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

**Umme Habiba Mustary, Ainun Nahar, Shahina Begum Rekha, Ariful Hasan and Nurul Islam**

### Abstract

Petroleum ether (Pet. ether), CHCl<sub>3</sub> and CH<sub>3</sub>OH 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, CHCl<sub>3</sub> and CH<sub>3</sub>OH gave LD<sub>50</sub> values 3.433, 2.347, 2.091, 1.879, 1.533 and 1.309mg/cm<sup>2</sup>; 2.179, 1.898, 1.532, 1.300, 1.136 and 1.055mg/cm<sup>2</sup>; and 2.598, 2.097, 1.892, 1.645, 1.476 and 1.357mg/cm<sup>2</sup> respectively. For *S. oryzae*, the same extracts gave LD<sub>50</sub> values 1.532, 1.341, 1.120, 1.010, 0.921 and 0.921mg/cm<sup>2</sup>; 1.860, 1.669, 1.444, 1.257, 1.240 and 1.114mg/cm<sup>2</sup>; and 2.758, 2.610, 2.289, 2.250, 2.040 and 1.947 mg/cm<sup>2</sup>; and for *C. chinensis* the LD<sub>50</sub> values for the same were 2.439, 1.536, 1.105, 0.810, 0.713 and 0.685mg/cm<sup>2</sup>; 2.439, 1.536, 1.534, 1.314, 1.095 and 1.069mg/cm<sup>2</sup>; and 3.017, 2.742, 2.570, 2.380, 2.164 and 1.985mg/cm<sup>2</sup> respectively after 6, 12, 18, 24, 30 and 36h of exposure. The Pet. ether and CHCl<sub>3</sub> extracts of the stem-bark of the test plant gave LD<sub>50</sub> values against *T. castaneum* were 3.402, 3.095, 3.066, 2.077, 1.699 and 1.419mg/cm<sup>2</sup>; and 3.283, 3.196, 3.047, 2.220, 2.056 and 1.756mg/cm<sup>2</sup> respectively; against *S. oryzae*, the LD<sub>50</sub> values were 3.140, 2.528, 2.147, 1.745, 1.398 and 1.182mg/cm<sup>2</sup>; and 3.408, 2.477, 2.284, 2.092, 1.860 and 1.636mg/cm<sup>2</sup>; and against *C. chinensis*, the LD<sub>50</sub> values were 3.433, 2.347, 2.091, 1.879, 1.533 and 1.309mg/cm<sup>2</sup>; and 3.711, 2.492, 2.327, 2.050, 1.866 and 1.674mg/cm<sup>2</sup> respectively after 6, 12, 18, 24, 30 and 36h of exposure. However, the CH<sub>3</sub>OH 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<sup>[1]</sup> and is found in tropical and subtropical regions as an ornamental shrub in gardens<sup>[2]</sup>. 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 Aloklot<sup>[3]</sup>. 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 antidote, 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 lighter in color, being white to yellowish. The head, appendages and the last abdominal segment are darker. The adult is a small reddish-brown beetle. The length of adult beetle is about 3.5mm in length and about 1.2mm in width. The antennae become bent and bear a distinct club and formed by three enlarged terminal joints [14]. *S. oryzae* is commonly known as rice weevil (Coleoptera: Curculionidae). The rice weevil, *S. oryzae* is one of the most widespread pests and causes heavy losses of stored grain both quantitatively and qualitatively throughout the world [15, 16, 17]. The rice weevil is small and about 1/10inch (2-3mm) size and stout in appearance. The colour of the rice weevil is reddish-brown to black with four light yellow or reddish spots on the corners of the elytra. The larvae are legless and stays inside the hollowed grain kernel. It is fat with a cream coloured body and dark head capsule [18]. The pupae is naked and the pupal stage lasts an average of 6 days. During hot summer months the full life cycle may take only 26 to 32, but requires a much longer period during cooler weather [19]. *C. chinensis* L. (Coleoptera: Bruchidae) has got great economic importance [20] and most destructive on stored pulses. *C. chinensis* is commonly known

as bean weevil and is known to be a pest to many stored legumes [21]. The female is larger and heavier than the male beetle and this species exhibits some sexual dimorphism. Males bear antennae which are pectinate while in females, the antennae are serrate [22]. The colour of larvae become yellowish-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 [23]. The life cycle completes within 25 to 34 days during summer, while 40 to 50 days in winter [24], but in the presence of grain protectants, life of the beetles found to be disturbed [25].

