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PHYTOCHEMICAL ANALYSIS AND SYNERGISTIC LARVICIDAL ACTION OF *ARGEMONE MEXICANA* AGAINST THIRD INSTAR LARVAE OF *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

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Abstract

Aedes aegypti is the principal dengue vector. A threat to public health exists globally due to the dengue vector's development of resistance as a result of the widespread use of chemical insecticides. Tropical and subtropical countries are especially susceptible to dengue vector infection. The focus of research has been on plant-based phytochemicals that are employed as larvicides against mosquitoes. Bioinsecticides made from plants are biodegradable, and safe for the environment. In the present investigation, *Argemone mexicana* leaf and seed extracts were successfully tested against the third instar of *Aedes aegypti*, in accordance with the guidelines of WHO. The aqueous extract of the leaf with LC_{50} and LC_{90} values of 133.25 and 311.68 ppm, ethanolic extract leaf with LC_{50} and LC_{90} values of 102.32 and 239.88 ppm, and petroleum ether extracts of the leaf with LC_{50} and LC_{90} values of 123.02 and 281 ppm. Similarly, aqueous, ethanolic, and petroleum ether seed extracts with LC_{50} values of 154.88, 120.22, and 104.71 ppm and LC_{90} values of 393.07, 281.83, and 239.88 ppm, respectively, in 24 h post-exposure. All the extracts were shown significant ($P<0.05$) larvicidal potential. Preliminary phytochemical analysis reveals the presence of different phytoconstituents. Our findings suggest that the leaves and seeds have larvicidal potential, this plant's metabolites may be a cost-effective and eco-friendly source that keeps the mosquito population below the threshold level.

***Keywords* :** Synergistic, *Aedes aegypti*, Ethanolic extract, petroleum ether extract, LC_{50}.

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Introduction

The family Culicidae generally comprises mosquitoes. Different mosquito species that are medically important are the carriers of a number of diseases and parasitic diseases, including chikungunya, zika, filariasis, schistosomiasis, dengue fever, yellow fever, and malaria. Globally, dengue fever is regarded as a severe public health issue, particularly in tropical nations where *Aedes aegypti* vectors are proliferating due to favorable environmental conditions. People in tropical and subtropical countries have been particularly affected by the *Aedes aegypti* mosquito (Das and Ansari, 2003). The disease can transmit to multiple people during a single gonotrophic cycle. Dengue infections are thought to affect around 400 million individuals per year, worldwide, and around four billion individuals in 128 countries are thought to be at risk of contracting the dengue virus (WHO, 2021). The Ministry of Health & Family Welfare-Government of India reports that, around two lakh cases of dengue infection and hundreds of deaths in India (MOHFW, 2021).

Some mosquitoes that are dangerous to humans have developed resistance to the synthetic chemical insecticides that the majority of individuals utilized to eradicate mosquitoes and other insects that act as carriers (Kona *et al*., 2018; Kumar and Pillai, 2011). The most efficient strategy to lessen or stop the spread of disease is to control the vectors using insecticides; the majority of vector control programs employ larvicides such as malathion, permethrin, deltamethrin, and temephos to target adult mosquitoes (Kona *et al*., 2018). The *Aedes aegypti* mosquito has increased its resistance to chemical insecticides like temephos, DDT, permethrin, cyfluthrin, and lambda-cyhalothrin (Ishak *et al*., 2015; Jangir and Prasad, 2022). Such chemical insecticides cause environmental contamination, have an impact on other organisms that are not their intended targets and may be hazardous to humans (Benelli, 2015; Elia-Amira *et al*., 2019). Inhibition of cholinesterase and chromosomal aberrations have been led on by the chemical insecticide in human peripheral leukocytes (Sharma *et al*., 2016). The need for novel insecticides has arisen as a result of insecticide resistance and unsatisfactory outcomes. There are numerous natural compounds in plants that work together to repel or kill insects. Phytochemicals (metabolites) are the best alternatives to synthetic insecticides because they act quickly, are biodegradable, and are less hazardous to species other than their intended targets (Ghosh *et al*., 2012).

Insecticides made from plants can be divided into the following categories: repellents, growth inhibitors, toxicants, etc (Rattan, 2010; Benelli, 2016) and affect the different phases of insect life (egg, larval, pupa, and adult). Plant metabolites have the power to stop larvae from growing or even killing them (Pavunraj *et al*., 2017).

