, *Tribolium castaneum*

1. Introduction

White mulberry is a fast-growing monoecious shrub or a medium sized tree with a cylindrical stem and rough, brown, vertically fissured bark, up to 8 to 15m high and 1.8m in girth. The scientific name of white mulberry is *Morus alba* (L.). Leaves are variable in size and shape, usually 5 to 7.5cm long, often deeply lobed, margins serrate or crenate-serrate, apex acute or shortly acuminate, base chordate or truncate; 3 basal nerves forked near the margins. Flowers are inconspicuous and greenish; male spikes (catkins) are broad, cylindrical or ovoid, female spikes are ovoid and stalked. Fruit (syncarp) consists of many drupes enclosed in a fleshy perianth, ovoid or subglobose, up to 5cm long, white to pinkish white, purple or black when ripe [1]. The genus *Morus* contains approximately 16 members of the Family Moraceae, occurring primarily in northern temperate regions with some extension into tropical areas of Africa and the South American Andes. There are 11 species distributed widely in China [2]. White mulberry is commonly called as Chinese white mulberry, Common mulberry, Russian mulberry, Silkworm mulberry and Moral blanco [3]. The plant has good therapeutic activity and low toxicity and has been extensively used in conventional Chinese medicine [4]. It is reported to have neuroprotective, skin tonic, antioxidant, antihyperglycemic, antibacterial, antihypertensive and antihyperlipidemic activities [5-7]. The strategy for the present investigation was designed to carry on screening of the crude extracts of the test plant species on three test organisms (*Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.)) for the detection of biological activity and keeping an option to show the extent of activity by analyzing the data statistically that read on various parameters during the course of the investigation.

Test insect *C. chinensis* (Family: Bruchidae) is a common species of beetle found in stored legumes [8]. The eggs are cemented to the surface of pulses and are smooth, domed structures with oval, flat bases. The larvae and pupae are normally only found in cells bored within the seeds of pulses [9]. The developmental period from egg to adult takes 20-25 days [10, 11].

S. oryzae (rice weevil) (Family: Curculionidae) is a serious stored product pest which attacks several crops and worldwide in distribution. The adult rice weevil is a dull reddish-brown to black in colour. The larval rice weevil must complete its development inside the seed kernel. The larva develops within the seed, hollowing it out while feeding. Total life cycle from egg to

adult took 34 to 49 days with an average of 42 days at 15 to 34 °C temperature and 58 to 89 per cent relative humidity [12].
T. castaneum (Family: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [13]. These beetles have chewing mouthparts, but do not bite or sting. The red flour beetle may elicit an allergic response [14]. The eggs are microscopic and the slender larvae are creamy yellow to light brown in colour. The adult is a small reddish-brown beetle. Total life cycle contain subsequently for egg incubation 8.8 days, larval development 22-100 days depending on temperature, pupal development 4.5 days, and for reproductive maturation 4-5 days [15].

2. Materials and Methods

2.1 Collection and preparation of test materials

White mulberry plant leaves, root and stem bark were collected from a mulberry cultivation garden just behind the Third Science Building, University of Rajshahi, Bangladesh in the month of April, 2016. Firstly, the plant was identified by the Department of Botany, University of Rajshahi where voucher specimens are kept in the Herbarium and then accordingly leaves, root and stem bark were separated while excess soil from the roots were removed without washing. Leaves, root and stem bark of the plant were then sliced and chopped into small pieces, dried under shade and powdered with the help of a hand grinder, weighed and placed in separate conical flasks to add solvents. Petroleum ether, $CHCl_3$ and CH_3OH (Merck, Germany) were used (200g × 600ml × 2 times) successively each of which took for 48h on a shaker. For each of the extract filtration was done by Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract was left as a scum. The extracts was then removed to glass vials and preserved in a refrigerator at 4 °C with proper labeling.

2.2 Collection and culture of test insects

Test insects *C. chinensis, S. oryzae* and *T. castaneum* were used in repellency activity tests in the crude extracts from the different parts of white mulberry. These test insects were selected because they are easy cultivable and noble laboratory animals. Moreover, they are important stored grain pests in a wide variety of cereal products. All the test insects of same age were used in this investigation and were collected from the stock cultures of the Crop Protection Laboratory, Department of Zoology, University of Rajshahi, Bangladesh.

