Pesticidal action of *Abutilon hirtum* (Lam.) sweet against *Tribolium castaneum* (Hbst.) and *Lymnaea acuminata* Lamarck through dose-mortality assay

Sahadat Hossain, Sadia Afrin Rimi, Hasan Ali, Rifat Ara Shawon, Mohammad Abdullah and Nurul Islam

Abstract

Petroleum ether, CHCl$_3$ and CH$_3$OH extracts of the aerial parts of *Abutilon hirtum* (Lam.) Sweet were subjected to dose-mortality tests against adult beetles of *Tribolium castaneum* (Hbst.) and *Lymnaea acuminata* Lamarck under laboratory conditions. The Pet. ether extract offered mortality to *T. castaneum* and gave LD$_{50}$ values 99.956, 89.910, 80.447 and 74.839 mg cm$^{-2}$ after 12, 18, 24 and 30h of exposure respectively, and the CH$_3$OH extract gave LD$_{50}$ values 218.427, 215.647, 209.361, 205.431, 201.792 and 199.855 mg cm$^{-2}$ after 6, 12, 18, 24, 30 and 36h of exposure respectively. While the CHCl$_3$ extract didn’t offer mortality against *T. castaneum*. In case of *L. acuminata*, the CHCl$_3$ and CH$_3$OH extracts offered LC$_{50}$ values 121.130, 112.784 and 110.369 ppm; and 66.323, 58.892 and 51.983 ppm both after 12, 18 and 24h of exposure respectively. The Pet. ether extract did not show mortality to *L. acuminata* up to 120 ppm.

Keywords: dose-mortality, *Abutilon hirtum*, *Tribolium castaneum*, *Lymnaea acuminata*

Introduction

*Abutilon hirtum* (Lam.) Sweet is a perennial herb or shrub, grows up to 0.5-2.5m in height and distributed throughout the tropical and subtropical regions of the Indian subcontinent. It is commonly known as “Indian mallow” or “Florida Keys Indian Mallow” and in Bengali it is known as “Vadathuthi” or “Belabenda” [1-2]. The stem, flower-stalks and leaf-stalks are velvety, hairy and sticky [3]. Flowers are comparatively small, and yellow in color. The leaves are heart shaped, serrate or dentate type and many rough ended grooves are present at the marginal side of the total leaves [4]. *A. hirtum* has been used traditionally for a long time in herbal medical treatment system. The leaves are used as demulcent and diuretic [5]. Leaf decoction of *A. hirtum* is used as mouth wash, against wounds, bladder inflammations and to treat ulcers [6-8]. Moreover, the fruits of this plant are eaten raw and the water extract of the bark is given to ease childbirth in Kenya [9-10]. It is also used as a poultice to ease the ache of kidney gravel and often mixed with glutinous rice and applied to ulcers in Malaysia. The leaves or flowers are applied in case of abscesses [11]. The present investigation was designed to find out the effect of the crude extracts of the test plant *A. hirtum* through dose-mortality assay against the stored grain pest, *T. castaneum* (Hbst.), and molluscicidal activity was done against the garden snail, *L. acuminata*. *T. castaneum* (Coleoptera: Tenebrionidae) is a worldwide pest of stored products and of Indo-Australian in origin [12]. The red flour beetle may produce an allergic response [13]. Eggs are microscopic and slender larvae are creamy yellow to light brown in color, while the adult is reddish-brown. 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 [14]. One of the test agent *L. acuminata* is a freshwater snail species of the family Lymnaeidae. This snail is native to South Asia and widespread in Bangladesh, Burma, India, Nepal, and Pakistan [15]. It is a host for many trematode species. It is the first intermediate host for the liver flukes, *Fasciola gigantica* and *F. hepatica*, which cause the infectious disease Fascioliasis in humans and other mammals [16-17].

