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Biological control of the *Aedes Aegypti* larvae by the aqueous extracts of *Argemone mexicana*

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Abstract
Mosquito-borne diseases are a significant public health concern in India, where 404 species and subspecies of mosquitoes belonging to 50 genera and 2 subfamilies have been identified to spread various diseases. Malaria was previously a major concern, however, recently, malaria cases have been decreasing, while Japanese encephalitis (JE) and dengue cases have been rapidly increasing, with a high case-fatality rate. *Aedes aegypti* mosquitoes spread Dengue viruses. This study aimed to evaluate the efficacy of the aqueous extracts of *Argemone mexicana* leaves, flowers, and roots against the fourth instar larvae of *Ae. aegypti*. The results showed that *A. mexicana* leaf extracts performed better than flower and root extracts. Concentration-dependent efficacy was observed, with leaf extracts exhibiting 69.57 ± 2.83% mortality at 10% concentration, while flower and root extracts showed 60.87 ± 1.76% and 56.52 ± 3.46% mortality, respectively. At 1.25% concentration, all the extracts were not effective, with leaf, flower, and root extracts showing 21.74 ± 2.67%, 17.39 ± 2.83%, and 13.04 ± 2.11% mortality, respectively. At 5% concentration, flower and root extracts delivered less than 50% mortality, while leaf extracts showed 60.87 ± 2.67% mortality. The study clearly showed that the aqueous extracts of *A. mexicana* are effective against the fourth instar larvae of *Ae. aegypti*.

Keywords: biopesticides, mosquito larvicides, biological control

Introduction

Mosquito-borne diseases are a significant public health concern not only in India but also worldwide. In India, 404 species and subspecies of mosquitoes belong to 50 genera, and 2 subfamilies were identified (Tyagi *et al*., 2015) [2]. Mosquitoes are vectors for many parasites of public health importance. Earlier malaria was a major concern. Malaria is a significant public health issue in India, with 1.6 million cases and 1100 deaths reported in 2009. Experts suggest that this is underestimated and the actual number of cases could be 9 to 50 times higher (Das *et al*., 2012) [3]. However, recently, malaria cases are decreasing, while Japanese encephalitis (JE) and dengue cases are rapidly increasing with a high case-fatality rate. To control these diseases, robust surveillance and integrated vector management strategies are necessary, based on sound epidemiological data (Dev *et al*., 2015) [1].

Using synthetic pesticides to control mosquito larvae is not advisable due to environmental and health reasons. Plant extracts have been proven to be used as an alternative to insecticides. The botanical groups with the most potential are Meliaceae, Rutaceae, Asteraceae, Annonaceae, Labiatae, Aristolochiaceae, and Malvaceae (Regnault-Roger, 1997) [7]. Some of these groups are characterized as aromatic plants. In 1933, Campbell *et al*., reported one of the earliest uses of plant extracts against mosquito larvae. They found that plant alkaloids like nicotine, anabasine, methylanabasine, and lupinine, extracted from the Russian weed *Anabasis aphylla*, killed larvae of *Culex pipiens, Culex territans,* and *Culex quinquefasciatus.* Haller (1940) [5] later discovered that extracts from Amur cork-tree fruit *(Phellodendron amurense)* also had a larvicidal effect on mosquitoes. Wilcoxon *et al*. (1940) [8] identified filicin, a toxic constituent of extracts from the male fern *Aspidium filixmas*, a phloroglucinol propyl ketone toxic to *C. quinquefasciatus.*

Hartzell and Wilcoxon (1941) [6] evaluated extracts from 150 plant species for their toxicities to mosquitoes and discovered several that were very effective. Ten plant species with carminative properties, including *Amomum krevanh, Carthamus tinctorius, Coriandrum sativum, Eugenia caryophyllata, Illicium vernum, Kaempferia galangal, Murraya paniculata, Myristica fragrans, Ocimum gratissimum*, and *Spilanthes acmella*, were screened for their larvicidal potential against *C. quinquefasciatus*.

