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Lethal action of *Argemone mexicana* L. extracts against *Culex quinquefasciatus* Say larvae and *Tribolium castaneum* (Hbst.) adults

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Abstract
Petroleum ether (Pet. Ether), Chloroform ($CHCl_3$) and Methanol (CH_3OH) extracts of *Argemone mexicana* L. were tested against adult beetles of *Tribolium castaneum* (Hbst.) and larvae of *Culex quinquefasciatus* Say through dose mortality assay. The seed extracts of Pet. Ether, $CHCl_3$ and CH_3OH were found active against adult beetles of *T. castaneum* and the LD_{50} values were 0.608, 0.428, 0.293, 0.192mg cm^{-2}; 1.261, 0.394, 0.241, 0.198, 0.194mg cm^{-2}; and 1.481, 1.212, 1.171, 1.099mg cm^{-2} after 1h, 6h, 12h, 18h; 1h, 6h, 12h, 18h, 24h and 12h, 18h, 24h, 30h of exposures respectively. The Pet. Ether extracts of seeds, aerial parts and roots were found active against *C. quinquefasciatus* larvae while the LC_{50} values were 503.161, 416.131, 355.398ppm; 621.789, 482.021, 387.385ppm and 694.589, 681.68, 628.119ppm after 6h, 12h and 18h of exposures respectively. The $CHCl_3$ extract of the same materials with the same exposures yielded LC_{50} values 950.604, 576.184 and 416.131ppm; 723.351, 637.999 and 621.789ppm and 694.589, 681.68 and 628.119ppm; while the CH_3OH extract yielded 950.604, 573.566 and 202.692ppm; 1431.852, 568.67 and 443.338ppm and 1465.043, 664.251 and 533.927ppm respectively.

Keywords: Dose mortality, Larvicidal activity, *Argemone mexicana, Culex quinquefasciatus, Tribolium castaneum*

1. Introduction
Argemone mexicana L. (Papaveraceae) is a weed of most cropping systems. *Argemone* is from the Greek argena, meaning 'cataract of the eye', and mexicana combines Mexico with the Latin suffix ana, belonging to, suggesting the country of origin [1]. It is commonly called Mexican poppy, prickly poppy or yellow thistle in English. In Bangladesh it grows in wheat, sugarcane, potato, pulses and tea fields. It is thought that its natural distribution is in Northern America included Mexico and Southern Florida [2]. In Southern India it occurs up to an altitude of 800m [3]. 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, 2.5–4.5cm in diam. and prickly; petals are 4–6, yellow to pale orange in color [4]. Fruit is prickly and oblong. Seeds are very numerous, nearly spherical, covered in a fine network of veins and brownish black in color.

The plant cures leprosy, skin diseases, inflammation and bilious fevers [5] 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* has been identified as an important allergen [6]. Extracts of *A. mexicana* readily kill the snail *Biomphalaria glabrata* and thus have potential as a molluscicide [7].

The present investigation was designed to find out the lethal action of the test plant against *Tribolium castaneum* adults and *Culex quinquefasciatus* larvae. *T. castaneum* (Hbst.) (Coleoptera: Tenebrionidae) commonly known as 'Rust-red flour beetle' is a worldwide pest of wheat flour, pulses, millets and cereals in the storage [8]. It is reddish brown in color and its antennae end in a three segmented club. It has complete a metamorphosis (egg, larva, pupa and adult) in its life cycle [9]; however, both the larvae and adults take part in damage. *C. quinquefasciatus* Say (Diptera: Culicidae) is the most common species of mosquitoes, while there are over 2500 different species of mosquitoes throughout the world. It has a complete metamorphosis (egg, larva, pupa and adult) in its life cycle while the egg, larval and pupal stages are aquatic. Hundred or more eggs are stuck together in rafts laid on one after another at a time and float on the surface of the water.

Most eggs hatch into larvae within 48 hours [10] and morphologically have the three body division common to insects: head, thorax, and abdomen. This medium-sized mosquito is found in tropical and subtropical regions of the world. It is the vector of *Wuchereria bancrofti*, avian malaria, and arboviruses including St. Louis encephalitis virus, Western equine encephalitis virus, Zika virus [11] and West Nile virus [12].

