Screening of Allamanda cathartica L. extracts against stored product pests, Tribolium castaneum (Herbst), Sitophilus oryzae (L.) and Callosobruchus chinensis (L.)

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Abstract

Petroleum ether (Pet. ether), CHCl₃ and CH₃OH extract of leaves and stem-bark of the medicinal plant Allamanda cathartica L. were tested for dose-mortality and repellent activity against three stored product pests, Tribolium castaneum (Herbst), Sitophilus oryzae (L.) and Callosobruchus chinensis (L.) adults. Against T. castaneum the leaf extracts of Pet. ether, CHCl₃ and CH₃OH gave LD₅₀ values 3.433, 2.347, 2.091, 1.879, 1.533 and 1.309mg/cm²; 2.179, 1.898, 1.532, 1.300, 1.136 and 1.055mg/cm²; and 2.598, 2.097, 1.892, 1.645, 1.476 and 1.357mg/cm² respectively. For S. oryzae, the same extracts gave LD₅₀ values 1.532, 1.341, 1.120, 1.010, 0.921 and 0.921mg/cm²; 1.860, 1.669, 1.444, 1.257, 1.240 and 1.114mg/cm²; and 2.758, 2.610, 2.289, 2.250, 2.040 and 1.947mg/cm²; and for C. chinensis the LD₅₀ values for the same were 2.439, 1.536, 1.105, 0.810, 0.713 and 0.685mg/cm²; 2.439, 1.536, 1.534, 1.314, 1.095 and 1.069mg/cm²; and 3.017, 2.742, 2.570, 2.380, 2.164 and 1.985mg/cm² respectively after 6, 12, 18, 24, 30 and 36h of exposure. The Pet. ether and CHCl₃ extracts of the stem-bark of the test plant gave LD₅₀ values against T. castaneum were 3.402, 3.095, 3.066, 2.077, 1.699 and 1.419mg/cm²; and 3.433, 3.047, 2.220, 2.056 and 1.726mg/cm² respectively; against S. oryzae, the LD₅₀ values were 3.140, 2.528, 2.147, 1.745, 1.398 and 1.182mg/cm²; and 3.408, 2.477, 2.284, 2.092, 1.860 and 1.636mg/cm²; and against C. chinensis, the LD₅₀ values were 3.433, 2.347, 2.091, 1.879, 1.533 and 1.309mg/cm²; and 3.711, 2.492, 2.327, 2.050, 1.866 and 1.674mg/cm² respectively after 6, 12, 18, 24, 30 and 36h of exposure. However, the CH₃OH extract of stem-bark didn’t offer mortality to the test beetles. All the extracts were subjected to repellent activity tests against the same three test agents, and no repellent activity was detected against any of the test agents.

Keywords: Allamanda cathartica L. Tribolium castaneum (Herbst), Sitophilus oryzae (L.) and Callosobruchus chinensis (L.)

Introduction

Allamanda cathartica L. is an evergreen shrub or vine belongs to the Family Apocynaceae and is one of the most studied species of the Allamanda genus. This species is popularly known as Allamanda big flower or thimble lady [1] and is found in tropical and subtropical regions as an ornamental shrub in gardens [2]. It is native to tropical South America (Brazil, French Guiana, Guyana and Surinam). It is also commonly known as Golden Trumpet, Yellow Allamanda, Golden Cup or Allamanda. In Bengali it is called Alkananda, or Aloklota [3]. It is a medium sized, fast growing plant that can reach a height of 20 feet (6m) or more tall. The simple leaves are arranged in groups of three or four along the branches. They are borne on very short stalks (petioles) only 2-5 mm long and have blades that are egg-shaped in outline (obovate) to somewhat elongated in shape (oblong-lanceolate). The leaves (5-17cm long and 2.5-6cm wide) have a narrowed (attenuated) base and a pointed tip (acuminate apex). They are somewhat shiny in appearance, relatively thick and leathery in nature, and hairless (glabrous). This evergreen, spreading and climbing vine is covered with vivid flowers in the warm months. Yellow trumpet shaped flowers explode into bloom during the warm months and cover the vine in vibrant color. The spiny, yellow green fruit follows and can be seen on the plant simultaneously with the spectacular blooms. The dark green, glossy leaves are produced on slender, green, twining stems which become woody with age. Allamanda should only be planted in frost-free locations, although it could be grown as an annual plant in colder climates.
due to its rapid growth rate. Requiring full sun locations for best flowering (some flowers are produced in locations receiving only 3h to 4h of sun), *Allamanda* is tolerant of various soil types and requires only moderate moisture. Regular, light fertilization during the growing season helps promote growth and flowering.