## 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 heaping in well ventilated room and powdered with the help of a grinder, weighed and placed in conical flasks to add solvents. Pet ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH (Merck, Germany) (200 g × 600 ml × 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/cm<sup>2</sup>; and 0.763, 1.018, 1.273, 1.527 and 1.782 mg/cm<sup>2</sup> of the leaf extracts collected in Pet. ether and CHCl<sub>3</sub> were applied on *T. castaneum*, *S. oryzae* and *C. chinensis*. The concentrations 1.018, 1.273, 1.527, 1.782 and 2.037 mg/cm<sup>2</sup> of the leaf extracts collected in CH<sub>3</sub>OH applied on *T. castaneum*, *S. oryzae* and the concentrations 1.527, 1.783, 2.037, 2.292 and 2.546 mg/cm<sup>2</sup> of the leaf extract collected in CH<sub>3</sub>OH applied on *C. chinensis*. Again, the concentrations 0.763, 1.018, 1.273, 1.527 and 1.782 mg/cm<sup>2</sup>; and 1.018, 1.273, 1.527, 1.782 and 2.037 mg/cm<sup>2</sup> of the stem-bark extracts collected in Pet. ether and CHCl<sub>3</sub> 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 (%) [26]. 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<sub>3</sub> and CH<sub>3</sub>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* 0.629, 0.314, 0.157, 0.0786 and 0.0393 mg/cm<sup>2</sup> concentrations were used. To set experiments half filter paper discs (Whatman No. 40, 9 cm diam.) were prepared and selected doses of all the extract separately applied onto each of the half-disc and allowed to dry out as exposed in the air for 10 minutes. Each treated half disc was then attached length wise, edge to edge, to a control half-disc with adhesive tape and placed in a Petri dish (9 cm diam.). *T. castaneum* beetles were released in the middle of the joint filter paper. For *S. oryzae* and *C. chinensis* each of the Petri-dishes was divided into three parts and marked with two narrow stick fixed with adhesive tape. Then both the sides were filled with food, where in one side was with the treated food and the other side was with the non-treated food no food was given in the middle one. Each concentration was tested five times. Ten adult insects were released in the middle of each filter-paper circle. Insects that settled on the non-treated half of the filter paper discs were counted for one-hour interval and up to five successive hours of exposure. The

average of the counts was converted to percent repulsion (PR) using the formula:  $PR = (Nc - 5) \times 20$ ; Where, Nc is the average hourly observation of insect on the untreated half of the disc [30, 32]. The values in the recorded data were then calculated for repellent activity, which was again developed by arcsine transformation for the calculation of ANOVA.

