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Dose-mortality and repellent potentials of *Argemone mexicana* L. extracts against *Sitophilus oryzae* L. and *Callosobruchus chinensis* L.

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Abstract
Petroleum ether (Pet. ether), Chloroform ($CHCl_3$) and Methanol (CH_3OH) extracts of seeds, aerial part, and roots of *A. mexicana* L. have been screened thoroughly for their insecticidal and repellent activity against the stored grain pests *Sitophilus oryzae* L. and *Callosobruchus* chinensis L. under laboratory conditions. The Pet. ether, $CHCl_3$ and CH_3OH extracts of the aerial part and roots did not offer any mortality at all, while the seed extracts of Pet. Ether, $CHCl_3$ and CH_3OH were found active and gave LD_{50} values against *S. oryzae* and *C. chinensis.* According to the intensity of activity of the seed extracts could be arranged in the descending order of: Pet. ether > $CHCl_3$ > CH_3OH against *S. oryzae* and $CHCl_3$ > Pet. ether > CH_3OH for *C. chinensis.* For repellency test against *S. oryzae* the Pet. ether extract of roots and seeds have shown significant result at 5% level of significance ($P<0.05$), and also the Pet. ether extract of roots and aerial parts, and CH_3OH extract of roots shown repellent activity against the adults of *C. chinensis* at 5% level of significance ($P<0.05$); but the $CHCl_3$ extract of the roots, aerial parts and seeds; CH_3OH extract of the aerial parts and seeds didn't show any repellent activity against *S. oryzae* and *C. chinensis.*

Keywords: Dose-mortality, repellency, *Sitophilus oryzae* L., *Callosobruchus chinensis* L. and *Argemone mexicana* L.

1. Introduction

The plant *Argemone mexicana* L. belonging to the family Papaveraceae, is a widely distributed plant throughout the subtropical and tropical regions of the world [1]. It is a weed of most cropping systems [2], especially in Tanzania, Australia, India and Pakistan [3]; and in Bangladesh it grows in wheat, sugarcane, potato, pulses and tea fields [4]. In Tanzania the plant is commonly found in the Lake Manyara National Park [5]. It is thought that its natural distribution is in the Northern America included Mexico and Southern Florida [6]. It is commonly called Mexican poppy, prickly poppy or yellow thistle in English. In Southern India it occurs up to an altitude of 800m and the plant flowers and fruits through the year [7]. It is an annual, herbaceous and seed propagated herb, grows up to 150cm in height with a slightly branched tap root. The stem is erect, branched, usually prickly and pale bluish-green in color. Leaves are alternate and without petioles. Flowers are solitary, 2.5 - 4.5 cm (in diam.) and prickly; petals are 4 - 6, yellow to pale orange in color [8]. Medicinal plants are believed to be an important source of new chemical entities with potential therapeutic effects [9]. Plant extracts as well as their primary and secondary metabolites have an important therapeutic role in the treatment of many human diseases [10, 11]. Plants make a significant contribution to health care due to the recognition of the value of traditional medicinal systems [12, 13]. Numerous useful drugs have been discovered from higher plants followed by ethnomedical practice [14]. *A. mexicana* L. possesses analgesic, narcotic, antispasmodic and sedative properties [15, 16]. The plant cures leprosy, skin diseases, inflammation and bilious fevers [17] and it is widely used in folk medicine to alleviate several ailments and narcotic effects. Seeds are useful in cough and asthma. In Northern and Central India *A. mexicana h*as been identified as an important allergen [18]. Extracts of *A. mexicana* readily kill the snail *Biomphalaria glabrata* and thus have potential as a molluscide [19]. Seeds are useful in cough and asthma. Seeds are laxative, nauseant, emetic, expectorant and demulcent. [20].

The present investigation was designed to find out the potentials of the crude extracts of the test plant species on the test organisms for the detection of insecticidal and repellent activities against *S. oryzae* L. and *C. chinensis* L.

