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Control potentials of the ornamental indoor plant *Epipremnum aureum* (Linden & Andre) G. S. Bunting against three stored product pests

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Abstract
Petroleum ether (Pet. ether), chloroform ($CHCl_3$) and methanol (CH_3OH) extracts of *Epipremnum aureum* (Linden & Andre) G. S. Bunting have been subjected to dose-mortality and repellent activity tests against the adult beetles of *Tribolium castaneum* (Hbst.), *Sitophilus oryzae* (L.) and *Callosobruchus chinensis* (L.). The Pet. ether extract of *E. aureum* gave the LD_{50} values 1.40, 1.21, 1.16, 1.10, 1.04, 0.96 and 0.84mg/cm^2 against *T. castaneum*; 1.73, 1.66, 1.32, 1.08, 1.00, 0.99 and 0.90mg/cm^2 against *S. oryzae*; 2.95, 2.02, 2.01, 1.91, 1.82, 1.72 and 1.65mg/cm^2 against *C. chinensis* after 6, 12, 18, 24, 30, 36 and 42h of exposures respectively. $CHCl_3$ and CH_3OH extracts did not show any mortality against all three test agents. In repellency tests, Pet. ether extract of *E. aureum* showed significant repellent activity against *T. castaneum* and *C. chinensis* at 5% level of significance (P<0.05); $CHCl_3$ extract showed significant repellent activity against *T. castaneum* and *C. chinensis* at 1% level of significance (P<0.01) and at 5% level of significance (P<0.05) respectively. However, the CH_3OH extract of *E. aureum* did not show repellent activity against *T. castaneum, S. oryzae* and *C. chinensis.*

Keywords: *Epipremnum aureum, Tribolium castaneum, Sitophilus Oryzae, Callosobruchus chinensis*

1. Introduction
The test insect *Tribolium castaneum* (Hbst.) is one of the most serious pests of stored products which is commonly known as the 'red flour beetle' (Coleoptera: Tenebrionidae). Because of their mouthparts they are adapted and feed on almost any kind of flour, cracked grains, etc. listed the specific food of them, includes whole-wheat flour, bran, rice flour, cornmeal, barley flour and oatmeal [1]. *Sitophilus oryzae* (L.) is known as rice weevil (Coleoptera: Curculionidae) and also one of the most damaging pests of stored cereals worldwide; causes severe loss in rice, barley, maize and wheat [2, 3, 4]. Another, destructive insect *Callosobruchus chinensis* (L.) is a common species of beetle found in the bean weevil subfamily and is known to be a pest to many stored legumes [5]. The test plant *Epipremnum aureum*, commonly known as money plant under the family Araceae [6]. It is an evergreen herbaceous shrub used as popular ornamental foliage [7]. *Epipremnum* species is reported as an after birth medicine belongs to Indonesian medicinal plants. Leaves of this plant are reported to be used as wound medicine, in the treatment of rheumatism and fractures and dysentery [8], piles, to treat wounds and abscesses [9]. Plant ingestions are among the chief cause of accidental poisonings in children younger than 5 years. Pets are also common victims of plant poisonings [10]. It has been found that each part of this plant possesses antibacterial and antioxidant properties. Moreover, apart from these it can also turn out to be anti-malarial, anti-cancerous, anti-tuberculosis, anti-arthritis and wound healing, etc. which are the severe problem globally [11]. *E. aureum* also has anti-termite properties [12]. Plant-based pesticides have been used as pesticides from ancient time [13]. To control insects, the use of conventional pesticides introduces many risks to the environment. Plant origin products with insecticidal properties have been tried for controlling of variety of insects [14]. The present investigation was carried out to find out the bio-active potentials of this test plant for its possible use in agriculture. For this purpose, the three stored product pests were used as the test organisms.

2. Materials and Methods

2.1. Collection and preparation of test materials

The aerial part of the plant *E. aureum* has been collected during the month of January from a flower garden near the Police Station, Binodpur, Rajshahi 6206, Bangladesh; and identified by the herbarium specialist of the Department of Botany, University of Rajshahi, Bangladesh. Both leaves and stem of the plant were collected together and chopped into small pieces, dried under shade and powdered 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 (50g × 150ml × 2 times) successively each of which took about 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 collected in glass vials and preserved with proper labeling.