Materials and Methods

Collection and preparation of test materials

The fresh materials of *A. hirtum* were collected from the bushy area of Station Bazaar (Both side of rail line), which is located near the University Railway Station of the University of Rajshahi, Bangladesh and identified by the Department of Botany, University of Rajshahi...
where a voucher specimen is kept in the herbarium. The areal parts of the plants (leaves, stem, fruit and flower) were collected together and chopped into small pieces, dried under shade and powered with the help of a hand grinder, weighed and placed in a conical flask to add solvents. The solvents Pet. ether, CHCl$_3$ and CH$_3$OH (Merck, Germany) were used (200g × 600ml × 3 times) successively each of which took for 48h on a shaker. For each of the extract filtration was done by using Whatman filter paper 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.

Collection and culture of test insects
The test insect T. castaneum of same age used in this investigation were received from the stock cultures of the Crop Protection Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. The adult snail Lymnaea acuminata (2.60±0.30 cm in length) were collected locally from ponds and low-lying submerged areas located at Rajshahi University campus. These snails were attached on the ventral surface of the leaves of aquatic plants. The collected snails were reared in pond water in an aquarium under laboratory conditions. By the maintenance of snails’ culture we got the supplies of snails of same size and age which were used in the experiments.

Dose-mortality tests on T. castaneum
For insecticidal activity tests Pet. ether, CHCl$_3$ and CH$_3$OH extracts were dissolved in its solvent of extraction at different concentrations to go through Ad Hoc experiments to set considerable mortality and that were considered as applicable doses. The final concentrations used in this experiment were 66.02, 69.16, 72.13, 75.45 and 78.60 mg cm$^{-2}$ for Pet. ether and CHCl$_3$ extract; 193.532, 198.625, 203.718, 208.811 and 213.904 mg cm$^{-2}$ for the CH$_3$OH extract. For each of the doses 1 ml of solvent was dropped on a Petri dish (50 mm) in such a way that it made a uniformed film over the floor of the Petri dish. The volatile solvent on the surface of the Petri dishes were evaporated out leaving a film made up of the extract on it. The actual extract present in 1 ml mixture was calculated just dividing the amount of extract by the surface area of the Petri dish and thus the dose per square centimeter was calculated. After drying 10 beetles (3-5 day 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 Petri dishes were placed on a plate with the same conditions of temperature and humidity as the insects were reared in stock cultures. For the Pet. ether and CHCl$_3$ extracts the mortality of the beetles was recorded after 6, 12, 18, 24 and 30h of exposure and for the CH$_3$OH extract it was recorded after 6, 12, 18, 24, 30 and 36h of exposure.

Table 1: LD$_{50}$ values of the Pet. ether extract of A. hirtum against T. castaneum.

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>LD$_{50}$ (mg cm$^{-2}$)</th>
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<tr>
<td></td>
<td>12h</td>
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<tr>
<td>Pet. ether</td>
<td>99.936</td>
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Table 2: LD$_{50}$ values of CH$_3$OH extracts of A. hirtum against T. castaneum.

<table>
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<tr>
<th>Solvent used</th>
<th>LD$_{50}$ (mg cm$^{-2}$)</th>
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<tbody>
<tr>
<td></td>
<td>6h</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>218.427</td>
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Molluscidal activity test
Adult L. acuminata were collected from the aquarium where they were cultured. Ten experimental snails were kept in each of the test tubes. Test extracts at different concentrations considered as doses were made hydrophilic by addition of calculated amount of DMSO (Dimethylsulfoxide) before adding half of the required amount of water in each. Then a certain amount of pond water was added to fill the pre-marked test-tubes with the help of a pipette. The snails were counted by visual inspection and were released in test-tubes containing 10 ml of water. Observation of mortality was made after 12, 18, and 24 h of exposure. Doses for the CHCl$_3$ extract were 100, 110 and 120ppm and for the CH$_3$OH extract were 50, 60 and 70ppm. But the Pet. ether extract did not exhibit any activity until 120ppm and was discarded from experimentation.