2. Materials and Methods

2.1 Collection and preparation of test materials

A. mexicana plants were collected from Chapai Nawabgonj, Bangladesh and identified by the Department of Botany, University of Rajshahi where voucher specimens are kept in the Herbarium. Accordingly seeds, aerial part and roots were arranged separate manner. The aerial part and roots were sliced and chopped into small pieces, dried under shade and powered with the help of a hand grinder, weighed and placed in separate conical flasks besides another flask containing crushed seeds to add solvents. Petroleum ether, $CHCl_3$ and CH_3OH (Merck, Germany) were used (200g × 600ml × 2 times) successively each of which took for 48h on a shaker. For each of the extract filtration was done by Whatman filter paper (made in USA) 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 ^{0}C with proper labeling.

2.2 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 and the culture of *C. quinquefasciatus* were maintained in the same laboratory throughout the experimental period for continuous supply of larvae.

2.3 Dose-mortality test

2.3.1 Dose-mortality tests on *T. castaneum*

For insecticidal activity test each of the three extracts was 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 concentrations for seed extract used in this experiment were 0.611, 0.509, 0.407, 0.305, 0.254mg cm^{-2} for Pet. E. and $CHCl_3$ extracts; and 1.375, 1.273, 1.120, 1.018 and 0.916mg cm^{-2} for the CH_3OH extract. For each dose 1ml 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 day old) were released in each of the Petri dishes in 3 replicates. A control batch was also maintained with the same number of insects after preparing the Petri dish by applying and evaporating the solvent only. The treated beetles were placed in an incubator at the same temperature as reared in stock cultures and the mortality of the beetles was counted after 30 min. 1h, 6h and more 4 times with 6h intervals.

2.3.2 Larvicidal activity test

Mosquito larvae hatched out from the rafts and start swimming in the pond water kept in a beaker. Test samples at different concentrations considered as doses were prepared in test tubes by addition of calculated amount of DMSO (Dimethylsulfoxide) to make them hydrophilic before adding half of the required amount of water in each. Then additional amount of water were added to fill the pre-marked test-tubes with the help of a pipette. The larvae were counted by visual inspection and were released in Test-tubes containing 10ml of water and the test-tubes along with a control batch were left for 30 hours. Observation of mortality was made after 6h, 12h, 18h, 24 and 30h of exposure.

2.3.3 Statistical analysis

The mortality (%) was corrected using Abbott's formula (1925): [13]

$P_r = \frac{P_o - P_c}{100 - P_c} \times 100$; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Mortality in the control (%). The data were then subjected to Probit analysis [14, 15].

3. Results and Discussion

3.1 Dose mortality effects on *T. castaneum* and *C. quinquefasciatus*

The results of the dose mortality assays of Pet. Ether, $CHCl_3$ and CH_3OH extracts of *A. mexicana* seeds against adult beetles of *T. castaneum* are represented in Table 1. The LD_{50} values were 0.608, 0.428, 0.293, 0.192mg cm^{-2}; 1.261, 0.394, 0.241, 0.198, 0.194mg cm^{-2}; and 1.481, 1.212, 1.171, 1.099mg cm^{-2} after 1, 6, 12 and 18; 1, 6, 12, 18, 24 and 12, 18, 24 and 30h of exposures respectively. The larvicidal activity against *C. quinquefasciatus* larvae for the Pet. Ether, $CHCl_3$ and CH_3OH extracts of seeds, aerial part and roots of *A. mexicana* are represented in Table 2. The Pet. Ether extracts gave LC_{50} values 503.161, 416.131, 355.398 and 196.416ppm; 621.789, 482.021 and 387.385ppm and 694.589, 681.68, 628.119, 518.246 and 435.509ppm after 6, 12, 18 and 24h; 6, 12 and 18h; and 6, 12, 18, 24 and 30h of exposures respectively. The $CHCl_3$ extract of the same materials with the same exposures yielded LC_{50} values 950.604, 576.184 and 416.131ppm; 723.351, 637.9995, 621.789, 495.484 and 482.021ppm and 1465.043, 751.397, 659.008, 260.155 and 245.445ppm; and the CH_3OH extract yielded 950.604, 573.566 and 202.692ppm; 1431.852, 568.67, 443.338, 282.737 and 234.128ppm; and 1465.043, 664.251, 533.927 and 19.492ppm respectively after 6, 12 and 18h; 6, 12, 18, 24 and 30h; and 6, 12, 18 and 24h of exposures respectively. According to intensity of activity the extracts of *A. mexicana* could be arranged in the following descending order: seed (CH_3OH) > aerial part (Pet. Ether) > seed (Pet. Ether) > aerial part (CH_3OH) > root (CH_3OH) > root (Pet. Ether) > aerial part ($CHCl_3$) root ($CHCl_3$) and seed ($CHCl_3$) extracts.

