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Evaluation of Larvicidal Action of Leaf and Seed of *Argemone Mexicana* Against the *Aedes Aegypti* (Diptera: Culicidae)

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Abstract

The primary carrier of dengue is *Aedes aegypti*. The dengue vector has developed resistance as a result of the extensive use of synthetic insecticide and poses a threat to public health worldwide. Dengue vector especially infects the people in tropical and subtropical nations. The plant natural phytochemicals used as a larvicide against the dengue vectors has been the prime concern for investigation. The plant-derived biopesticide is biodegradable, non-toxic, and eco-friendly.

In accordance with WHO recommendations, the current study assesses the larvicidal potential of ethanolic and petroleum ether extract of *Argemone mexicana's* leaves and seeds against the third instar of *Aedes aegypti*. Seed extract exhibits significant larvicidal potential with LC_{50} and LC_{90} values of 125.89, 72.44 ppm, and 478.63, 398.10 ppm, respectively. Similarly, leaf extract with LC_{50} and LC_{90} values of 194.98, 134.89, and 776.24, 645.65 ppm, in 24 h. Both the extracts achieved 100% mortality with higher concentrations at 1000 ppm and 500 ppm. At 95% confidence interval log probit analysis revealed the $LC_{50,}$ $LC_{90,}$ and regression analysis showed the dependent variable (Y-mortality) was positively correlated with the independent variable (X-concentration). The leaves and seeds extracts were found to be safe for *Poecilia reticulata*, selected as a non-target organism. Our outcomes suggest that the ethanolic and petroleum ether extract of leaves and seed have larvicidal potential and metabolites of this plant could serve as an ecofriendly and economical source for the eradication of dengue vector.

Keywords: Larvicidal, *Aedes Aegypti*, Non-Target Organism, Ethanolic Extract, Petroleum Ether Extract, LC50

Introduction

Mosquitoes are included in the class Insecta, the order Diptera (two-winged flies), and the family Culicidae. Different medically significant species of mosquitoes are the vector of various pathogens and parasitic diseases like dengue fever, yellow fever, malaria fever, Japanese encephalitis, chikungunya, zika, filariasis, and schistosomiasis. *Aedes aegypti* mosquito has affected people mostly in urban and semiurban areas of tropical and subtropical nations (Ansari, 2003) [9]. In a single gonotrophic cycle dengue vector can spread dengue virus to more than one individual. The key vector of dengue and hemorrhagic fever is *Aedes aegypti* (Machenzie et al., 2004).

According to the WHO, an estimated 100-400 million people get infected with dengue thorough out the world. Combating the disease-carrying mosquitoes is the only way to stop the spread of the dengue virus (WHO 2022) and a total of 193245 cases and 306 deaths were reported only in India (NVBDCP 2021) [26].

Since most people used synthetic chemicals to get rid of mosquitoes and other vectors, some mosquitoes that are harmful to humans have become resistant to those chemicals [19,20]. Controlling vectors with insecticides is the most effective way to reduce or prevent disease transmission, most dengue vector control programs target adult mosquitoes with malathion, permethrin, deltamethrin, and Bacillus thuringiensis israelensis, and temephos used as larvicides [15]. Resistance to permethrin and temephos has developed in Aedes species [10]. A review on insecticide-resistant and susceptibility revealed that in most areas of India's dengue vector, *Aedes aegypti*, has developed stronger resistance to DDT, permethrin, cyfluthrin, and lambda-cyhalothrin [16].

However, the intensive use of chemical insecticide has caused cholinesterase inhibition as well as chromosomal abnormalities in human peripheral leukocytes [35]. Aside from the development of a resistant mosquito population, the use of such insecticides increases environmental pollution, affects the non-target organism, and risks toxicity in human beings [5]. Because of insecticide resistance and the poor results, the search for new insecticides has become essential. Natural chemicals found in plants are abundant and act synergistically against insects. Phytochemicals act quickly, degrade quickly, and have low toxicity against the non-target organism [12, 36]. Plant-derived bioinsecticides are the most suitable alternative to synthetic chemical insecticides and sustainable solutions against the dengue vector.