Out of the 11 plant-derived oils tested, *Daucus carota* L. oil was highly toxic to two strains (v) of *Aedes aegypti* mosquitoes. The Liverpool strain is considered a useful tool for identifying potent natural insecticides, but caution should be taken when extrapolating the efficacy of these oils to other mosquito strains. The potential use of these oils in integrated pest management strategies is also discussed (Tare, *et al*. 2004) [10]. *Curcuma zedoaria* rhizome volatile oil was found to contain β-tumerone, 1,8-cineole, and 7-zingiberene as major constituents. Zedoary oil demonstrated significant potential against the fourth instar larvae of A. aegypti (LC 50 and LC90 values were 33.45 and 83.39 ppm, respectively) (Champakaew, *et al*. 2007) [9].

Argemone mexicana has been found to possess antimicrobial, wound healing, larvicidal and chemosterilant, nematicidal and allelopathic potential, antimalarial, antibacterial and antifungal, molluscicidal, anticancer, hepatoprotective, anti-HIV, and neuropharmacological activity. Chemical investigations of the plant have revealed the presence of alkaloids, amino acids, phenolics, and fatty acids. The major alkaloid found in *A. mexicana* is sanguinarine, which has been reported to possess a wide range of biological activities including antimicrobial, anti-

inflammatory, and anticancer properties (Sharanappa *et al*., 2014) [11].
In the present study larvicidal efficacy of the aqueous extracts of *A. Mexicana* plant leaves, flowers, and roots were investigated against the 4th instar larva of *Ae. aegypti.*

Materials and methods
In this study, the early instar larvae of *Ae. aegypti* were collected from Sangareddy town. They were identified by Prof. M. Madhavi, Department of Zoology, Osmania University, Hyderabad, Telangana, India. The mosquito larvae were reared on dog biscuits and yeast powder in glass troughs. The fourth instars were used for the larvicidal bioassay.
The leaves, flowers and roots of the test plant *A. Mexicana* were collected from the Zaheerabad region, Telangana State. The collected plant materials were washed with distilled water, dried for two weeks, powdered using an electric grinder and stored in air-tight containers.
50 grams of the prepared powders were added to 250 mL of distilled water separately and boiled at 40^0C for 30 minutes. Then the broth was filtered and the collected aqueous solution was used to prepare 1.25%, 2.5%, 5% and 10% test solutions.
The larvicidal experiments were conducted in the Department of Zoology, tara Government Degree and PG College, Sangareddy. Clean plastic bottles were used for the larvicidal bioassays. Three replicates were made for each concentration, along with three control replicates in each experiment. 20 larvae of *Ae. aegypti* were introduced into each replicate and the number of dead larvae was observed after 12, 24, 36, and 48 hours of exposure. Abbott's formula (1925) was used to calculate corrected mortality percentages.

Results & discussion
In this study, the obtained results of the larvicidal bioassays are given in Table No. 1 and Figure No.1. *A. Mexicana* leaf extracts performed better than flower and root extracts. Concentration dependant efficacy was observed. At 10% concentration, leaf extracts exhibited 69.57 ± 2.83% Mortality, whereas 60.87 ± 1.76% and 56.52 ± 3.46% larval mortalities were observed with A. Mexicana flower and root extracts, respectively. At 1.25% concentration, all the extracts were proved not to be effective with 21.74 ± 2.67%, 17.39 ± 2.83, and 13.04 ± 2.11% mortalities with leaf, flower, and root extracts, respectively. At 5% concentration, flower and root extracts delivered less than 50% mortality. However, leaf extracts showed 60.87 ± 2.67% mortality at the same concentration.

Table 1: Larvicidal efficacy of *A. Mexicana* plant extracts against the 4th instar larva of *Ae. Aegypti*

Conc.	Leaf extracts	Flower extracts	Root extracts
0	0 ± 1.76	0 ± 1.76	0 ± 1.76
1.25%	21.74 ± 2.67	17.39 ± 2.83	13.04 ± 2.11
2.50%	30.43 ± 4.47	26.09 ± 2.40	21.74 ± 3.33
5%	60.87 ± 2.67	47.83 ± 2.91	47.83 ± 2.91
10%	69.57 ± 2.83	60.87 ± 1.76	56.52 ± 3.46

