Impact of the Argemone mexicana Stem Extracts on the Reproductive Fitness and Behavior of Adult Dengue Vector, Aedes aegypti L. (Diptera: Culicidae)

Authors: Warikoo, Radhika, and Kumar, Sarita

Source: International Journal of Insect Science, 6(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/IJIS.S19006

Libertas Academica
FREEDOM TO RESEARCH

Open Access: Full open access to this and thousands of other papers at http://www.la-press.com.

International Journal of Insect Science

Impact of the *Argemone mexicana* Stem Extracts on the Reproductive Fitness and Behavior of Adult Dengue Vector, *Aedes aegypti* L. (Diptera: Culicidae)

Radhika Warikoo and Sarita Kumar

Department of Zoology, Acharya Narendra Dev College, University of Delhi, New Delhi, India.

ABSTRACT: Present investigations evaluated the impact of *Argemone mexicana* stem extracts on the reproductive fitness of dengue vector, *Aedes aegypti*, assessed in terms of oviposition deterrent and ovicidal potential. The oviposition deterrent studies of the extracts, prepared using petroleum ether, hexane, benzene, acetone, and ethanol as the solvents, revealed the maximum deterrence potency of the petroleum ether extracts with a significant 15.6% ED at 60 ppm rising by 83.8% at 1000 ppm to 99.4% effective deterrence (ED). Other stem extracts were found to be ineffective at 60 ppm, though resulted in 85.3–96.2% ED and diminished fecundity in *A. aegypti* at 1000 ppm. Further, *A. mexicana* stem extracts exhibited moderate ovicidal potential against *A. aegypti* eggs causing only 42.65–67.85% egg mortality at 1000 ppm, the lowest hatch of 32.15% caused by the benzene extract. Other stem extracts also failed to express effective ovicidal potency with the percent egg hatch ranging between 96.6 and 99.0 at 400 ppm, and 78.8 and 99.0 at 600 ppm. Our results suggest the significant but variable efficacy of *A. mexicana* stem extracts causing reproductive disadvantage in *A. aegypti*.

KEYWORDS: *Argemone*, oviposition deterrence, ovicidal, egg hatchability, effective deterrence

CITATION: Warikoo and Kumar. Impact of the *Argemone mexicana* Stem Extracts on the Reproductive Fitness and Behavior of Adult Dengue Vector, *Aedes aegypti* L. (Diptera: Culicidae). *International Journal of Insect Science* 2014:6 71–78 doi:10.4137/IJIS.S19006.

RECEIVED: July 28, 2014. **RESUBMITTED:** September 9, 2014. **ACCEPTED FOR PUBLICATION:** September 14, 2014.

ACADEMIC EDITOR: Helen Hull-Sanders, Editor in Chief

TYPE: Original Research

FUNDING: RW received a Senior Research Fellowship from the University Grants Commission, New Delhi. The authors confirm that the funder had no influence over the study design, content of the article, or selection of this journal.

COMPETING INTERESTS: Authors disclose no potential conflicts of interest.

CORRESPONDENCE: sarita.sanjay90@gmail.com; saritakumar@andc.du.ac.in

Paper subject to independent expert blind peer review by minimum of two reviewers. All editorial decisions made by independent academic editor. Prior to publication all authors have given signed confirmation of agreement to article publication and compliance with all applicable ethical and legal requirements, including the accuracy of author and contributor information, disclosure of competing interests and funding sources, compliance with ethical requirements relating to human and animal study participants, and compliance with any copyright requirements of third parties.

Introduction

Vector-borne diseases are a major source of infirmity worldwide. Mosquitoes constitute a major public health problem as vectors of severe human diseases, such as malaria, filariasis, yellow fever, dengue, and Japanese encephalitis, contributing significantly to an increasing number of fatalities in tropical and subtropical countries of the world.[1,2] In India, with an annual incidence of 700 million clinically manifested cases and a death toll of 1.1–2.7 million, dengue is considered as one of the most important diseases.[3] As till date there is neither any specific medication nor an effective vaccine available against dengue virus, the only way to control the disease is to control the mosquito vector, *Aedes aegypti*, agent of disease transmission.