Argemone mexicana is included the in Papaveraceae family, also known as the Mexican poppy. The plant Mexican poppy is a blessing because it has countless advantages in the field of drug research. Various pharmacological properties viz., "Antidiabetic activity, Anti-Cancer activity, Anti-HIV activity, CNS-related activities, antimicrobial activity, Antioxidant activity, Anti-inflammatory, analgesic, antipyretic activity, Hepatoprotective activity, Anti-fertility activity, Antiallergic activity, Nematocidal activity, Allelopathic effect, Anthelmintic activity, Larvicidal activity, Antifeedant action" (Nancy and Praveena, 2017). This is why we decided to investigate its components as probable sources of vector control agents, using the mosquito species *Aedes aegypti*. The plant *Argemone mexicana's* leaf and seeds exhibit insecticidal or larvicidal potential against *Cx. quinquefasciatus* (Sakthivadivel *et al*., 2012), *Tribolium castaneum* (Patil and Zambare, 2019) *Spodoptera litura fab* (Vetal and Pardeshi, 2019), and *Chrysoperla carnea* (Aragon *et al*., 2020).

Material and Methodology

Collection of plant material

Plant samples were gathered from fully grown plants on farmer lands in the Bulandshahr region (28.4070° N, 77.8498° E). We made sure *Argemone mexicana* wasn't endemic, endangered, or under threat. During the flowering period, fully formed, healthy leaves and seeds were harvested, cleaned with distilled water, and then dried for 15 days at 30°C in a dimly lit space. Using an electric grinder, the dried materials were ground into powder.

Fig. 1: *Argemone mexicana* (leaf and seed)

Preparation of plant extracts

The solvents were ethanol, and petroleum ether (200 ml of each), and 10 g of powder of leaf and seed was extracted in a glass Soxhlet apparatus, at the temperature of the solvents' boiling point. Following separation, the extracted material was placed in a beaker to let the solvent evaporate over a water bath. After complete evaporation, the whole residue was weighed and redissolved in distilled water. In order to be used later, a 2000 ppm stock solution was stored at 4^{o}C. (Kasiramar, 2018).

Preliminary phytochemical analysis and physicochemical parameters of leaf and seed

The qualitative phytochemicals analysis of *Argemone mexicana* was performed by following standard procedure (Harborne, 1998; Rajasudha and Manikandan, 2019). The physicochemical characteristics of the leaf and seed like "ash values, loss on drying, water-soluble ash, acid-insoluble ash, extraction values," etc. were determined as per the standard procedure outlined by WHO (2011).

Mosquito culture

Aedes aegypti eggs and larvae were gathered from mosquito ovitraps installed on the college campus and various locations of the district Bulandshahr. A binocular microscope was used to identify the larvae of *Aedes aegypti* (Figure 2) by following the identification key and monograph (Christophers, 1960; Rueda, 2004). A plastic tray that measured 20x15x5 cm with tap water was used to culture the larvae. A mixed powder of brewer's yeast, rice, and soybean in a ratio of 3:1:1 was provided as a diet to the larvae. laboratory setup was maintained at 27±2°C temperature, Related humidity of 75±5, and a light and dark period of 14:10 h (Kamraj *et al*., 2009). On moist filter paper, the eggs were gathered and hatched in dechlorinated tap water and the remaining dried eggs were stored at room temperature for further use. It was determined whether larval instars had reached the third instar stage. Larvae were collected and put to the test utilizing the natural extracts of the chosen plant for their mortality.

Larvicidal bioassay

Argemone mexicana leaf and seed extracts were tested for their larvicidal action on the dengue vector, in accordance with the WHO (2005) standard recommendations with some changes. 2000 ppm extract (from the stock solution) was added to 10 ml of 0.1% Tween-20. During the preliminary testing plant extract that achieved 100% larval mortality within 24 h were selected and diluted in distilled water to prepare the desired concentrations, 50, 100, 200, 400, and 500 ppm, and LC_{50} and LC_{90} values were calculated. For the larvicidal bioassay test, 20 larvae in five replicates were put in a 250 ml plastic beaker with 150 ml of extract. The larvae did not get any food during the larvicidal bioassay. The control consisted of distilled water, 5% ethanol, and 0.1% Tween-20.

Data analysis

The average larval mortality data were put through probit analysis to determine LC_{50}, LC_{90}, and other statistics at 95% confidence limits (Finney, 1971), and regression equation using the software "MS Excel 2021." When needed, the mortality in control was corrected using Abbott's formula (Abbott, 1925).