In this context, plant-derived insecticides have been reported to be biodegradable, making them effective and environmentally friendly tools against mosquitoes [6, 37, 38]. They are therefore more environmentally friendly, cost-effective, long-lasting alternatives to synthetic insecticides, nontoxic to non-target organisms, and have high specific activity against mosquitoes. Oils and extracts from plants can kill mosquito larvae [12]. Plant-derived insecticides can be divided into six groups according to how they affect the various insects, as attractants, repellents, growth inhibitors, chemosterilants, antifeedants, and toxicants [30, 31]. Plant-derived insecticides act of different stages, which comprise the egg, larvae, pupa, and adult stages. Some plant compounds are capable of inhibiting growth or killing the larvae. The plant *Acalypha fruticose* crude (ethyl acetate) extract of the leaves exhibits significant larvicidal potential [27].

In addition to providing a powerful barrier against diseases spread by mosquitoes, phytochemicals have emerged as viable alternatives to synthetic chemical insecticides. Many anti-vector effects including larvicidal activity, growth inhibition, fecundity suppression, oviposition deterrence, and ovicidal activity of phytochemicals against mosquitoes have been demonstrated [21].

Due to the growing demand for natural insecticides made from plants rather than chemical ones, The purpose of the current study was to evaluate the larvicidal potential of ethanolic and petroleum ether extracts of the leaves and seeds of *Argemone mexicana* against the third instar larvae of *Aedes aegypti*.

Material and Methodology

Collection of Plant Material

The current study was carried out from May - October 2021. Plant materials were collected from the Bulandshahar region (28.4070° N, 77.8498° E), Utter Pradesh, India. It was ensured that the plant *Argemone mexicana* (Papaveraceae) was not endemic, threatened, or endangered. Leaf and seed were taken from healthy plants and brought to the research lab for further processing. The leaf and seed were cleaned through distilled water and allowed to dry in a dark place at 30°C in the laboratory room for 15 days. The dried leaf and seed were powdered mechanically using an electric grinder and kept in clean trays.

Preparation of Plant Extracts

10 g of powdered materials (leaf and seed) were extracted in 200 ml of solvents (ethanol and petroleum ether), serially in glass Soxhlet apparatus, separately. The extraction was done constantly for six hours each day (4 days). The Soxhlet apparatus temperature was set in accordance with the solvent's boiling point. Finally, the extracted material was separated out in a small beaker for the evaporation of the solvent. The extracted material was made free of solvent on the water bath. The total residue of leaf and seed was weighted after the complete evaporation and redissolved in distilled water to obtain the required concentration. As a stock solution of 2000 ppm was stored at 4°C for further use.

Mosquito Culture

Aedes aegypti larvae were collected from the district Bulandshahar and sub-district region Khurja, Utter Pradesh, India, and colonized in the research laboratory of the department of zoology N.R.E.C. College Khurja (Bulandshahar), at temperature 27±2 °C, Related humidity 75±5%, and Light/Dark photoperiod 14:10 hours. A wet cloth was dropped over the mosquito rearing cage to maintain the relative humidity. The larvae were cultured in a plastic tray (20 cm X 15 cm X 5 cm) filled with tap water. Brewer's yeast powder, and dog biscuits powder in a ratio of 2:1, respectively, were provided to larvae as food. The pupae were collected in a two-liter plastic container and placed into a mosquito rearing cage (50 × 50 × 50 cm) in order for adults to emerge. 10% sugar solution in four sterilized Petri dishes with a cotton wick was provided to the adults. In order to provide the blood meal for the nourishment of females (eggs follicles). Albino rats were introduced into the mosquito cage for one night per week. Eggs were collected on moist filter paper, a small enamel bowl was lined with 3.5-inch-wide strips of filter paper, and dechlorinated water was added to a depth of 3 cm. Every day, egg papers were removed. After that, A plastic tray containing dechlorinated water was then used to hatch the eggs. Larval instar was checked for their attainment of the third instar stage. Larvae were collected and tested for their mortality using the natural extracts of the selected plant.