During the past few decades, worldwide efforts have been made to control the mosquito population. The synthetic chemical insecticides have been used since long as the quickest and most sought-after approach to deal with the increasing and widely spreading mosquito population. However, the indiscriminate and persistent use of insecticides has produced serious repercussions, such as insect resistance to insecticides, mammalian toxicity, harm to non-target organisms, bioaccumulation, and environment damage.[4,5] These problems have directed the attention of researchers toward the immediate need for novel insecticides.[6]

Over the past few years, the botanical insecticides have caught researchers' attention as alternate mosquito control agents, being environmentally safe and non-hazardous to non-target organisms.

Several phytochemicals isolated from various botanical sources have been reported to have detrimental effects on

different species of mosquitoes, inducing multiple effects such as larvicidal, fecundity suppression, ovicidal activity, oviposition deterrence, growth inhibition, etc.[7–12]

Weeds, the so-called nuisance plants, belong to one such group, which has attracted researchers' attention as eco-friendly substitutes to chemical insecticides for the mosquito management.[9,10] The larvicidal potential of the extracts prepared from the different parts of Mexican prickly poppy, *Argemone mexicana*, has been reported against the early fourth instars of *A. aegypti*.[10] These extracts have also been shown to induce behavioral and morphological modifications in the larvae of *A. aegypti*. Researchers have also reported the larvicidal and chemosterilant activity of phytochemicals derived from *A. mexicana* seeds against *A. aegypti*.[13]

The literature available, however, reveals that the impact of this plant on the reproductive fitness and behavior of mosquito vectors, in terms of ovicidal and oviposition deterrent potential, has not been explored extensively. Hence, the present study was undertaken to assess the ovicidal efficacy and oviposition deterrence potential of the extracts prepared from the stems of *A. mexicana* against *A. aegypti*. These investigations may provide significant information in formulating a new agent modifying the reproductive fitness of the mosquito adversely and formulating an appropriate mosquito management strategy against the dengue vector.

Materials and Methods

Mosquito rearing. Different stages of the dengue fever mosquito, *A. aegypti*, were collected from seven different locations across Delhi, India, and the surrounding areas (Fig. 1). The colony was maintained in a rearing laboratory at 28 ± 1°C, 80 ± 5% Relative Humidity (RH), and 14:10 Light/Dark (L/D) photoperiod.[14] The adults were reared in screened cloth cages (45 × 40 × 40 cm) with a moist cotton pad placed on the top of cage to provide water for them. Water-soaked split raisins were placed in the cage as a source of food primarily for the male mosquitoes. Intermittent blood meals were provided to the females, for the maturation of egg follicles, by keeping restrained albino rats in the cages. The eggs were collected in an enamel bowl half-filled with dechlorinated tap water and lined with Whatman filter paper on all the sides. The eggs were transferred to the trays (12″ × 16″) filled with dechlorinated water and allowed to hatch. Hatched larvae were fed upon a mixture of finely ground dog biscuits (procured from M/s Lal Pet Products, India) and yeast (procured from M/s Solar Sales, India) in the ratio of 3:1 by weight with utmost care in order to prevent formation of any scum on the surface of water. The pupae formed were collected in an enamel bowl and transferred to the cages for adult emergence.