In traditional systems of medicine, different parts (leaves, stem, flower, root and even whole plant) of *A. cathartica* L. have been used to treat different diseases. Studies have indicated the potential anti-inflammatory and antioxidant properties of *Allamanda* flower extracts. In traditional medicine, an infusion of the stem bark and leaves is used as a purgative [4]. The leaf extract has displayed anti-inflammatory and healing activities [4, 5]. Phytochemical studies of flowers have reported the isolation of iridoid, plumieride and flavonoids such as rutin and sugars [6]. Iridoids are secondary metabolites with potential therapeutic applications [7, 8]. Plumieride is the major compound of the extracts from flowers of *A. cathartica* with potential anti-inflammatory and antihypernociceptive activities in models of neuropathic and inflammatory pain [9]. Leaves are also used as an antitode, and for relieving coughs and headaches. It has been used as a laxative, febrifuge, as well as for the treatment of jaundice and enlarged spleen resulting from malaria. Studies have indicated the potential antibacterial, antifungal and *in vitro* hepatoprotective properties of *Allamanda* flower extracts. It has a long history as a medicinal plant for the treatment of varied conditions, such as feverish infections like gonorrhoea, dysentery and hepatitis [10]. The leaf extract has displayed antifertility potency in male, antimicrobial activity against multiple drug resistant clinical pathogen and also exhibits membrane stabilizing property. The milky sap (latex) is also known to possess antibacterial and possibly anticancer properties [11].

The red flour beetle, *T. castaneum* (Herbst.) is the most destructive pest of stored products and is cosmopolitan in distribution (Coleoptera: Tenebrionidae) [12]. The larvae destroy 12.5-14.60 per cent of the individual seeds and during their development some 88 grains are attacked by per larva. It leads to considerable loss in quantity of grains and reduce its viability [13]. The slender young larva is yellowish white and measures 1mm in length. When mature, it turns reddish yellow, becomes hairy and measures over 6mm in length. The pupae are legless and stays inside the hollowed out head capsule [14]. The pupal stage lasts for about 20 days. The pupae are whitish with reduced legs. The pupae are dark brown and pupation occurs inside the legume seeds. The eggs are yellow in colour and occur singly which become opaque when hatched [24]. The life cycle completes within 25 to 34 days during summer, while 40 to 50 days in winter [25], but in the presence of grain protectants, life of the beetles found to be disturbed [26].

**Materials and Methods**

**Collection and preparation of test materials**

The medicinal plant of *A. cathartica* was identified by a plant taxonomist in the Department of Botany University of Rajshahi, Bangladesh and was collected from a roadside garden of Rajshahi City, Bangladesh. The collected plants were cleaned, chopped into small pieces with a cutter, air dried under shade without heating in well ventilated room and powdered with the help of a grinder, weighed and placed in conical flasks to add solvents. Pet ether, CHCl₃ and CH₃OH (Merck, Germany) (200 g x 600 ml x 2 times) were used successively, each of which kept 48h on a shaker. For each of the extract filtration was done by Whatman filter paper (USA) at 24h interval in the same flask followed by evaporation until the extract was left. The extracts were transferred to glass vials and preserved in refrigerator at 4°C with proper labelling.

**Collection and culture of test insects**

The test insects *T. castaneum*, *S. oryzae* and *C. chinensis* used in this investigation to carry out dose-mortality and repellent activity tests were obtained from the Crop Protection and Toxicology Laboratory of the Department of Zoology, University of Rajshahi, Bangladesh. The culture was maintained in the laboratory at an ambient condition.