The larvicidal activity of different extracts of *A. mexicana* against *C. quinquefasciatus* in the present study receive supports from the findings of Sakthivadivel and Daniel [16] who reported that the LC_{50} values for leaves and seed extracts of *A. mexicana* were 30.47ppm and 24.17ppm; and LC_{90} were 246.33 and 184.99 respectively. The root extracts of *A. mexicana* was found to be the most effective while the LD_{50} value was 91.331ppm after 24h of exposure [17]. However, Elawad and the group showed the activity of *Solenostemma argel* extract on *Anopheles* larvae significantly toxic with LC_{50} value 161.1ppm after 24h exposure [18]. Sakthivadivel and Thilagavathy [19] also revealed 100% failure of the egg hatching on treatment with ethanolic extracts of *A. mexicana* seeds against *A. aegypti*. Root extracts of *A. mexicana* has

oviposition altering and ovicidal efficacy against dengue vector *A. aegypti* and it also has altered reproductive fitness and behavior of the same [20], and appeared as the source of the active ingredient of mosquito larvicide [21-24]. The acetone fraction of the petroleum ether extract of seeds from *A. mexicana* L. exhibited larvicidal and growth inhibiting activity against the second instar larvae of *A. aegypti* (Linn). This activity occurred at higher concentrations (200, 100, 50 and 25 ppm). Petroleum ether extracts of *A. mexicana* along with other plant materials were tested for larvicidal activity against 3rd instar larvae of important vector mosquitoes i.e. *C. quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* (Diptera: Culicidae) to yield LC_{50} value of less than 100 ppm against all three vector mosquitoes tested [25]. The plant has free radical scavenging, antibacterial and antimutagenic activity [26-28,]; and insecticidal potential as well. The repellent activity of plant powders of *A. mexicana* was revealed by Pugazhvendan [29, 30].

Table 1: LD_{50} values of Pet E, $CHCl_3$ and CH_3OH extracts of *A. mexicana* seed extracts against *T. castaneum.*

Extract	solvent	LD_{50} mg cm^{-2} at different exposures (in hours)					
		1h	6h	12h	18h	24h	30h
Seed	Pet. E.	0.608	0.428	0.293	0.192	All dead	All dead
	$CHCl_3$	1.261	0.394	0.241	0.198	0.194	All dead
	CH_3OH	-	-	1.481	1.212	1.171	1.099

Table 2: LD_{50} values of Pet E, $CHCl_3$ and CH_3OH extracts of *A. mexicana* seed, aerial part and root extracts against *C. quinquefasciatus.*

Extracts	solvent	LD_{50} mgcm^{-2} at different exposures (in hours)				
		6h	12h	18h	24h	30h
Seed	Pet. E.	503.161	416.131	355.398	196.416	All dead
	$CHCl_3$	576.184	416.131	416.131	All dead	All dead
	CH_3OH	950.604	573.566	202.692	All dead	All dead
Aerial part	Pet. E.	621.789	482.021	367.385	All dead	All dead
	$CHCl_3$	723.351	637.9995	621.789	495.484	482.021
	CH_3OH	1431.852	568.677	443.338	282.737	234.128
Root	Pet. E.	694.589	681.687	628.119	518.246	435.509
	$CHCl_3$	1465.043	751.397	659.008	260.155	245.445
	CH_3OH	1465.043	664.251	533.927	19.492	All dead

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