The "rice weevil", *Sitophilus oryzae* L. (Coleoptera: Curculionidae) is a serious and severe insect pest of stored cereals and their products [21, 22]. One of the major pests of stored commodities in Tropics is *Sitophilus* spp., causing considerable economic loss to stored wheat grain [23, 24]. Heavy infestation of these pests due to lack of proper food hygiene and storage may cause weight loss of as much as 30–40% [25, 26]. Different biological activities of plant derivatives have been demonstrated for the control of this stored grain pest [27, 28]. A number of workers have delved into the life cycle of *S. oryzae* [29, 30, 31], but the results do not show uniformity as far as the time taken in completion of life cycle [29], the features of different developmental stages encountered [31] and the generations completed per year [30] are concerned.

C. chinensis L. (Coleoptera: Bruchidae) is a common species of beetle found in the bean weevil subfamily, and is known to be a pest to many stored legumes [32]. It is a small insect, growing to be about 5 mm in length as an adult. It has no snout and the adult stage is described as being brown in color with black and grey patches over the body. This species exhibits some sexual dimorphism [33]. The maximum duration of egg, larval and pupal period was 5, 17 and 6 days, respectively on survival of different stages of *C. chinensis* [34, 35]. The developmental period from egg to adult takes 20-25 days [36, 37].

2. Materials and Methods

2.1 Collection and preparation of test materials: The fresh materials of *A. mexicana* were collected from Kansat, Chapai Nawabganj. Seeds, aerial part and roots were collected separately and excess soil form the roots were removed without washing. A herbarium of the plant was prepared, identified and kept in the herbarium of the Department of Botany, University of Rajshahi, Bangladesh. The aerial part and roots of plant materials then cut into small pieces using a knife and spread out to dry without heaping the material together. This was done under the shade of the sun or in a well-ventilated room. Then materials were powdered by a grinder avoiding excess heat (up to 40ºC) during grinding. Whatman filter paper (made in USA) at 24h interval in the same flask followed by evaporation until the extract was left. The extracts was then removed to glass vials and preserved in a refrigerator at 4 ^{0}C with proper labeling.

2.2 Collection and culture of test insects: To carry on insecticidal and repellent activity tests of the extractives of *A. mexicana* L. two stored grain pests *S. oryzae*, and *C. chinensis* were selected, because they are economically dangerous pests to infest a wide variety of cereal products and also because they are rapidly reproducing species. The life histories made these insects as popular choice as test insects for biological studies. The test insects used in the present investigation were collected from the stock cultures of the Crop Protection Laboratory of the Department of Zoology, University of Rajshahi, Bangladesh.

2.3 Dose-mortality tests: To test the extracts of *A. mexicana* parts against the test beetles same aged individuals were used provided with their food as a unit of volume-by-volume measurement. Doses established through *Ad Hoc* experiments were dissolved in 1ml of solvent for each and mixed with the prepared food in Petri dishes. However, being volatile the solvent was evaporated out shortly. To have a dose-effect to calculate toxicity through Probit analysis five successive doses were applied. For *S. oryzae* the concentrations of the Pet. ether extract were 5, 4, 3, 2 and 1 mg/g; for the $CHCl_3$ extract were 6, 5, 4, 3 and 2 mg/g; and for the CH_3OH extract were 8, 7, 6, 5 and 4 mg/g. For *C. chinensis* the concentrations of the Pet. ether, $CHCl_3$ and CH_3OH extract were 18, 15, 13, 10 and 8 mg/g. The mortality was observed for 0.5h, 6h, 12h, 18h, 24h, 30h, 36h and 42h of exposure respectively.