2.3 Repellent activity

The repellency test was adopted from the method of McDonald *et al*., (1970) with some modifications. [16]. A general concentration for each of the extracts (of Pet. ether, $CHCl_3$ and CH_3OH) was selected as stock dose for repellency applied against the adults of *C. chinensis* and *S. oryzae* to make other successive doses by serial dilution to give 1.415, 0.707, 0.354, 0.177 and 0.088mg/cm^2 and for *T. castaneum* the doses were established as 0.629, 0.314, 0.157, 0.080 and 0.039mg/cm^2.
For the application on *C. chinensis* and *S. oryzae* Petri dish (9cm in diam.) was divided into three parts and marked with two narrow stick through adhesive tape. Then the both side filled with food where in one side treated food and other side with non-treated food followed by the concentration except the middle one. Then ten adult insects were released into the middle of the petri-dish.
Whereas, in case of *T. castaneum* half filter paper discs (Whatman No. 40, 9cm diam.) were prepared and selected doses of all the extracts separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 20 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (9cm diam.). For each of the test samples three replicates were maintained. Being volatile the solvent was evaporated out within a few minutes. Then ten insects were released in the middle of each filter paper circles.
Repellency was observed for one-hour interval and up to five successive hours of exposure for the all three insect species population. In case of *C. chinensis* and *S. oryzae* just by counting the number of insects from the non-treated part and the middle part of the 90mm Petri dish floor. While, for *T. castaneum* just by counting the number of insects from the non-treated part of the filter paper spread on the floor of the 90mm Petri dish. The values in the recorded data were then calculated for percent repellency, which was again developed by arcsine transformation for the calculation of analysis of variance (ANOVA). The average of the counts was converted to percentage repellency (PR) using the formula of Talukder and Howse (1993, 1995) [17, 18]: $PR = (Nc-5) \times 20$; where, Nc is the average hourly observation of insects on the untreated half of the disc.

3. Results

The Pet. ether, $CHCl_3$ and CH_3OH extracts of white mulberry leaves, stem bark and only the Pet. ether extracts of root showed repellency to *C. chinensis*. The $CHCl_3$ extracts of stem bark showed repellency to *S. oryzae*. On the other hand, the Pet. ether and $CHCl_3$ extracts of leaves, root and stem bark and only the CH_3OH extracts of stem bark showed repellent activity to *T. castaneum*. Against *C. chinensis* the CH_3OH extracts of leaves offered highly repellent activity at 0.1% level of significance ($P<0.001$); the Pet. ether extracts of leaves showed moderate repellency at 1% level of significance ($P<0.01$); while the Pet. ether extracts of root and stem bark, the $CHCl_3$ extracts of leaves and stem bark and the CH_3OH extracts of stem bark gave mild repellency at 5% level of significance ($P<0.05$). However, the $CHCl_3$ and CH_3OH extracts of root did not show repellent activity against this test insect. In case of *S. oryzae* only the $CHCl_3$ extracts of stem bark offered moderate repellency at 1% level of significance ($P<0.01$); however, no other extracts showed any kinds of repellent activity against this particular test organism. Another repellency tests against *T. castaneum* the Pet. ether and $CHCl_3$ extracts of stem bark gave moderate repellency at 1% level of significance ($P<0.01$); while the Pet. ether extracts of leaves and root, the $CHCl_3$ extracts of leaves and root and the CH_3OH extracts of stem bark showed mild repellent activity at 5% level of significance ($P<0.05$). However, the CH_3OH extracts of leaves and root did not offer repellent activity against *T. castaneum*.
According to intensity of repellency white mulberry extracts against *C. chinensis* the result could be arranged in a descending order: leaves (CH_3OH) > leaves (Pet. ether) > stem bark (CH_3OH) > root (Pet. ether) > stem bark ($CHCl_3$) > stem bark (Pet. ether) > leaves ($CHCl_3$) extracts. In case of *S. oryzae* the intensity of repellency could not be arranged because only one extract ($CHCl_3$ of stem bark) offered repellency. However, the intensity of the result of repellency by the plant extracts against *T. castaneum* could be arranged in a descending order: stem bark (Pet. ether) > Stem bark ($CHCl_3$) > leaves (Pet. ether) > leaves ($CHCl_3$) > root (Pet. ether) > stem bark (CH_3OH) > root ($CHCl_3$) extracts.

Table 1: ANOVA results of the repellency against *C. chinensis* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of white mulberry (leaves, root and stem bark).