Plant collection. The *A. mexicana* plant was collected from New Delhi, India and surrounding regions, and brought

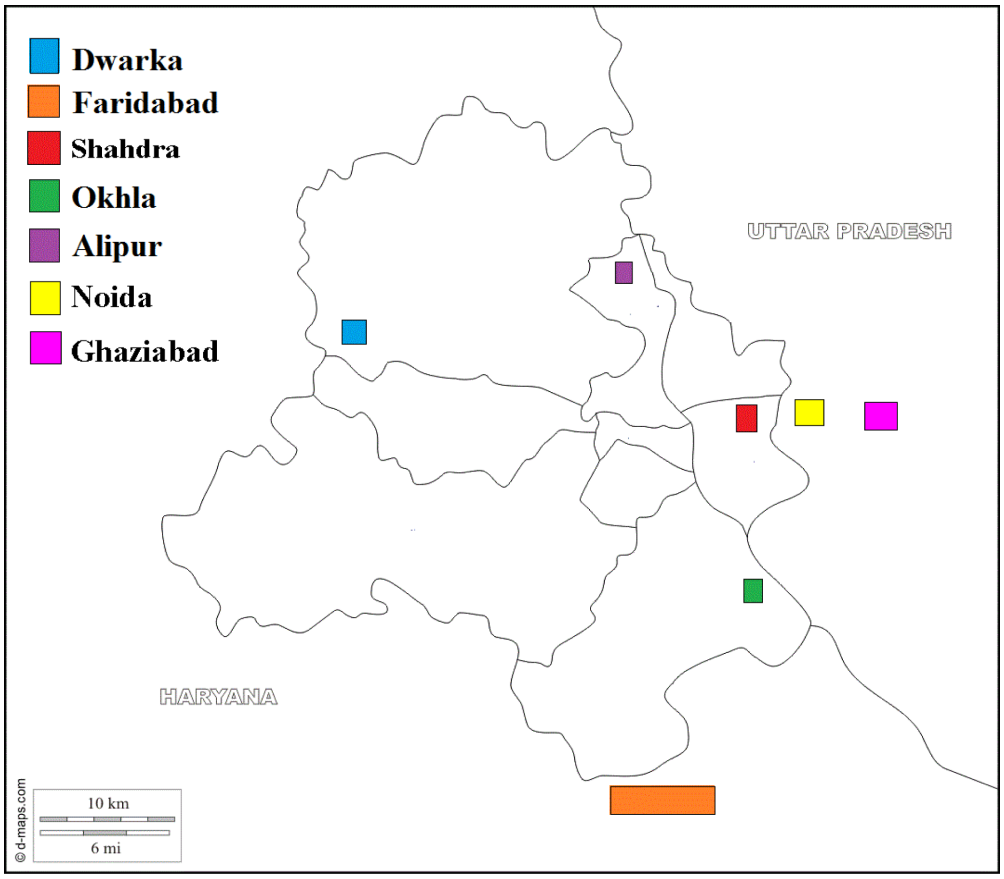

Figure 1. Areas selected in Delhi and NCR for the collection of mosquito larvae. http://d-maps.com/carte.php?num_car=16531&lang=en.

to the laboratory. The stems were carefully cut from the plant and thoroughly washed with dechlorinated tap water to remove traces of dust. The stems were critically scrutinized under microscope, and disease-free, healthy stems were separated. The selected stems were kept under shade at room temperature of 27 ± 2°C for about 20 days till they dried completely. The dried stems were crushed into powdered form and sieved through a nylon strainer to obtain fine powder.

Preparation of the extract. The powdered stem material was weighed and 200 g of the material was extracted separately with 1000 mL of five different solvents of varying polarities, viz, petroleum ether, hexane, benzene, acetone, and ethanol, using a Soxhlet extraction apparatus. The extraction continued for 3 days, 8 hours per day, at a temperature not beyond the boiling point of the respective solvent. The five crude extracts, thus formed, were concentrated using a rotary vacuum evaporator at 45°C under low pressure and stored in a refrigerator at 4°C as the stock solution of 1000 ppm for investigating their oviposition deterrent and ovicidal potential against *A. aegypti*. The dilutions of each extract were prepared using ethanol as the solvent.