Result

Table 1 shows the physicochemical parameters of the seed and leaf of *Argemone mexicana* (total ash values 11.42, 13.56 mg, water-soluble ash 2.34, 6.81 mg, acid insoluble ash 3.27, 0.98 mg and extraction value 6.41, 33.53, 34.50 mg and 15.86, 26.50, 5.58 mg, respectively) and table 2 shows the result of the qualitative phytochemical screening. The ethanolic extracts displayed more phytoconstituents than the other extracts.

At 500 ppm, the leaf's (aqueous, ethanolic, and petroleum ether) extracts were shown the strongest larvicidal efficacy (table 3-4). The significant larvicidal activity was observed in the aqueous extract of the leaf and seed at concentrations of 500 ppm and 600 ppm, respectively, with LC50 and LC90 values of 133.25, 154.88 ppm, and 311.68, 393.07 ppm, and the maximum larvicidal activity was demonstrated by the ethanolic extracts of the leaf and seed at a concentration of 500 ppm, with LC50 and LC90 values of 102.32, 120.22 ppm and 239.88, 281.83 ppm, respectively. Similarly, petroleum ether extract of the leaf and seed were shown the highest larvicidal activity at a concentration of 500 ppm with LC_{50} and LC_{90} values of 123.02, 104.71, and 281.83, 239.88 ppm, respectively in 24 h post-exposure. Ethanolic extract from leaf and petroleum ether extract from seed were shown significantly more larvicidal activity than the other extracts. In the control setting, no mortality was noted.

Table 3 shows that the rate of mortality significantly increases with the increase in concentration. At a 95% level of confidence, the regression analysis's findings indicate a positive correlation between the independent variable (concentration) and the dependent variable (mortality) (table 3-4). The outcome of probit analysis showed that the ethanolic extract of leaf and petroleum ether extracts of seed were shown more larvicidal potential. To the third instar of the dengue vector, all extracts were shown significant ($P<0.05$) larvicidal potential (table 4).

Table 1 : Physicochemical parameters of seeds and leaf of *Argemone mexicana.*

Parameters	Sub-parameters	Result (%) of seed	Result (%) of leaf
Loss on drying		11.42±0.39	13.56±0.55
Ash value	Total ash value	8.75±0.12	8.80±0.24
	Water soluble ash	2.34±0.10	6.81±0.24
	Acid soluble ash	3.27±0.12	0.98±0.16
	Sulphated ash value	4.34±0.17	3.50±0.32
Extractive value	Aqueous	6.41±0.21	15.86±0.49
	Ethanolic	33.53±0.60	26.50±0.64
	Petroleum ether	34.50±0.64	5.58±0.40

*Values presented in the table are the mean ± SD of three replicates.

Table 2 : Results of phytochemical analysis of seed and leaf of *Argemone mexicana*.

Phytoconstituents	Seed			Leaf		
	Aqueous	Ethanolic	Petroleum ether	Aqueous	Ethanolic	Petroleum ether
Flavonoids	+	+	-	+	+	-
Glycosides	+	+	+	+	-	-
Steroids	-	+	-	+	+	-
Oils and fats	-	+	+	-	+	+
Amino acids	+	+	-	-	+	-
Anthraquinones	-	-	+	-	-	-
Tannins	+	+	-	+	+	+
Terpenoids	-	+	+	-	+	-
Alkaloids	-	+	+	-	+	+
Carbohydrates	-	+	-	+	+	-
Phenols	+	+	+	-	+	+
Saponins	+	-	+	-	-	+

+; Present, -; Absent.

Table 3 : larvicidal action of different extracts at different concentrations.

Plant	Part used	Aqueous Conc. (ppm/L)	Aqueous % Mortality and SD	SE*	Ethanolic Conc. (ppm/L)	Ethanolic % Mortality and SD	SE	Petroleum ether Conc. (ppm/L)	Petroleum ether % Mortality and SD	SE
Argemone mexicana	Leaf	500	100±0.0	0.0	500	100±0.0	0.0	500	100±0.0	0.0
		400	80±1.41	1.24	400	95±0.89	0.78	400	85±1.41	1.24
		200	55±1.41	1.24	200	70±0.89	0.78	200	65±0.89	0.78
		100	25±0.89	0.78	100	45±0.89	0.78	100	30±0.89	0.78
		50	15±0.63	0.55	50	20±1.09	0.96	50	15±0.89	0.78
	Seed	600	100±0.0	0.0	500	100±0.0	0.0	500	100±0.0	0.0
		400	70±1.41	1.24	400	85±0.96	0.89	400	95±0.63	0.55
		200	45±0.89	0.78	200	65±1.09	0.96	200	70±1.41	1.24
		100	20±0.89	0.78	100	35±0.63	0.55	100	40±1.41	1.24
		50	10±1.09	0.96	50	15±1.89	1.6	50	20±0.89	0.78
Control	No mortality was observed in the control during the larvicidal bioassay.									