Larvicidal Bioassay

Following the WHO standard guidelines with some modifications, ethanolic and petroleum ether extracts of leaf and seed of *Argemone mexicana* were tested for larvicidal activity against the dengue vector, *Aedes aegypti*. From the stock solution of leaf and seed, 2000 ppm extract was added in 10 ml Tween-20. Various concentrations were prepared from this solution, diluted in distilled water 50, 100, 200, 400, 500, 1000 ppm, and 25, 50, 100, 200, 400, 500 ppm for the leaf and seed, respectively. In five replicates, 20 larvae were placed in 250 ml of the plastic beaker with 150 ml of extract for the in-vitro larvicidal bioassay test. During the in-vitro larvicidal bioassay, the larvae received no food. The control consisted of Tween-20 and 5% ethanol dissolved in distilled water. To determine the leaf and seed extracts' LC_{50} and LC_{90} values. bioassays at various concentrations were performed on the treatment that demonstrated at least 100% mortality within 24 h.

Toxicity Test

The non-target organism, *Poecilia reticulata* was preferred as the test animal to evaluate the toxic effect of the leaf and seed extracts. In accordance with the procedure outlined by Promsiri et al., (2006) [29]. For 10 days, *Poecilia reticulata* were acclimated in the glass aquarium at 28±2 °C and given an artificial diet (Taiyo Guppy Bit). The leaf and seed extract LC_{50} and LC_{90} values were used to evaluate their toxicity, respectively. A glass aquarium with 500 ml of leaf and seed extract in water solution was administered to each group of 10 fishes (in 4 Replicates). 10 fish were used as the control in dechlorinated tap water. In 24 and 48 h after the treatments were applied, the percentage of deaths was computed.

Data Analysis

For each concentration of leaf and seed extracts, the percentage larval mortality was computed and subjected to log-probit analysis (Finney's probit model) [14] for calculating LC50, LC90 (at 95% confidence level), regression equation, and other statistics by using the software "MS Excel 2021". Abbott's formula [1] was applied to correct mortality in control (Abbot 1925).

Results

Statistical data presented in table 1 and 2 show that the ethanolic and petroleum ether extracts of leaves of *Argemone mexicana* showed significant larvicidal potential. In 24 h after the treatments were applied, 1000 ppm concentration achieved 100% mortality ($F= 197.179$, $P < 0.001$, $R^2 = 0.9801$ and $F= 180.233$, $P < 0.001$, $R^2 = 0.9783$) with LC_{50} and LC_{90} values of 194.98, 134.89 and 776.24, 645.65 ppm, respectively. Similarly, the seed extract 500 ppm concentration achieved 100% mortality in 24 h after the treatment were applied ($F= 95.766$, $P < 0.006$, $R^2 = 0.9599$ and $F= 220.610$, $P < 0.001$, $R^2 = 0.9822$) with LC_{50} and LC_{90} values of 125.88, 72.44 ppm and 478.63, 398.10 ppm. The concentrations of 50 ppm and 25 ppm of leaf and seed showed the lowest larval mortalities, respectively (table 1).