Oviposition deterrence studies. The oviposition deterrence studies were performed against *A. aegypti* under controlled temperature, humidity, and photoperiod conditions using multiple concentration tests of *A. mexicana* stem extract prepared in a particular solvent. The experiment was conducted on 25 blood-fed females of *A. aegypti* released in the cage along with an equal number of males. For each extract, a total of 10 small bowls lined with Whatman filter paper were prepared for oviposition. Nine of these bowls were filled with 99 mL of dechlorinated distilled water and 1 mL of a particular concentration of the selected stem extract. The tenth oviposition bowl served as the control and contained 99 mL of dechlorinated distilled water and 1 mL of ethanol. These oviposition bowls were placed in the cage at random places for egg laying. The position of each cup was changed each day to negate the position effect, if any. The bowls were kept till no further egg laying was noticed, for at least 48 hours. The eggs laid into each cup were collected every day separately and scored using a dissecting microscope at the magnification of ×40. Identical protocol was followed for investigation on the oviposition deterrent efficacy of other stem extracts against *A. aegypti*. Three replicates of each concentration of each extract were carried out for evaluating the deterrent efficacy of extracts.

The impact of the stem extracts of *A. mexicana* on the egg-laying capacity, and thus, the reproductive fitness of female *A. aegypti* was assessed by calculating the fecundity rate of females at each concentration of every extract using the following formula:

$$\text{Fecundity Rate} = \frac{\text{Total number of eggs laid in a gonotrophic cycle}}{\text{Total numbers of females}}$$

The percent effective deterrence of each extract was evaluated by the following formula in order to evaluate their potential to reduce the reproductive fitness of females and deter them for egg laying.

$$\text{ED\%} = \frac{(\text{NC} - \text{NT})}{\text{NC}} \times 100$$

where ED = effective deterrence, NC = total number of eggs laid in control bowl, and NT = total number of eggs laid in an experimental bowl. Based on the above observations, the Oviposition Activity Index (OAI) of female *A. aegypti* was also calculated in each case using the following formula:

$$\text{OAI} = \frac{(\text{NT} - \text{NC})}{(\text{NT} + \text{NC})}$$

The results obtained were analyzed by one-way analysis of variance (ANOVA), and means were separated using Tukey's test for statistical significance considered for $P \leq 0.05$.

Ovicidal studies. The potential of all the five extracts prepared from the stems of *A. mexicana* was also estimated for their efficacy as ovicidal agents against *A. aegypti*. The investigations were performed at 28 ± 1°C on the freshly laid eggs of *A. aegypti* collected in oviposition bowls filled with dechlorinated distilled water and lined with Whatman filter paper. The eggs were counted using a dissecting microscope at the magnification of 40×.

A total of 250 freshly laid eggs of *A. aegypti* were counted and transferred to a 250 mL glass jar containing homogenous test solution of 1 mL of a particular concentration of the extract and 99 mL of distilled water. Likewise, nine glass jars were prepared, each containing 250 eggs and 1 mL of a particular concentration of the extract and 99 mL of distilled water. Control treatments of the eggs were carried out by replacing the extract with only ethanol. After the exposure period of 24 hours, the treated eggs from each jar were carefully transferred to another glass jar containing distilled water and were washed thoroughly to ensure the removal of any traces of the extract. Thereafter, the eggs were submerged in trays containing only dechlorinated water and were allowed to hatch. The hatched larvae were counted, and the percent hatch was calculated in each instance.

$$\text{Percent hatch} = \frac{\text{Number of hatched larvae}}{\text{Number of eggs exposed to an extract}}$$

The percent hatch in control treatment was compared with the percent hatch observed in each concentration of the stem extract to assess the ovicidal potential of the extract. Identical protocol was followed for all the stem extracts, and three replicates were carried out simultaneously for each concentration of the extract making a total of 750 eggs for

each test. The results obtained were analyzed by one-way ANOVA, and means were separated using Tukey's test for statistical significance considered for $P \leq 0.05$. After treatment, the eggs unable to hatch were scrutinized under light microscopy for any morphological alterations.

Results

The present investigation evidently indicates the potential of stem extracts of *A. mexicana* to deter the oviposition by *A. aegypti* and diminish their reproductive fitness (Tables 1–3 and Fig. 2). Conversely, these extracts were found only moderately effective as ovicidal agents as they could not establish significantly high ovicidal efficacy even at very high concentrations (Tables 4 and 5).