Name of the test organism	Name of the parts of White mulberry	Name of the Solvents used for extraction	Sources 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
C. chinensis	Leaves	Pet. ether	4	4	16	26.261**	1.258	7.4E-07	0.327
		$CHCl_3$	4	4	16	8.854*	0.288	0.001	0.882
		CH_3OH	4	4	16	77.925***	1.183	2.78E-10	0.356
	Root	Pet. ether	4	4	16	16.973*	3.492	1.31E-05	0.031
		$CHCl_3$	4	4	16	6.198	0.821	0.003	0.531
		CH_3OH	4	4	16	2.862	1.092	0.058	0.394
	Stem bark	Pet. ether	4	4	16	13.122*	1.242	6.33E-05	0.333
		$CHCl_3$	4	4	16	15.965*	0.689	1.92E-05	0.610
		CH_3OH	4	4	16	18.704*	3.014	7.05E-06	0.050

*** = Significant at 0.1% level (*P<0.001*); ** = Significant at 1% level (*P<0.01*); * = Significant at 5% level (*P<0.05*)

Table 2: ANOVA results of the repellency against *S. oryzae* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of white mulberry (leaves, root and stem bark).

Name of the test organism	Name of the parts of White mulberry	Name of the Solvents used for extraction	Sources 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
S. Oryzae	Leaves	Pet. ether	4	4	16	0.486	0.459	0.746	0.765
		$CHCl_3$	4	4	16	0.995	1.024	0.439	0.425
		CH_3OH	4	4	16	0.613	0.619	0.660	0.656
	Root	Pet. ether	4	4	16	7.416	0.876	0.001	0.500
		$CHCl_3$	4	4	16	6.677	0.538	0.002	0.710
		CH_3OH	4	4	16	6.262	0.567	0.003	0.691
	Stem bark	Pet. ether	4	4	16	5.259	1.454	0.007	0.262
		$CHCl_3$	4	4	16	22.690**	1.425	1.9E-06	0.271
		CH_3OH	4	4	16	1.381	0.393	0.285	0.810

** = Significant at 1% level (*P<0.01*)

Table 3: ANOVA results of the repellency against *T. castaneum* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of white mulberry (leaves, root and stem bark).

Name of the test organism	Name of the parts of White mulberry	Name of the Solvents used for extraction	Sources 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
T. castaneum	Leaves	Pet. ether	4	4	16	18.812*	3.320	6.8E-06	0.037
		$CHCl_3$	4	4	16	17.372*	0.340	1.1E-05	0.847
		CH_3OH	4	4	16	3.364	1.348	0.035	0.296
	Root	Pet. ether	4	4	16	16.796*	0.056	1.4E-05	0.994
		$CHCl_3$	4	4	16	14.214*	2.056	3.9E-05	0.135
		CH_3OH	4	4	16	1.201	1.853	0.348	0.168
	Stem bark	Pet. ether	4	4	16	22.962**	1.750	1.8E-06	0.189
		$CHCl_3$	4	4	16	22.824**	1.456	1.9E-06	0.262
		CH_3OH	4	4	16	15.462*	1.881	2.3E-05	0.163

** = Significant at 1% level (*P<0.01*); * = Significant at 5% level (*P<0.05*)

4. Discussion

Though works on repellency by white mulberry is scanty it was found tested for controlling various bacterial agents. The antibacterial and antifungal phytoconstituents were established by Ayoola *et al.* [19] from the aqueous and ethanolic extract of white mulberry. Mulberry extracts are rich in phytochemicals and have antimicrobial potential against harmful pathogens [20]. The compounds isolated from *M. alba* leaves were active against oral pathogens commonly *Streptococcus mutans* [21]. Hypoglycemic activity by the dose at 600mg/kg/day of 70% alcohol extract of *M. alba* root bark to diabetic rats for 10 consecutive day reduced the amount of the glucose by 59% as compared to control and increased insulin production by 44% [22]. The present investigation was carried out against *C. chinensis*, *S. oryzae* and *T. castaneum* to yield promising repellency activity. All the three insects are stored product pests and they cause a huge damage in stored products and ultimately causing economic damage, the extracts of white mulberry leaves, root and stem bark can be used in control of these stored product pests as these extracts showed repellency against those insect pests. Moreover, repellency is the system tends to dissuade pests away from a susceptible crop (repellent) [23] what can be called as a push approach. Thus, plants are natural source of these repellent agents, reported in numerous ethnobotanical information. Plant-derived repellents do not pose hazards of toxicity to humans and domestic animals, and are easily biodegraded compared to synthetic compounds, natural products are presumed to be safer for humans [24]. This study was attempted to highlight white mulberry claimed to be used or associated with insect repellent activity, and it was found considerable. However, test result on other attributes also support the present finding, such as repellency for the extracts of white mulberry against stored product pests were also established.

5. Conclusion

Components of plants have the potentials to play a major role in pest management in sustainable agriculture production since these products are traditionally used by the farmers in developing countries appear to be most promising and quite safe. The findings of the present study indicate the repellent effects of white mulberry extractives on the adults of *C. chinensis, S. oryzae* and *T. castaneum.* Being natural in origin these plant materials might be biodegradable and thus safe and sustainable to the environment.

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