Plant	Part used	Ethanolic extract		Standard error (SE)*	Petroleum ether extract		Standard error (SE)
		Conc. (ppm or mg/L) and % mortality ± SD*			Conc. (ppm or mg/L) and % mortality ± SD		
Argemone mexicana	Leaf	1000	100 ± 0.0	0.0	1000	100 ± 0.0	0.0
		500	85 ± 0.89	0.78	500	90 ± 0.74	0.65
		400	70 ± 0.89	0.78	400	75 ± 1.09	0.96
		200	55 ± 1.83	1.60	200	60 ± 1.09	0.96
		100	30 ± 0.89	0.78	100	35 ± 0.63	0.55
		50	15 ± 0.63	0.55	50	20 ± 0.78	0.65
	Seed	500	100 ± 0.0	0.0	500	100 ± 0.0	0.0
		400	85 ± 1.16	1.02	400	90 ± 1.01	0.89
		200	60 ± 0.89	0.78	200	75 ± 0.89	0.78
		100	35 ± 0.89	0.78	100	55 ± 1.09	0.96
		50	15 ± 0.89	0.78	50	40 ± 0.74	0.65
		25	10 ± 0.4	0.35	25	25 ± 0.89	0.78
Control	In the control group, there was no mortality observed.						

*Values are mean ± Standard deviation of five replicates.

*Standard error of five replicates.

Table 1: Larvicidal action of different concentrations of leaves and seeds of *Argemone mexicana*

Data presented in table 1 show that the mortality rate increase with the concentration increase. The average mortality of five replicates is presented in table 1. Results of regression analysis demonstrate that the mortality rate (Y= % mortality) and concentration of exposure (X= log. concentration) were positively correlated. At 95% confidence level the result of probit analysis confirmed that the seed extract was more efficient compared to leaf extract (Tables 1 and 2). The upper bound, lower bound and regression equations are presented in table 2. The toxic effect of plant extracts on Poecilia reticulata, a non-target organism was further screened for in leaf and seed extracts. In 24 and 48 h after the treatments were applied, the ethanolic and petroleum ether extract of leaf and seed did not exhibit a noticeable effect.

Mosquito species	Plant Part used	Solvents	% Mortality (24 h)	LC50 (ppm)	LC90 (ppm)	95% confidence interval		Regression equation	R^2 value	P-value (P<0.05)
						Lower bound	Upper bound			
*Aedes aegypti*L. (Diptera: Culicidae)	Leaf	Ethanol	100%	194.98	776.24	1.91	2.85	Y = 2.3825x - 0.4778	R^2 = 0.9801	P<0.0001
		Petroleum ether	100%	134.89	645.65	1.56	2.37	Y = 1.9701x + 0.7393	R^2 = 0.9783	P<0.0001
	Seed	Ethanol	100%	125.89	478.63	1.58	2.84	Y = 2.2125x + 0.3538	R^2 = 0.9599	P<0.0006
		Petroleum ether	100%	72.44	398.10	1.40	2.05	Y = 1.7293x + 1.8067	R^2 = 0.9822	P<0.0001

Tabe 2: Evaluation of the larvicidal efficacy of different solvents extracts against *Aedes aegypti* third instar larvae

