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Lethal efficacy of indoor ornamental plant *Aglaonema marantifolium* (Schott.) against three economically important stored product pests *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (HBST.)

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Abstract
Petroleum ether (Pet. ether), chloroform ($CHCl_3$) and methanol (CH_3OH) extracts of the test plant *Aglaonema marantifolium* (Schott.) have been subjected to dose-mortality and repellent activity tests against adult beetles of *Callosobruchus chinensis* (L.), *Sitophillus oryzae* (L.) and *Tribolium castaneum* (HBST.). The Pet. ether extract of *A. marantifolium* gave the LD_{50} values 1.04, 0.91, 0.87, 0.83, 0.80, 0.78 and 0.77mg/cm^2 against *S. oryzae*; 1.34, 1.19, 1.14, 1.12, 1.07, 1.03 and 1.02mg/cm^2 against *T. castaneum*; and the CH_3OH extract gave the LD_{50} values 2.34, 2.15, 1.93, 1.86, 1.83, 1.82 and 1.81mg/cm^2 against *C. chinensis* after 6, 12, 18, 24, 30, 36 and 42h of exposures respectively. The $CHCl_3$ extract did not show any mortality against all the three test insects. In repellency tests, the $CHCl_3$ extract was found to show significant repellent activity against the adult beetles of *C. chinensis* at 5% level of significance ($P<0.05$). However, Pet. ether and CH_3OH extracts of *A. marantifolium* did not show repellent activity against any of the three test beetles.

Keywords: Dose-mortality, Repellency, *Aglaonema marantifolium*, *Tribolium castaneum*, *Sitophilus oryzae*, *Callosobruchus chinensis*

1. Introduction
The genus *Aglaonema*, belongs to the family Araceae Juss. and comprises 21 species [1]. The common name for *Aglaonema* is Chinese evergreen because Chinese were the first to cultivate the dark green *Aglaonema* for centuries [2]. The Genus *Aglaonema* has been appeared as an ornamental foliage plant due to its attractive foliage, easiness to grow, tolerance to low relative humidity and low light conditions [3, 4]. Besides, *Aglaonema* species have been cultivated in China and other Asian countries for centuries as indoor ornamental foliage plants or houseplants [5]. All species are herbaceous evergreens native to South-east Asia, Northeastern India, across Southern China, and into Indonesia and New Guinea [1, 6]. *Aglaonema* plants have been widely used in recent years because of its anti-aging and longevity properties, natural anti-allergic and anti-inflammatory properties [7]. Moreover, decoction of the roots is drunk to treat dropsy and fever [8]. In addition, the genus *Aglaonema* has capability to remove pollutants from indoor air such as benzene, toluene, TCE, m-xylene, hexane etc. [9]. The present investigation was designed to find out the effect of the crude extracts of the test plant *A. marantifolium* to dose-mortality and repellent activity against the stored product pests *Callosobruchus chinensis* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Hbst.). The *T. castaneum*, commonly known as 'red flour beetle' (Coleoptera: Tenebrionidae) are not adapted to feed on hard whole grains but almost any kind of flour, cracked grains, etc. including whole-wheat flour, bran, rice flour, cornmeal, barley flour and oatmeal. They also feed upon dried fruits, dried plant roots, nuts, chocolates, drugs, snuff, cayenne pepper, pulses and prepared cereal foods such as corn flakes [10]. In addition, cereals are also being damaged by this beetle [11]. Rice weevil, *S. oryzae* known as the most destructive pest of stored grain throughout the world because they feed directly on grain kernels [12, 13]. They are the primary insect pest of stored rice in warm climate areas [14]. Another test beetle, *C. chinensis* belonging to the family, Chrysomelidae and are commonly known as the pulse beetle, Chinese bruchid and cowpea bruchid [15]. This species is one of the most damaging pests to the stored legumes for their general diets and wide distribution [16].

2. Materials and Methods

2.1. Collection and preparation of test materials

A. marantifolium has been collected as whole plant during the month of January from three different places of the Rajshahi University campus; and identified by the herbarium management by the voucher specimens which were kept in the herbarium in the Department of Botany, University of Rajshahi, Bangladesh. Both leaves and root of the plant were collected together and chopped into small pieces, dried under shade and powdered together with the help of electric grinder, weighed and placed into a conical flask to add solvents. The solvents Pet. ether, $CHCl_3$ and CH_3OH were used (100g × 300ml × 2 times) successively each of which took for 48h on a shaker. For each of the extract filtration was done by filter paper at 24h of interval in the same flask followed by the evaporation until the extract was left as a scum. The extracts were then removed to glass vials and preserved with proper labeling.

2.2. Collection and culture of test insects

The test insects *C. chinensis*, *S. oryzae* and *T. castaneum* adults were collected from the mass cultures of the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. By the maintenance of insects' culture the supplies of the same age individuals of three different test insects were available and were used for experiments in this investigation.