2.2. Collection and culture of test insects

The test insects *T. castaneum*, *S. oryzae and C. chinensis* adults were collected from the mass cultures of the Crop Protection and Toxicology Laboratory, Department of Zoology, University of Rajshahi, Bangladesh. In this investigation we got the supplies of the same aged individuals of three different test insects by the maintenance of insect's culture and were used for the experiments.

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

For insecticidal activity test Pet. ether, $CHCl_3$ and CH_3OH 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 doses. The final doses used in this experiment were 1.43, 1.22 and 1.02mg/cm^2 for Pet. ether extract. For each dose 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 (3-5 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 placed in an incubator at the same temperature as reared in stock cultures and for Pet. ether, $CHCl_3$ and CH_3OH extract*s* the mortality of the beetles w*ere* counted after 6h, 12h, 18h, 24h, 30h, *36h and 42h* of exposures *respectively*. However, the $CHCl_3$ and CH_3OH extract*s* did not show any mortality at all.

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

The experiment for insecticidal test on *S. oryzae* or *C. chinensis* are not the same as on *T. castaneum* since the feeding are different. Here also the *Ad Hoc* experiments were set to find out the final concentrations for dose selection. The doses of Pet. ether extract used against *S. oryzae* in this experiment were 1.27, 1.15 and 1.02mg/cm^2; for *C. chinensis* the doses of Pet. ether extract were 2.29, 2.16 and 2.04mg/cm^2. For each of the doses 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 as like as affected beetles of *T. castaneum* were counted.

2.5. Statistical analysis

The mortality (%) was corrected using Abbott's formula [15]. 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 [16, 17].

2.6. Repellent activity

The repellency test was adopted from the method of McDonald *et al*. [18] with some modifications. 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 the 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 all the 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 [19, 20]: 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 *T. castaneum*, *S. oryzae* and *C. chinensis*

The results of the dose mortality assays Pet. ether extract of *E. aureum* against beetles of *T. castaneum* are represented in Table 1. The LD_{50} values were 1.40, 1.21, 1.16, 1.10, 1.04, 0.96 and 0.84mg/cm^2 after 6h, 12h, 18h, 24h, 30h, 36h and 42h of exposures respectively. The LD_{50} values were 1.73, 1.66, 1.32, 1.08, 1.00, 0.99 and 0.90mg/cm^2 after 6h, 12h, 18h, 24h, 30h, 36h and 42h of exposures respectively. The LD_{50} values were 2.95, 2.02, 2.01, 1.91, 1.82, 1.72 and 1.65mg/cm^2 after 6h, 12h, 18h, 24h, 30h, 36h and 42h of exposures respectively. According to intensity of the sensitivity of the beetles to the extracts it could be arranged in the following descending order: *T. castaneum*> *S. oryzae*> *C. chinensis.*

Table 1: LD_{50} values of Pet. ether extract of *E. aureum* against *T. castaneum*, *S. oryzae* and *C. chinensis*

Solvent of extraction	LD_{50} (mg/cm^2) after different duration of exposure						
	6h	12h	18h	24h	30h	36h	42h
Pet. ether	1.40	1.21	1.16	1.10	1.04	0.96	0.84
	1.73	1.66	1.32	1.08	1.00	0.99	0.90
	2.95	2.02	2.01	1.91	1.82	1.72	1.65

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

The Pet. ether extracts of *E. aureum* showed repellency against *T. castaneum* both at 5% level of significance (P<0.05) and the $CHCl_3$ of *C. chinensis* showed repellency at 1% level of significance (P<0.01) and at 5% level of significance (P<0.05) respectively. However, the CH_3OH extract of *E. aureum* against *T. castaneum*, *S. oryzae* and *C. chinensis* did not show repellency. Moreover, none of the extracts showed repellent activity against *S. oryzae*. According to intensity of repellency, Pet. ether and $CHCl_3$ extracts were almost equal in efficacy against *C. chinensis*. On the other hand, the intensity of repellency against *T. castaneum* the result could be arranged in a descending order: $CHCl_3$> Pet. ether extracts.