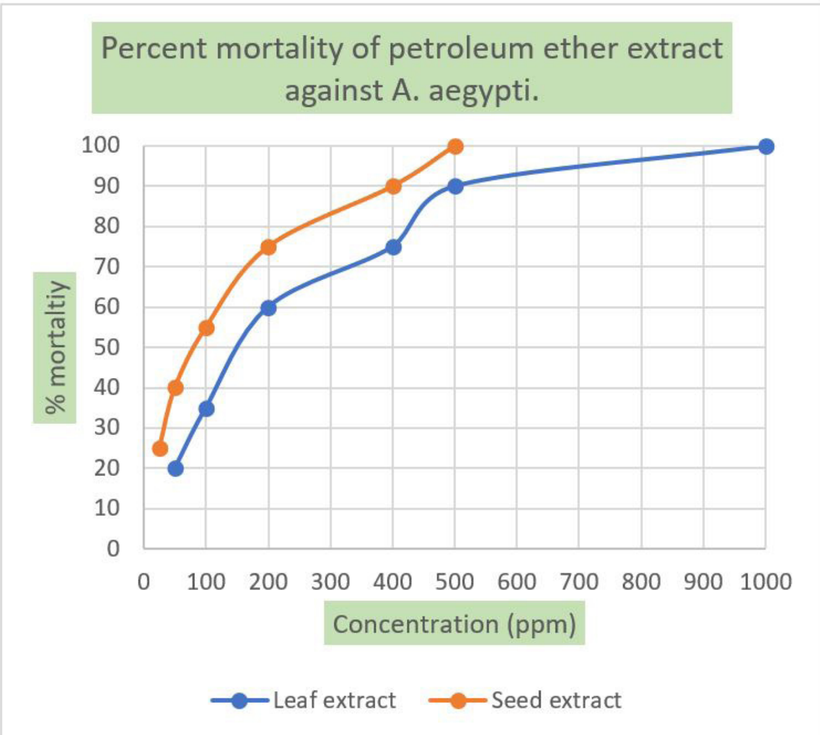

Figure 1: Graph showing the percentage mortality of petroleum ether extracts of leaf and seed against the third instar larvae of Aedes aegypti

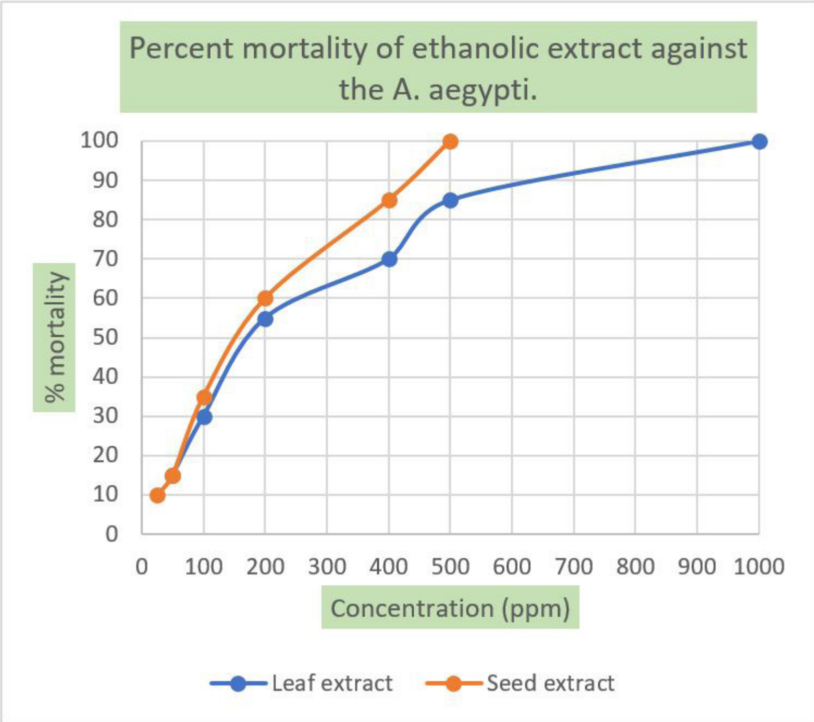

Figure 2: Graph showing the percentage mortality of ethanolic extracts of leaf and seed against the third instar larvae of Aedes aegypti

Discussion

Plant extract demonstrates different biological activities on mosquitoes, including repellent, larvicidal, ovicidal deterrents, insect growth regulator, growth inhibitor, chemosterilant, etc. This could be a result of a complicated mixture of phytochemicals found in plants that may be working together to produce such a response. Because of their synergistic complex biomolecules, plant-derived pesticides rarely cause the pest to become resistant to them [24].

In the present investigation, the third instar larvae of *Aedes aegypti* were treated with ethanolic and petroleum ether extract of the leaf and seeds of Argemone mexicana. Both extracts demonstrate significant larvicidal potential.