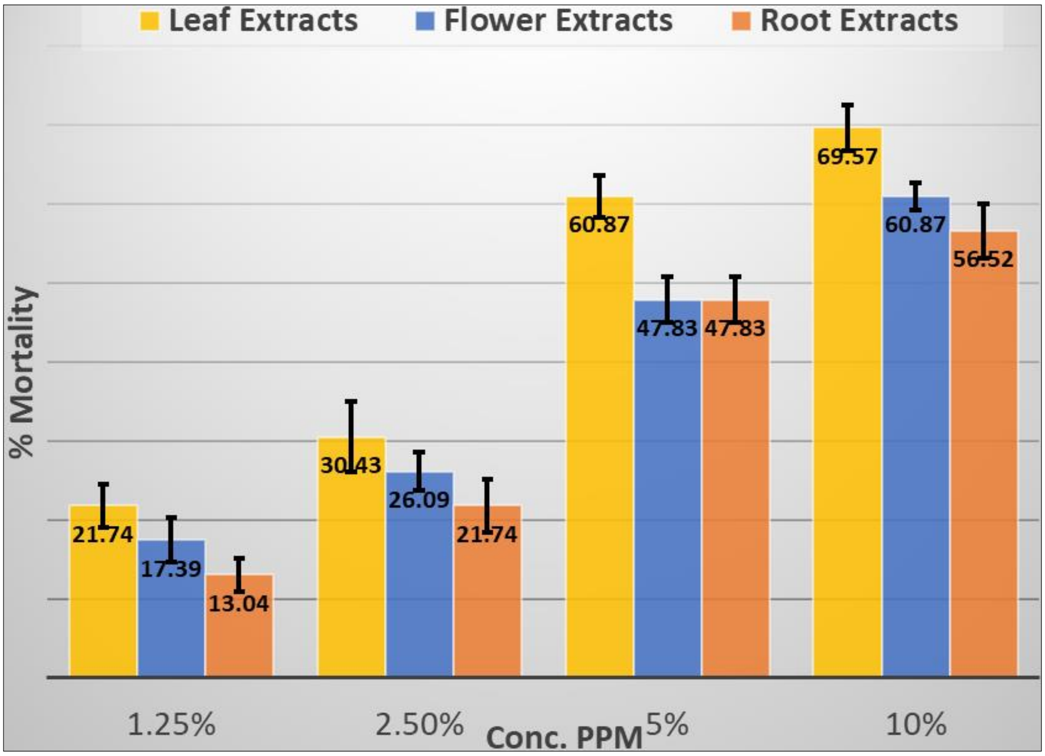

Fig 1: Larvicidal efficacy of *A. Mexicana* plant extracts against the 4th instar larva of *Ae. aegypti*

The study clearly showed that the aqueous extracts of *A. mexicana* are effective against the 4th instar larvae of *Ae. aegypti*. Same resukts were obtaine din another study where, Elawad *et al*. (2014) [12] found that acetone extracts of *A. mexicana* leaere effective as larvicides against 2nd and 4th instar larvae of *Culex quinquefasciatus* and *Anopheles arabiensis* mosquitoes. Sharanappa *et al*. (2014) [11] reported that *A. mexicana* plant extracts possess antimicrobial, wound healing, pesticidal and chemosterilant, nematicidal and allelopathic potential, antimalarial, antibacterial and antifungal, molluscicidal, anticancer, hepatoprotective activities Chemical investigations of the plant have revealed the presence of alkaloids, amino acids, phenolics, and fatty acids. The major alkaloid found in *A. mexicana* is sanguinarine, which has been reported to possess a wide range of biological activities including antimicrobial, anti-inflammatory, and anticancer properties.

Conclusion

In the present study, the leaf extracts of *A. mexicana* showed efficient larvicidal properties against the 4th instar larvae of *Ae. aegypti.* Flower and leaf extracts of *A. mexicana* also showed better larvicidal properties at 10% concentration. *A. mexicana* leaf extracts were already proven to be effective against the larvae of *Cx. quinquefasciatus* and *An. Arabiensis.* Earlier studies reported the presence of alkaloids such as sanguinarine in the extracts of *A. mexicana.* The same phytochemicals may be responsible for the larvicidal efficacy of *A. mexicana* against the 4th instar larvae of *Ae. aegypti.*

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