2.3. Dose-mortality tests on *C. chinensis* and *S. oryzae*

The experiment for insecticidal test on *C. chinensis* or *S. oryzae* are the same for their feeding habit. Here the *Ad Hoc* experiments were set to find out the final concentrations for dose selection. The concentrations of CH_3OH extract used against *C. chinensis* in this experiment were 2.14, 2.04 and 1.94mg/cm^2; for *S. oryzae* the concentrations of Pet. ether extract were 1.02, 0.92 and 0.81mg/cm^2. For each of the experiments 1ml of the prepared dose was mixed with the prepared food and being volatile the solvent was evaporated out shortly. The actual extract present in 1ml mixture was calculated just dividing the value by the amount of used calculated food. After drying 10 insects of the same age were released on the food in 3 replicates. A control batch was also maintained with the same number of insects after preparing the food by applying and evaporating the solvent only. The treated insects were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the insects were counted after 6h, 12h and more 5 time's up to 42h of exposures with 6h intervals. However, the Pet. ether and $CHCl_3$ extracts for *C. chinensis*; $CHCl_3$ and CH_3OH extract*s* for *S. oryzae* did not show any mortality at all.

2.4. Dose-mortality tests on *T. castaneum*

The experiment for insecticidal test on *T. castaneum* are not the same as *C. chinensis* or *S. oryzae* since the feeding are different. Here also the *Ad Hoc* experiments were set to find out the final concentrations for dose selection. For insecticidal activity tests, the Pet. ether extract w*as* dissolved in its solvent of extraction at different concentrations to go through *Ad Hoc* experiments to set considerable mortality and that were considered as doses. The final concentrations used in this experiment were 1.27, 1.17 and 1.07mg/cm^2 for Pet. ether. For each of the experiments 1ml of solvent was dropped on a Petri dish (50mm) in such a way that it made a uniform film over the Petri dish. Then the Petri dishes were air-dried leaving the extract on it. The actual extract present in 1ml mixture was calculated just dividing the value by the area of the Petri dish and thus the dose per square centimeter was calculated. After drying 10 beetles (5**-**7 days old) were released in each of the Petri dishes in 3 replicates. After preparing the Petri dish by applying and evaporating the solvent a control batch was also maintained with the same number of insects. The treated beetles were then placed in the incubator at the same temperature as reared in stock cultures and the mortality was recorded.

2.5. Statistical analysis

The mortality (%) was corrected using Abbott's formula [17]. The formula is $P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Mortality in the control (%). The data were then subjected to probit analysis [18, 19].

2.6. Repellent activity

The repellency test was adopted from the method of McDonald *et al*. with some modifications [20]. A general concentration for each of the extracts (Pet. ether, $CHCl_3$ and CH_3OH) was selected as stock dose for repellency applied against the adults of *S. oryzae* and *C. chinensis* to make other successive doses by serial dilution to give 0.628, 0.314, 0.157, 0.078 and 0.039mg/cm^2 and for *T. castaneum* the doses were established as same as the previous one. For the application of the extracts on *S. oryzae* and *C. chinensis* Petri dish (of 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 exposures for the all three insect species population. In case of *S. oryzae* and *C. chinensis* 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 [21, 22]: PR = (Nc-5) × 20; where, Nc is the average hourly observation of insects on the untreated half of the disc.

3. Results

3.1. Dose mortality effects on *C. chinensis*, *S. oryzae* and *T. castaneum*

The results of the dose mortality assays, the CH_3OH extract of *A. marantifolium* against beetles of *C. chinensis* are represented in Table 1. The LD_{50} values were 2.34, 2.15, 1.93, 1.86, 1.83, 1.82 and 1.81mg/cm^2 after 6, 12, 18, 24, 30, 36 and 42h of

exposures respectively. The lethal activity of Pet. ether extract of *A. marantifolium* against the weevils of *S. oryzae* are represented in Table 2. The LD_{50} values were 1.04, 0.91, 0.87, 0.83, 0.80, 0.78 and 0.77mg/cm^2 after 6, 12, 18, 24, 30, 36 and 42h of exposures respectively. The insecticidal activity against *T. castaneum* for the Pet. ether extract of *A. marantifolium* is also represented in Table 2. The LD_{50} values were 1.34, 1.19, 1.14, 1.12, 1.07, 1.03 and 1.02mg/cm^2 after 6, 12, 18, 24, 30, 36 and 42h of exposures respectively. According to intensity of sensitivity of the beetles could be arranged in the following descending order: *S. oryzae* > *T. castaneum* > *C. chinensis*.

Table 1: LD_{50} values of the CH_3OH extract of *A. marantifolium* against *C. chinensis* adults.

Solvent of extraction	Test agent	LD_{50} (mg/cm^2) after hours of exposure						
		6h	12h	18h	24h	30h	36h	42h
CH_3OH	***C. chinensis***	2.34	2.15	1.93	1.86	1.83	1.82	1.81

Table 2: LD_{50} values of the Pet. ether extract of *A. marantifolium* against adult beetles of *S. oryzae* and *T. castaneum*.