Table 2: ANOVA results of the repellency against *T. castaneum*, *S. oryzae* and *C. chinensis* by the Pet. ether, $CHCl_3$ and CH_3OH extracts of *E. aureum*.

Plant	Name of the beetles	Solvent of Extraction	Source of variation	SS	df	MS	F	P-value
E. aureum	*T. castaneum*	Pet. ether	Between doses	1650.07	4	412.52	11.46*	0.0001
			Between time interval	336.05	4	84.01	2.33^{ns}	0.0999
		$CHCl_3$	Between doses	11114.3	4	2778.59	22.27**	2.25E-06
			Between time interval	2625.46	4	656.36	5.26^{ns}	0.0067
		CH_3OH	Between doses	7247.08	4	1811.77	5.40^{ns}	0.0060
			Between time interval	2077.21	4	519.30	1.55^{ns}	0.24
	S. oryzae	Pet. ether	Between doses	1508.42	4	377.10	8.44^{ns}	0.0007
			Between time interval	295.08	4	73.77	1.65^{ns}	0.21
		$CHCl_3$	Between doses	895.04	4	223.76	2.99^{ns}	0.05
			Between time interval	162.66	4	40.66	0.54^{ns}	0.70
		CH_3OH	Between doses	468.39	4	117.10	5.10^{ns}	0.0076
			Between time interval	107.41	4	26.85	1.170^{ns}	0.36
	C. chinensis	Pet. ether	Between doses	1129.92	4	282.48	11.29*	0.0002
			Between time interval	201.31	4	50.33	2.01^{ns}	0.14
		$CHCl_3$	Between doses	1839.61	4	459.90	14.42*	3.59E-05
			Between time interval	33.66	4	8.42	0.26^{ns}	0.90
		CH_3OH	Between doses	919.23	4	229.81	3.11^{ns}	0.05
			Between time interval	56.63	4	14.16	0.19^{ns}	0.94

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

4. Discussion

The findings of the present investigation of *E. aureum* got support from the works of the previous researchers. The significant antioxidant activity of the methanol extract of the plant *E. aureum* was established [21]. The aqueous and methanol extracts of the plant found as a source of natural antioxidants and folk medicines [22]. The locomotor and diuretic activities were evaluated from the aqueous and alcoholic extracts of the leaves of *E. aureum* on experimental rats [23]. Both of the extracts were having a significant CNS depression action by reducing locomotor and diuretic action in experimental animals. According to Meshram and Srivastava this money plant is involved in the phytoremediation process that removes the Cobalt and Cesium, and purify the air against formaldehyde [24]. Anti-termite and anti-repellant activities were also established against subterranean termites *Odontotermes obesus* [25]. The bioactive phytoconstituents were recorded from the methanol extract of the plant having efficacy against highly pathogenic bacteria i.e. *Salmonella* species as well as resistant pathogenic bacteria like *P. aeruginosa*. For the presence of secondary metabolites such as alkaloids, tannins, flavonoids, triterpenoids and saponins- the plant may responsible for the antimicrobial activity [26]. According to the previous reports these findings have got supports and the extracts of *E. aureum* can be used in the control of these stored product pests that showed both mortality and repellency against the test insects. This study was attempted to evaluate *E. aureum* which was claimed to be used or associated with insect mortality and insect repellent activity, and the result was found considerable. However, test results on other attributes also support the present findings, such as mortality and repellency of the extracts of *E. aureum* against the stored product pests.

5. Conclusion

The findings of the present study indicate the repellency and mortality effects of the extracts of *E. aureum* on the adult beetles of *T. castaneum*, *S. oryzae* and *C. chinensis*. Being natural in origin these materials could be biodegradable and thus safe and sustainable for the environment. So, attention should be given to the plant to find out new botanical insecticides which could prove useful in pest control and since these are traditionally used by the farmers in developing countries appear to be the most promising and safe.

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