*Mean mortality (%) of five replicates ± Standard deviation of five replicates.

Table 4 : Larvicidal efficacy of aqueous, ethanolic, and petroleum ether against the third instar of dengue vector.

Mosquito species	Plant Part used	Solvents	% Mortality	LC_{50}	LC_{90}	95% confidence interval Lower bound	95% confidence interval Upper bound	Regression equation	R^2	(P<0.05)
Aedes aegypti	Leaf	Aqueous	100%	133.25	311.68	0.0866	6.8505	Y = 3.4646x – 2.3697	0.78	0.04
		Control	0.0							
		Ethanol	100%	102.32	239.88	1.3549	5.6878	Y = 3.5214x – 2.103	0.89	0.01
		Control	0.0							
		Petroleum ether	100%	123.02	281.83	0.6031	6.4491	Y = 3.5261x - 3.3778	0.83	0.03
		Control	0.0							
	Seed	Aqueous	100%	154.88	393.07	0.2503	6.7483	Y = 3.4993x – 2.6927	0.80	0.04
		Control	0.0							
		Ethanol	100%	120.22	281.83	0.5867	6.3680	Y = 3.4774x – 2.2416	0.83	0.03
		Control	0.0							
		Petroleum ether	100%	104.71	239.88	1.3699	5.7627	Y = 3.5664x – 2.2288	0.89	0.01
		Control	0.0							

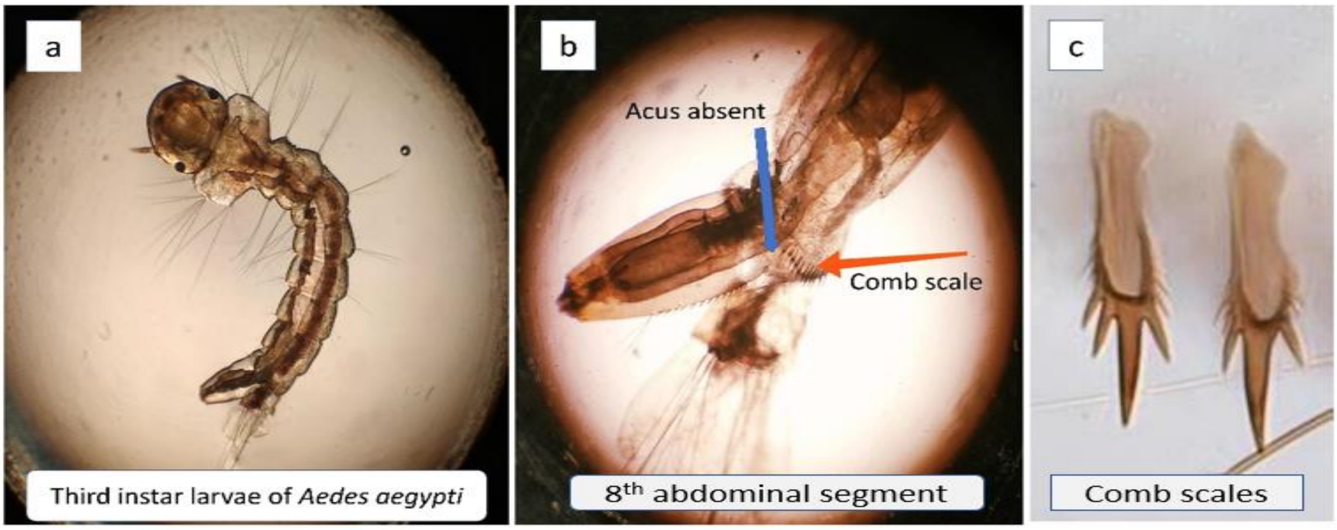

Fig. 2 : Microscopy identification of *Aedes aegypti* larvae. a) Third instar larvae of *Aedes aegypti*. b) Single row of comb scales indicated by the orange arrow and Acus absent. c) Comb scales with a long medial spine and short subapical spines (200X magnification).