Solvent of extraction	Test agents	LD_{50} (mg/cm^2) after hours of exposure						
		6h	12h	18h	24h	30h	36h	42h
Pet. ether	*S. oryzae*	1.04	0.91	0.87	0.83	0.80	0.78	0.77
	T. castaneum	1.34	1.19	1.14	1.12	1.07	1.03	1.02

3.2. Repellent effects on *C. chinensis*, *S. oryzae* and *T. castaneum*

Among Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. marantifolium*, only the $CHCl_3$ extract was found repellent at 5% level of significance ($P<0.05$) against the test beetle *C. chinensis*. However, the Pet. ether and CH_3OH extract of *A. marantifolium* did not show the repellent activity against *C. chinensis*, *S. oryzae* and *T. castaneum*. In addition, the $CHCl_3$ extract also did not show repellency to *S. oryzae* and *T. castaneum* at all.

Table 3: ANOVA results of repellency of the adult beetles of *C. chinensis*, *S. oryzae* and *T. castaneum* by *A. marantifolium* extracts.

Plant	Name of test agents	Solvent of extraction	Source of variation	SS	df	MS	F	P-value
A. marantifolium	*C. chinensis*	Pet. ether	Between doses	390.36	4	97.59	2.65^{ns}	0.07
			Between time interval	85.13	4	21.28	0.58^{ns}	0.68
		$CHCl_3$	Between doses	1134.20	4	283.55	9.62*	0.0004
			Between time interval	163.64	4	40.91	1.39^{ns}	0.28
		CH_3OH	Between doses	205.25	4	51.31	3.72^{ns}	0.02
			Between time interval	599.37	4	149.84	10.85*	0.0002
	S. oryzae	Pet. ether	Between doses	984.87	4	246.22	5.75^{ns}	0.0046
			Between time interval	482.70	4	120.68	2.82^{ns}	0.06
		$CHCl_3$	Between doses	342.59	4	85.65	2.97^{ns}	0.05
			Between time interval	217.20	4	54.30	1.88^{ns}	0.16
		CH_3OH	Between doses	717.96	4	179.49	3.61^{ns}	0.03
			Between time interval	123.52	4	30.88	0.62^{ns}	0.65
	T. castaneum	Pet. ether	Between doses	3687.09	4	921.77	2.35^{ns}	0.10
			Between time interval	4377.12	4	1094.28	2.79^{ns}	0.06
		$CHCl_3$	Between doses	985.39	4	246.35	2.16^{ns}	0.12
			Between time interval	949.87	4	237.47	2.08^{ns}	0.13
		CH_3OH	Between doses	1782.47	4	445.62	1.54^{ns}	0.24
			Between time interval	4951.92	4	1237.98	4.28^{ns}	0.02

** = Significant at 1% level ($P<0.01$), * = Significant at 5% level ($P<0.05$), ns= Non significant

4. Discussion

In the present investigation, the results achieved for the test plant *A. marantifolium* got supports from the previous works. There are many evidences of antioxidant, antimicrobial, phytotoxic, etc. properties of *Aglaonema* spp. The species *A. simplex* have anti-aging and longevity properties that has been widely used in recent years [7]. They also described the cellular anti-oxidant activity of this plant, 95% ethanol macerated extract and 95% ethanol fresh fruit extract effectively attenuated *t* BuOOH-induced intracellular oxidative stress. The plant has the capability to remove pollutants from indoor air [9]. The indoor plant *A. modestum* species has been screened for their ability to remove VOCs such as benzene, toluene, TCE, m-xylene, hexane etc. From the Malaysian biodiversity, investigators found five pheophorbide-related compounds from the leaves and stems of *A. simplex* [23]. Their detailed spectroscopic analyses showed that there were pheophorbide and hydroxypheophorbide derivatives of chlorophyll 'a' and 'b'. Another compound identified as 151-hydroxypurpurin-7-lactone ethyl methyl diester, was isolated for the first time from the Araceae family. The isolated compounds exhibited moderate-to-strong photo-cytotoxic activities towards human leukemia (HL60) and two oral squamous carcinoma cell lines (HSC-2 and HSC-3). The Genus *Aglaonema* contains polyhydroxy alkaloids that exhibit the glycosidase inhibitor activity [24]. The extracts contained secondary metabolites belonging to terpenoids, steroids, phenolics, alkaloids and glycosides. They also described *A. simplex* is one of the potential source of the phytochemicals for the treatment of atherosclerosis. The ethanol extract of leaves of the medicinal plant *A. hookeri anum* possessed cytotoxic activity against the brine shrimp nauplii. They also described, the extracts of the plant were having antibacterial properties compared with the commercially available standard antibiotic amoxicillin and might be a useful source for the development of new potent antibacterial agents [25]. However, there are still no studies of ethno-pharmacological properties or toxicity of the genus *Aglaonema* [7]. According to the previous reports findings of

this investigation got supports and the extracts of *A. marantifolium* could be used in the control of these stored product pests while its extracts showed both mortality and repellency against the test insects.

5. Conclusion

By analyzing the results of dose-mortality and repellency against *C. chinensis*, *S. oryzae* and *T. castaneum* it can be finally concluded that, *A. marantifolium* contains potent bioactive compounds, which may be effective in controlling the stored product pests.

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