Our studies clearly concluded the increasing oviposition deterrence of the stem extracts of *A. mexicana* with their increasing concentration. The petroleum ether extract Petroleum ether Stem Extract (PSE) was found to be the most effective extract to deter the egg laying and cause reproductive disadvantage in female *A. aegypti*. The extract established a significant %ED of 15.6% ($P < 0.001$; $F = 121.28$) at as low as 60 ppm (Fig. 2) causing a total of 202 eggs laid in the PSE bowls as against 239 eggs laid in control bowl (Table 1). The percent effective deterrence of the extract rose by 83.8% at 1000 ppm causing significant reproductive disadvantage resulting in only two eggs being laid by female *A. aegypti* (Table 1). The efficacy of other extracts was found in the decreasing order of the moderately significant oviposition deterrence exhibited by the hexane extract followed by that shown by benzene and ethanol stem extracts. The hexane stem extract caused an appreciable increase in the %ED against *A. aegypti* ranging between 18.98 and 96.1% ($P < 0.001$; $F = 435.42$) with the concentration of extract increasing from 80 to 1000 ppm. Likewise, Benzene Stem Extract (BSE) resulted in a significant 83% increase in reproductive disadvantage of *A. aegypti* females with 13.2% ($P < 0.01$) ED at 80 ppm rising up to 96.2% ($P < 0.01$; $F = 397.93$) at 1000 ppm. A more drastic decrease in reproductive fitness was seen in Ethanol Stem Extract (ESE)-containing oviposition medium, which revealed an 88% decreased egg laying with increase in concentration from 80 to 1000 ppm ($P < 0.01$, $F = 291.03$). On the other hand, the Acetone Stem Extract (ASE) was found to be the least effective affecting reproductive fitness of *A. aegypti* being the only extract with a positive OAI of 0.01 at 80 ppm (Table 3).

The stem extracts of *A. mexicana*, however, could establish just moderately effective ovicidal potential against *A. aegypti* eggs failing to cause high levels of egg mortality even at 1000 ppm. The results of the ovicidal potential of different stem extracts of *A. mexicana* against *A. aegypti* eggs are presented in Tables 4 and 5. Among the five different stem extracts tested, the BSE caused the lowest percent egg hatch of 32.15% at 1000 ppm reducing by 21% at 800 ppm though still causing toxicity to 46.85% eggs ($P < 0.05$; $F = 51.76$).

Table 1. Oviposition deterrent activity of the stem extracts of *Argemone mexicana* against female *Aedes aegypti*.

SOLVENT	CONCENTRATION (ppm)									
	CONTROL	40	60	80	100	200	400	600	800	1000
Petroleum ether	239.0 ± 13.4*ab (9.56)	254.0 ± 4.2a (10.16)	201.7 ± 9.7bc (8.07)	176.7 ± 14.5cd (7.07)	146.0 ± 10.7d (5.84)	105.6 ± 3.7e (4.22)	86.3 ± 2.5e (3.45)	66.0 ± 2.3e (2.64)	18.0 ± 2.2f (0.72)	1.33 ± 0.2f (0.05)
Hexane	335.0 ± 12.2a (13.4)	356.7 ± 5.8a (14.27)	340.9 ± 1.0a (13.64)	271.4 ± 2.4b (10.86)	246.9 ± 1.6b (9.88)	211.6 ± 6.8c (8.46)	139.0 ± 10.4d (5.56)	62.3 ± 4.7e (2.49)	43.6 ± 4.3ef (1.74)	13.0 ± 1.2f (0.52)
Benzene	344.6 ± 18.2a (13.78)	371.9 ± 8.0a (14.88)	353.7 ± 12.2a (14.15)	299.1 ± 1.8b (11.96)	276.3 ± 2.5b (11.05)	109.3 ± 5.1c (4.37)	68.0 ± 2.2d (2.72)	31.3 ± 1.3de (1.25)	19.3 ± 0.8e (0.77)	13.0 ± 0.3e (0.52)
Acetone	360.3 ± 14.0ab (14.41)	382.9 ± 9.3a (15.32)	375.1 ± 3.1ab (15.00)	369.8 ± 2.2ab (14.79)	351.2 ± 7.5ab (14.05)	328.0 ± 16.7b (13.12)	221.0 ± 12.5c (8.84)	142.6 ± 11.8d (5.71)	106.0 ± 5.8de (4.24)	53.0 ± 9.2e (2.12)
Ethanol	302.3 ± 12.4abc (12.09)	331.2 ± 2.0ac (13.25)	313.9 ± 2.4abc (12.57)	283.5 ± 7.6cd (11.34)	275.1 ± 1.6cd (11.00)	252.0 ± 14.7d (10.08)	167.0 ± 3.2e (6.68)	103.3 ± 2.7f (4.13)	51.3 ± 1.0g (2.05)	18.0 ± 1.6g (0.72)