Several researchers have tested crude extracts of *Argemone mexicana*, leaf, seed, roots, and flower against various species of mosquitoes in the past. The petroleum ether extract of this plant's leaf and seed has previously been demonstrated to have larvicidal potential against the *Anopheles stephensi*, with LC_{50} and LC_{90} values of 30.47, 24.17, and 246.33, 184.99 ppm, respectively [33]. *Argemone mexicana* leaf extract achieved 100% mortality against *Cx. Quinqusfasciatus* (Karmegan 1997) and its larvicidal and chemosterilant effects on *Aedes aegypti* have been demonstrated by acetone extract of seed [33]. Antioxidant and expectorant properties have previously been demonstrated by flower extract of this plant [7, 34] and a GII of 0.01 has been recorded for the plant's flower extract at concentrations of 50 and 25 ppm. Showed growth inhibition and larvicidal potential against *Cx. quinqusfasciatus*, in 24 h after the treatment was applied achieved 100% mortality at 100 and 200 ppm concentrations [13].

The previous research on *Argemone mexicana* indicated that it produced useful inhibitory compounds, and ethanolic extract of leaves showed inhibitory effects against the bacterium *Bacillus subtilis, Proteus mirabilis*, and *Escherichia coli* [3]. Phytochemicals extracted from this plant, *Aedes aegypti* larvae exhibit altered behavior and morphological modification [39], and *Argemone mexicana* root extracts prepared with different solutions of petroleum ether, benzene, ethanol, hexane, and acetone were tested for their effectiveness as oviposition deterrents and ovicidal agents against the *Aedes aegypti* mosquito [40]. The seed Chloroform extract of *Argemone mexicana* exhibited larvicidal potential against *Cx. pipiens*, [43]. According to Elawad et al. (2014) [11], the second and fourth instars of *Cx. quinquefasciatus* were both prone to the larvicidal effects of acetone extract of *Argemone mexicana* leaves. In another example, the larvicidal efficacy of a methanolic extract of seeds, and roots of this plant were effective against *Cx. Quinquefasciatus.*

The respective LC50 values are 282.73 ppm and 19.49 ppm. [2].

Researchers have found that various phytochemicals derived from different plants are effective insecticides against mosquito species that are medically significant. More than 10 plant species were used in the study. Kumar et al., (2012) [21] have used 15 plant species. At a concentration of 1000 ppm, only 10 plants showed larvicidal activity against *Aedes aegypti*, demonstrating that they have significant larvicidal potential. Methanolic extract prepared from *Vitex ovata* showed a larvicidal potential and achieved 76% and 84% mortality within 24 h at the concentrations tested 5000 ppm and 10000 ppm, respectively, against the *Aedes aegypti* [4]. *Ricinus communis* leaf and seed extract in methanol demonstrated significant mortality against the larvae of *Ae. aegypti*, with LC_{50} and LC_{90} values of 9.37, 31.1 ppm, and 15.52, 45.24 ppm respectively [25]. *V. trifolia* oils have LC_{50} and LC_{90} values of 57, 77 ppm, 55, 78 ppm, against *Aedes aegypti* and *C. quinquefasciatus*, respectively [8] and Clitoria ternatea methanol extract of the flower have been exhibited larvicidal activity against *Aedes aegypti* with LC_{50} and LC_{90} values of 1056 ppm and 2491 ppm, respectively [32].

In our investigation, after the 24 h of exposure *Argemone mexicana* ethanolic and petroleum ether extract of leaf and seed demonstrated significant larvicidal activity against the third instar larvae of *Aedes aegypti* with LC_{50} values of 194.98, 134.89 ppm, and 125.89, 72.44 ppm, respectively.

Alkaloids, tannins, triterpenes, and anthraquinones were found in the crude extracts of *Argemone mexicana roots*; these phytochemicals have long been known to have a negative impact on insect development. They might interfere with the natural processes of growth, and this interference can be identified by observing changes in GII. Isoquinoline alkaloids make up the majority of the potent compounds that have been isolated from the different parts of this plant [18].

In conclusion, our research demonstrated that *Argemone mexicana's* leaf and seed extract can be developed as environmentally friendly larvicides and a promising substitute to eradicate the *Aedes aegypti*. Additionally, more investigation is required to identify, purify, and isolate the active ingredients that kill mosquitoes.

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