2.4 Statistical analysis: The mortality (%) was corrected by using Abbott's formula [38]: $P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Control mortality (%). The data were then subjected to Probit analysis [39, 40]

2.5 Repellent activity tests: The method of repellency test used in this investigation was adopted from the method No. 3 [41] 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 0.629, 0.314, 0.157, 0.078 and 0.039 mg/cm^2. For the application of the extracts the Petri dishes (of 9cm in diam.) were divided into three parts and marked with two narrow sticks through adhesive tape. Then both the sides were filled with treated and non-treated food and the middle part was kept without food supplement. Ten adult insects were released in the middle of the petri-dish. The same was done for each of the doses at least in three replicates. Observations were made five times with 1h interval.

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) [42, 43]: 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 *S. oryzae* and *C. chinensis*: The results of the dose mortality test of the Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. mexicana* seeds against *S. oryza and C. chinensis* are represented in Table 1. Against *S. oryza* the Pet. ether extract gave LD_{50} values 13.9, 11.8, 10.7, 9.7, 9.0 and 6.2 mg/g, the $CHCl_3$ extract gave LD_{50} values 18.2, 17.8, 14.7, 12.9, 12.0 and 10.6 mg/g and the CH_3OH extracts gave LD_{50} values 22.1, 17.4, 16.0, 12.4, 12.9 and 11.6 mg/g after 12h, 18h 24h, 30h, 36h and 42h of exposure respectively. Against *C. chinensis* the Pet. ether extract gave LD_{50} values 4.0, 3.6, 3.4, 3.0, 3.0 and 2.9 mg/g; the $CHCl_3$ extract gave LD_{50} values 6.6, 5.7, 5.5, 3.9, 3.2 and 3.1 mg/g; and the CH_3OH extracts gave LD_{50} values 9.8, 7.5, 6.9, 6.3, 6.0 and 5.7 mg/g after 12h, 18h 24h, 30h, 36h and 42h of exposures respectively. According to intensity of activity the seed extracts of *A. mexicana* could be arranged in the following descending order: Pet. ether > $CHCl_3$ > CH_3OH extracts against both the test insects *S. oryzae* L. and *C. chinensis* L.

Table 1: LD_{50} values of the Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. mexicana* seed against *S. oryzae* and *C. chinensis*.

Plant part	Insect Used	Solvent of extraction	LD_{50} mg/g at different exposures (in hours)					
			12h	18h	24h	30h	36h	42h
Seed	*S. oryzae*	Pet. ether	13.9	11.8	10.7	9.7	9.0	6.2
		$CHCl_3$	18.2	17.8	14.7	12.9	12.0	10.6
		CH_3OH	22.1	17.4	16.0	12.4	12.9	11.6
	C. chinensis	Pet. ether	4.0	3.6	3.4	3.0	3.0	2.9
		$CHCl_3$	6.6	5.7	5.5	3.9	3.2	3.1
		CH_3OH	9.8	7.5	6.9	6.3	6.0	5.7

3.2 Repellent effects on *S. oryzae* and *C. chinensis*: For repellency test against *S. oryzae* L. the Pet. ether extract of roots and seeds were showed significant result at the 5% level of significance ($P<0.05$), and against *C. chinensis* the Pet. ether extract of roots, aerial parts and the CH_3OH extract of roots were also gave repellent activity at 5% level of significance ($P<0.05$); however the $CHCl_3$ extract of roots, aerial parts and seeds; the CH_3OH extract of aerial parts and seeds didn't show any repellent activity against none of the test insects (Table 2).

Table 2: ANOVA results of the repellent activities of the Pet. ether, $CHCl_3$ and CH_3OH extracts of *A. mexicana* (seed, aerial part and roots) against the test agents *C. chinensis* and *S. oryzae*.