Discussion

Plant extracts have been shown to have a variety of biological effects on mosquitoes, including repellent, larvicidal, ovicidal deterrents, insect development regulators, growth inhibitors, chemosterilants, etc. This could be the result of a complex blend of phytochemicals that are found in plants and may be working together to produce such a result. Due to their complex synergistic interactions, plant-derived insecticides rarely cause the insect to acquire resistance to them (Maurya et al., 2012). Anthraquinone, alkaloids, flavonoids, glycosides, saponins, phenols, tannins, and other compounds were noticed in the preliminary phytochemical screening of *Argemone mexicana* leaf and seed in the current study. There is evidence that the components of plants, including flavonoids, alkaloids, tannins, and saponins, are what give them their poisonous and insecticidal qualities. (Akinyemi *et al*., 2005, Azmathullah, 2011).

In the present study, In 24 h post-exposure, the aqueous, ethanolic, and petroleum ether extracts of the leaf with LC_{50} and LC_{90} values of 133.25, 102.32, 123.02 ppm, and 311.68, 239.88, 281.83 ppm, and the aqueous, ethanolic and petroleum ether extracts of seed with LC_{50} and LC_{90} values of 154.88, 120.22, 104.71 ppm and 393.07, 281.83, 239.88 ppm, respectively against the 3rd instar of the dengue vector, *Aedes aegypti.*

According to previous research on the leaf, seed, root, and flower crude extracts have been tested in the past against various mosquito species. Acetone extract of the seed and ethanolic extract of the flower of *Argemone mexicana* both showed larvicidal potential against *Cx. quinqusfasciatus*, with LC_{50} and LC_{90} values after 24 hours of exposure of 18.61 and 39.86 ppm, respectively (Sakthivadivel and Thilagavathy, 2003), and Granados-Echegoyen *et al*., (2018) reported that growth-inhibiting activity of flower extracts of this plant on second instar larvae of *Aedes aegypti*. According to Warikoo and Kumar (2013) leaves, stem, and roots extract of this plant exert a negative impact on the morphology and behavior of the dengue vector, *Aedes aegypti* larvae, and they have oviposition inhibitory and ovicidal potential (Warikoo and Kumar, 2014). *Argemone mexicana* seed extract in chloroform showed larvicidal efficacy against *Cx. pipiens* (Zeinab and Abou-Elnaga, 2015) and the larvicidal effects of an acetone fraction of *Argemone mexicana* leaves were effective against the 2nd and 4th instar of *Cx. quinquefasciatus*, according to Elawad et al. (2014). Ali *et al*., (2017) reported the effectiveness of a methanolic extract of this plant's seeds with LC_{50} value of 282.73 ppm and roots with LC_{50} value of 19.49 ppm against *Cx. quinquefasciatus*.

Numerous phytochemicals produced from diverse plants have been shown by researchers to be useful insecticides against different mosquito species and to have medical significance. The ethanolic extract made from *Vitex ovata* showed larvicidal ability against *Aedes aegypti* (Aziz *et al*., 2021). Nagpal *et al*., (2018) report significant mortality was shown by leaf and seed of *Ricinus communis* extract in methanol against *Ae. aegypti* larvae, with LC_{50} values of 9.37, 31.1 ppm and LC_{90} values of 15.52, 45.24 ppm, respectively. Furthermore, the methanolic extract of the flower of *Clitoria ternatea* has been shown to have larvicidal activity against *Aedes aegypti* with LC_{50} value of 1056 ppm and LC_{90} values of 2491 ppm (Ravindran *et al*., 2020). According to Chandrasekaran et al. (2019), *Vitex trifolia* oils had larvicidal activity against *Aedes aegypti* and *C. quinquefasciatus* with LC50 values of 57 and 77 ppm and LC90 values of 55 and 78 ppm, respectively.

Conclusion

The results of our study showed that the leaf and seed extract of *Argemone mexicana* can be used to create larvicides that are safe for the environment and a promising alternative for *Aedes aegypti* eradication. The great variety of aromatic plants in India's flora offers the potential for the formulation of natural insecticides for the control of mosquitoes and other pests. These findings may encourage researchers to look for novel, naturally occurring active chemicals that can replace synthetic insecticides derived from other medicinal plants. To isolate, and purify the active components that kill mosquitoes, more research is also necessary.

Conflict of interest

The authors claim that there was no conflict of interest during the course of the research.

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