Table 3: LC$_{50}$ values of CHCl$_3$ and CH$_3$OH extracts of A. hirtum against L. acuminata.

<table>
<thead>
<tr>
<th>Solvent used</th>
<th>LC$_{50}$ (ppm)</th>
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<tr>
<td></td>
<td>12h</td>
</tr>
<tr>
<td>CHCl$_3$</td>
<td>121.130</td>
</tr>
<tr>
<td>CH$_3$OH</td>
<td>66.323</td>
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Statistical analysis
The mortality (%) was corrected using Abbott’s formula [18]. The formula is $P_t = \frac{P_o - P_c}{100 - P_o} x 100$; Where, $P_t$ = Corrected mortality (%), $P_o$ = Observed mortality (%), $P_c$ = Mortality in the control (%). The data were then subjected to Probit analysis [19-20].

Results and Discussion
Dose mortality effects on T. castaneum and molluscidal activity on L. acuminata
The results of the dose-mortality assays of Pet. ether and CH$_3$OH extracts of A. hirtum against adult beetles of T. castaneum are represented in Table 1 and Table 2. The Pet. ether extract offered promising mortality and the LD$_{50}$ values were 99.956, 89.910, 80.447 and 74.839 mg cm$^{-2}$ after 12h, 18h, 24h and 30h of exposure respectively. The CH$_3$OH extract also offered a promising result and the LD$_{50}$ values were 218.427, 215.647, 209.361, 205.431, 201.792 and 199.855 mg cm$^{-2}$ after 6h, 12h, 18h, 24h, 30h and 36h of exposure respectively. But the CHCl$_3$ extract did not show any activity against T. castaneum. The molluscidal activity against L. acuminata for the CHCl$_3$ and CH$_3$OH extracts of A. hirtum are represented in Table 3. The CHCl$_3$ extract offered LC$_{50}$ values 121.130, 112.783 and 110.369 ppm after 12, 18 and 24h of exposure respectively, and the CH$_3$OH extract gave LC$_{50}$ values 66.323, 58.892 and 51.983ppm. However, the Pet. ether extract did not show mortality to L. acuminata even up to 120ppm. According to the intensity of dose-mortality against T. castaneum the extracts could be arranged in a descending order as Pet. ether > CH$_3$OH extracts; and against L. acuminata, the extracts could be arranged in a descending order as CH$_3$OH > CHCl$_3$ extracts.

The findings of the present investigation receive supports from the experiment done on A. hirtum and its related species by previous researchers. Works on A. hirtum extracts for evaluating cytotoxicity, insect mortality and repellency is scanty, and however there are some evidences of research that have been done to find out the medicinal, phytochemical, pharmacognostical and structural properties. It has been already proved that A. hirtum is highly effective in medicinal
and traditional usage for the treatment of various human ailments [33]. The leaf extracts of A. hirtum (Lam.) Sweet can positively use in the treatment against many treatment for the Herbal Medicine Industry [34]. Also leaf extract of A. hirtum possess significant hepatoprotective activity [29]. Its related species A. indicum was recorded to be effective as the anti-ulcer and anti-snake venom agents [35]. And several essential phytoconstituents like tocopherol oil and β- Sitosterol were isolated from this plant [25-28]. Another study suggested that the hydroethanolic extract of A. hirtum provided antioxidant and antitumor activity which might be due to the presence of any active potentials, such as alkaloids, tannins, flavonoids, phenols [27]. Methanolic diethy ether, petroleum ether and ethyl acetate extract of A. hirtum yielded promising antiarthritic activity [28].

**Conclusion**

By analyzing the results of dose mortality against T. castaneum and molluscidal activity against L. acuminata it can be finally concluded that, A. hirtum has some bioactive potentials, which could be used as tools in the pest control strategy. If those specific chemical substances could be identified properly and commercial application could be made successful it might help the integrated pest management program.

**References**