**Dose-mortality tests**

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

The concentration of extractives used in this experiment as doses were set through *Ad Hoc* experiments, while the concentrations 0.509, 0.763, 1.018, 1.273 and 1.527 mg/cm²; and 0.763, 1.018, 1.273, 1.527 and 1.782 mg/cm² of the leaf extracts collected in Pet. ether and CHCl₃ were applied on *T. castaneum, S. oryzae* and *C. chinensis*. The concentrations 1.018, 1.273, 1.527, 1.782 and 2.037 mg/cm² of the leaf extracts collected in CH₃OH applied on *T. castaneum, S. oryzae* and the concentrations 1.527, 1.783, 2.037, 2.292 and 2.546 mg/cm² of the leaf extract collected in CH₃OH applied on *C. chinensis*. Again, the concentrations 0.763, 1.018, 1.273, 1.527 and 1.782 mg/cm²; and 1.018, 1.273, 1.527, 1.782 and 2.037 mg/cm² of the stem-bark extracts collected in Pet. ether and CHCl₃ applied on *T. castaneum, S. oryzae* and *C. chinensis*. For the dose mortality on *T. castaneum* each of the doses one ml was dropped on a Petri dish (50 mm) and spread on the surface and left open for a while dry the solvent out before releasing the test insects on it. For *S. oryzae* and *C. chinensis* the doses poured into the Petri dishes containing food grains shook well to make coat of extract on each of the grains. After drying out the grains 10 insects (3-5 days old) were released in each of the Petri dishes and the whole
experiment was set in 3 replicates. Released insects within this captivity might have contacts with the substance distributed on the floor or on the surface of the grains. A control batch was also maintained with the same number of insects after preparing the Petri dish in the same way as done for three stored product pests only with the solvent. The mortality of the beetles was counted after 6, 12, 18, 24, 30 and 36 h of exposure respectively.

Statistical analysis
The mortality (%) was corrected using Abbott’s formula: where, \( Pr = \) Corrected mortality (%), \( Po = \) Observed mortality (%), \( Pc = \) Control mortality (%). Data were then subjected to probit analysis according to Finney [27] and Busvine [28].

Repellent activity test
The repellent activity test used was adopted from the method of McDonald and co-authors [29] with some modifications by [30, 31]. A general concentration for each of the extracts (Pet. ether, CHCl\(_3\) and CH\(_2\)OH) was selected as stock dose for repellent activity test application to make other successive doses by serial dilution. For the repellent activity test on T. castaneum, S. oryzae and C. chinensis. The stem-bark extracts of the same collected in Pet. ether, CHCl\(_3\), shows mortality on three stored product pest, while the CH\(_2\)OH extract did not show mortality against any of the test agents. However the leaf and stem-bark extracts collected in Pet. ether, CHCl\(_3\), and CH\(_2\)OH did not show any repellent activity against the adult beetles of T. castaneum, S. oryzae and C. chinensis.

Dose mortality effects on T. castaneum, S. oryzae and C. chinensis
The dose mortality assay of Pet. ether, CHCl\(_3\) and CH\(_2\)OH extracts of A. cathartica are represented in Table 1 and Table 2. The Pet. ether extract of leaves gave LD\(_{50}\) values against T. castaneum. For the CHCl\(_3\) extract of leaves the LD\(_{50}\) values were between 2.179 to 1.055 mg/cm\(^2\); and for the CH\(_2\)OH extract of leaves the LD\(_{50}\) values ranged between 2.598 to 1.357 against T. castaneum. For S. oryzae the Pet. ether, CHCl\(_3\) and CH\(_2\)OH extracts of leaves the LD\(_{50}\) values were between 1.532 to 0.921 mg/cm\(^2\); 1.860 to 1.114 mg/cm\(^2\) and 2.758 to 1.947 mg/cm\(^2\) respectively. For C. chinensis the Pet. ether, CHCl\(_3\) and CH\(_2\)OH extracts of leaves gave LD\(_{50}\) values ranged between 2.439 to 0.685 mg/cm\(^2\), 2.439 to 1.069 mg/cm\(^2\) and 3.017 to 1.985 mg/cm\(^2\) respectively. On the other hand, all the extracts of the stem-bark offered promising insecticidal activity. The Pet. ether and CHCl\(_3\) extracts of stem-bark gave LD\(_{50}\) values ranged between 3.402 to 1.419 mg/cm\(^2\) and 3.283 to 1.756 mg/cm\(^2\); 3.140 to 1.182 mg/cm\(^2\) and 3.408 to 1.636 mg/cm\(^2\); and 3.433 to 1.309 mg/cm\(^2\) and 3.711 to 1.674 mg/cm\(^2\) against T. castaneum, S. oryzae and C. chinensis respectively.