Plant part	Solvents of extraction	Insects used	Sources of variation (df)			F-ratio with level of significance		P- value	
			Between doses	Between time interval	Error	Between doses	Between time interval	Between doses	Between time interval
Seed	Pet. ether	*S. oryzae*	4	4	16	10.48*	20.94	0.00023	3.38E-06
		C. chinensi	4	4	16	0.98	7.25	0.448	0.002
	$CHCl_3$	*S. oryzae*	4	4	16	7.27	4.48	0.002	0.013
		C. chinensis	4	4	16	4.62	2.27	0.0114	0.107
	CH_3OH	*S. oryzae*	4	4	16	3.54	0.03	1.784	0.182
		C. chinensis	4	4	16	6.76	6.60	0.002	0.002
Aerial part	Pet. ether	*S. oryzae*	4	4	16	4.34	2.04	0.014	0.136
		C. chinensis	4	4	16	10.58*	2.19	0.001	1.75E-02
	$CHCl_3$	*S. oryzae*	4	4	16	0.65	0.47	0.634	0.758
		C. chinensis	4	4	16	3.59	16.20	0.028	0.032
	CH_3OH	*S. oryzae*	4	4	16	0.89	0.87	0.493	0.504
		C. chinensis	4	4	16	5.82	3.47	0.004	0.023
Root	Pet. ether	*S. oryzae*	4	4	16	14.83*	3.83	3.03E-	0.183
		C chinensis	4	4	16	12.29*	0.36	9.32E-	O.832
	$CHCl_3$	*S. oryzae*	4	4	16	3.54	1.78	0.0297	0.007
		C chinensis	4	4	16	3.82	1.66	0.023	0.207
	CH_3OH	*S. oryzae*	4	4	16	4.67	8.46	0.011	0.207
		C. chinensis	4	4	16	12.51*	1.83	8.4E-01	0.173

* = Significant at 5% level ($P<0.05$)

4. Discussion

Now a days the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable [44]. The findings of the present investigation receive support from experiments done on *A. mexicana* and its related species by previous researchers.

The leaves and seed extracts of *A. mexicana* offered larvicidal activity of different extracts of *A. mexicana* against *C. quinquefasciatus* larvae [45]. The root extracts of *A. mexicana* was found to be the most effective after 24h of exposure and gave LC_{50} 91.331 ppm [46]. It was also revealed that 100% failure of the egg hatching on treatment with ethanol extracts of *A. mexicana* seeds against *A. aegyptii* [47]. Root extracts of *A. mexicana* has oviposition altering and ovicidal efficacy against dengue vector *A. aegyptii* and it also has altered reproductive fitness and behavior of the test agent [48] and appeared as the source of the active ingredient of mosquito larvicide [49-52]. The plant has free radical scavenging, antibacterial and antimutagenic activity [53-55] and insecticidal potential as well. The repellent activity of *A. mexicana* plant powder was also approved [56, 57]. The seed extracts of Pet. ether, $CHCl_3$ and CH_3OH were found active against adult beetles of *T. castaneum* [58]. Though works on repellency by *A. mexicana* is scanty it was found tested on *T. castaneum* [59] and the plant powder was found repellent. Bhumij tribes in Odisha, India use seed oil of *A. mexicana* as mosquito repellent [60]. Against *T. castaneum* the $CHCl_3$ extracts of the aerial part and roots offered moderate repellency at 1% level of significance ($P<0.01$), and the Pet. ether extract of the aerial part, root and seed gave mild repellency at 5% level of significance ($P<0.05$) [61]. The *Saraca indica* leaf extracts of Pet. ether and $CHCl_3$ against *S. oryzae* and the root extracts of Pet. ether, $CHCl_3$ and CH_3OH *against C. chinensis* gave repellency at 5% level of significance ($P<0.05$) [62]. After studying the findings of the previous researchers and analyzing the outcome of the present investigation it is certain that *A. mexicana* is a promising plant for potential chemical substances to show biological activity against different test agents under laboratory conditions. So, this plant is a potential source of promising biologically active compounds and thus further investigation should be attempted on this natural resource.

3. Conclusion

The present study reveals that *A. mexicana* extracts have lethal effect on *C. chinensis* and *S. oryzae* adults, and also have potentials to repel the test insects at 1% (moderate) to 5% (mild) level of significance. It could be promising to go through a thorough investigation of this plant for the possible use it's medicinal and pest control essences.

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