### Results

Pet. ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of the leaves of *A. cathartica* offered dose mortality against *T. castaneum*, *S. oryzae* and *C. chinensis*. The stem-bark extracts of the same collected in Pet. ether, CHCl<sub>3</sub>, shows mortality on three stored product pest, while the CH<sub>3</sub>OH extract did not show mortality against any of the test agents. However the leaf and stem-bark extracts collected in Pet. ether, CHCl<sub>3</sub>, and CH<sub>3</sub>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<sub>3</sub> and CH<sub>3</sub>OH extracts of *A. cathartica* are represented in Table 1 and Table 2. The Pet. ether extract of leaves offered highest mortality giving LD<sub>50</sub> values (Table -1) ranged between 3.433 to 1.309 mg/cm<sup>2</sup> against *T. castaneum*. For the CHCl<sub>3</sub> extract of leaves the LD<sub>50</sub> values ranged between 2.179 to 1.055 mg/cm<sup>2</sup>; and for the CH<sub>3</sub>OH extract of leaves the LD<sub>50</sub> values ranged between 2.598 to 1.357 against *T. castaneum*. For *S. oryzae* the Pet. ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of leaves the LD<sub>50</sub> values were ranged between 1.532 to 0.921 mg/cm<sup>2</sup>; 1.860 to 1.114 mg/cm<sup>2</sup> and 2.758 to 1.947 mg/cm<sup>2</sup> respectively. For *C. chinensis* the Pet. ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of leaves gave LD<sub>50</sub> values ranged between 2.439 to 0.685 mg/cm<sup>2</sup>, 2.439 to 1.069 mg/cm<sup>2</sup> and 3.017 to 1.985 mg/cm<sup>2</sup> respectively. On the other hand, all the extracts of the stem-bark offered promising insecticidal activity. The Pet. ether and CHCl<sub>3</sub> extracts of stem-bark gave LD<sub>50</sub> values ranged between 3.402 to 1.419 mg/cm<sup>2</sup> and 3.283 to 1.756 mg/cm<sup>2</sup>; 3.140 to 1.182 mg/cm<sup>2</sup> and 3.408 to 1.636 mg/cm<sup>2</sup>; and 3.433 to 1.309 mg/cm<sup>2</sup> and 3.711 to 1.674 mg/cm<sup>2</sup> against *T. castaneum*, *S. oryzae* and *C. chinensis* respectively.

**Table 1:** LD<sub>50</sub> values of Pet. ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *A. cathartica* leaf against *T. castaneum*, *S. oryzae* and *C. chinensis*.

Plant parts	Insects	solvents	LD <sub>50</sub> (mg/ cm <sup>2</sup> ) at different hours					
			6h	12h	18h	24h	30h	36h
Leaf	<i>T. castaneum</i>	Pet. ether	3.433	2.347	2.091	1.879	1.533	1.309
		CHCl <sub>3</sub>	2.179	1.898	1.532	1.300	1.136	1.055
		CH <sub>3</sub> OH	2.598	2.097	1.892	1.645	1.476	1.357
	<i>S. oryzae</i>	Pet. ether	1.532	1.341	1.120	1.010	0.921	0.921
		CHCl <sub>3</sub>	1.860	1.669	1.444	1.257	1.240	1.114
		CH <sub>3</sub> OH	2.758	2.610	2.289	2.250	2.040	1.947
	<i>C. chinensis</i>	Pet. ether	2.439	1.536	1.105	0.810	0.713	0.685
		CHCl <sub>3</sub>	2.439	1.536	1.534	1.314	1.095	1.069
		CH <sub>3</sub> OH	3.017	2.742	2.570	2.380	2.164	1.985

**Table 2:** LD<sub>50</sub> values of Pet. ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *A. cathartica* stem-bark against *T. castaneum*, *S. oryzae* and *C. chinensis*.

Plant parts	Insects	solvents	LD <sub>50</sub> (mg/ cm <sup>2</sup> ) at different hours					
			6h	12h	18h	24h	30h	36h
Stem-bark	<i>T. castaneum</i>	Pet. ether	3.402	3.095	3.066	2.077	1.699	1.419
		CHCl <sub>3</sub>	3.283	3.196	3.047	2.220	2.056	1.756
	<i>S. oryzae</i>	Pet. ether	3.140	2.528	2.147	1.745	1.398	1.182
		CHCl <sub>3</sub>	3.408	2.477	2.284	2.092	1.860	1.636
	<i>C. chinensis</i>	Pet. ether	3.433	2.347	2.091	1.879	1.533	1.309
		CHCl <sub>3</sub>	3.711	2.492	2.327	2.050	1.866	1.674

## Discussion

Pet ether, CHCl<sub>3</sub> and CH<sub>3</sub>OH extracts of *A. cathartica* leaves and the Pet ether and CHCl<sub>3</sub> 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 [33, 35, 36, 37, 38]; antifungal activity [39]; antibacterial activity [35, 40, 41, 49]; antidermatophyte and wound healing activity [5, 54]; algicidal activity [42]; antioxidant potential [4, 10, 34, 36, 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*.

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