### Table 1: LD\(_{50}\) values of Pet. ether, CHCl\(_3\) and CH\(_2\)OH extracts of A. cathartica leaf against T. castaneum, S. oryzae and C. chinensis.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Insects</th>
<th>solvents</th>
<th>LD(_{50}) (mg/cm(^2)) at different hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>T. castaneum</td>
<td>Pet. ether</td>
<td>3.433</td>
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<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>2.179</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH(_2)OH</td>
<td>2.598</td>
</tr>
<tr>
<td></td>
<td>S. oryzae</td>
<td>Pet. ether</td>
<td>1.532</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>1.860</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH(_2)OH</td>
<td>2.758</td>
</tr>
<tr>
<td></td>
<td>C. chinensis</td>
<td>Pet. ether</td>
<td>2.439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>2.439</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH(_2)OH</td>
<td>3.017</td>
</tr>
</tbody>
</table>

### Table 2: LD\(_{50}\) values of Pet. ether, CHCl\(_3\) and CH\(_2\)OH extracts of A. cathartica stem-bark against T. castaneum, S. oryzae and C. chinensis.

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>Insects</th>
<th>solvents</th>
<th>LD(_{50}) (mg/cm(^2)) at different hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>3.283</td>
</tr>
<tr>
<td></td>
<td>S. oryzae</td>
<td>Pet. ether</td>
<td>3.140</td>
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<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>3.408</td>
</tr>
<tr>
<td></td>
<td>C. chinensis</td>
<td>Pet. ether</td>
<td>3.433</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CHCl(_3)</td>
<td>3.711</td>
</tr>
</tbody>
</table>
Discussion

Pet ether, CHCl₃ and CH₃OH extracts of A. cathartica leaves and the Pet ether and CHCl₃ of its stem bark showed dose-mortality effects against T. castaneum, S. oryzae and C. chinensis; and none of the above extracts offered repellent activity against any of the test insects. Being a heavily studied plant there are so many reports available even on its bioactive potentials, however no report was found on bioactivity against two of the test agents S. oryzae and C. chinensis used in this investigation, and works on repellent activity is lacking. While these findings receive supports from the previous researchers mostly on antimicrobial activity [53, 55, 36, 37, 39], antifungal activity [50]; antibacterial activity [38, 40, 41, 49]; antidermatophytic and wound healing activity [15, 54]; algicidal activity [42]; antioxidant potential [4, 10, 34, 56, 40, 43, 44, 45]; nematocidal activity [46, 47]; cytotoxic activity or brine shrimp lethality [33, 36, 45, 55, 56]; antidiabetic activity [48]; anti-inflammatory activity [50]; antifertility activity [51]; Toxicity [38, 52]; antileukemic activity [53]; antidermatophytic activity [54]; antimalarial activity [10, 44, 45]; genotoxic activity [55, 56]. Only Mannan and co-authors [36] showed insecticidal activity of the test plant against one of the test agents T. castaneum in this investigation. However, the report on antimalarial activity could be mentioned as mosquito larvicidal activity as well. The huge potentials of the test plant is undoubtedly depicted by many researchers, and this investigation clarifies its potentials by adding insecticidal activity against the storage pests S. oryzae and C. chinensis and also added that the leaves and the stem bark of A. cathartica extracts contain no components that repel insects. The wound healing activity and the antidermatophytic activities might advocate in favour of antifungal or antibacterial activity, and again the same could be referred to as antimicrobial activity since both the pathogens are considered as microbes. Maroyi [52] used the term toxicity of the test plant only for the cause that all parts of this beautiful flowering plant cause dermatitis.

Conclusion

Different parts of Allamanda cathartica plant possess biologically active compounds, and the leaf and stem bark contains no insect repellent potentials while tested against three major stored product pests T. castaneum, S. oryzae and C. chinensis.

Acknowledgement

The authors are grateful to the University Grant Commission (UGC) of Bangladesh and to the University of Rajshahi for research grants. They would like to express their gratitude to the Chairman, Department of Zoology, University of Rajshahi for providing laboratory facilities.

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