Notes: *The figures represent mean ± S.E.M. Figures in parentheses indicate the fecundity rate of female adults at that particular concentration. Figures in each row followed by different letters are significantly different ($P < 0.05$, one way ANOVA followed by Tukey's all pair wise multiple comparison test).

Table 2. Statistical analysis (ANOVA) for Oviposition deterrence activity of the stem extracts of *Argemone mexicana* against female *Aedes aegypti.*

S.NO.	EXTRACT	SUM OF SQUARES (SS)	MEAN OF SQUARES (MS)	*F* VALUE	PROBABILITY (*P* VALUE)
1.	PSE	211598.746	23510.972	121.289	<0.001
2.	HSE	456406.392	50711.821	435.422	<0.001
3.	BSE	630505.215	70056.135	397.929	<0.001
4.	ESE	362265.483	40251.720	291.034	<0.001
5.	ASE	433411.647	48156.850	153.486	<0.001

Notes: *Df (numerator) = 8. Df (denominator) = 66.

Other stem extracts also failed to express effective ovicidal potency resulting in 98–99% egg hatch till 400 ppm and only 51–58% at 1000 ppm (Table 4).

Discussion

The rapid increase in global population of mosquitoes has caused alarming rise in the mosquito-borne diseases, which have become a source of major annoyance and fatalities. The synthetic chemical insecticides have been used since long as the quickest and most sought-after approach to deal with the increasing and widely spreading mosquito population. However, the indiscriminate and persistent use of insecticides has elicited detrimental effects, such as development of resistance amongst the target mosquito population, and everlasting toxicity to non-target organisms, environment, and humans. To address and overcome these issues, botanical insecticides are now being used widely as mosquito control agents, being environmentally safe and non-hazardous to non-target organisms. Researchers have reported use of botanicals as larvicides, ovicides, growth regulators, and behavioral modifiers against mosquitoes.[4,9,10,12] Despite various efforts, the mosquito population is on continual rise leading to increasing incidences of mosquito-borne diseases. *A. aegypti*, a dengue vector, has become the major nuisance in tropical and subtropical countries.

Scientists have reported that oviposition is one of the most critical events in the life cycle of mosquitoes that chiefly determines several life parameters, such as population densities, species propagation, and dispersal of species in different geographical zones.[15] In some mosquito species, female choice in oviposition site is adaptive.[16] Thus, selection of the appropriate egg-laying site can dramatically increase the survival, causing increased egg hatch and efficient development of their offspring. Keeping in view that assessment of the oviposition decisions of mosquitoes may provide additional information on the factors influencing reproductive fitness and regulation of the *Aedes* population, further assisting in predicting the response of mosquito population to various control measures, the impact of the extracts prepared from the weed *A. mexicana* stems was evaluated against the reproductive fitness of the females of *A. aegypti*.

Our study demonstrated the significant oviposition deterrent potential of the five different stem extracts of *A. mexicana* against gravid females of *A. aegypti* leading to appreciable reproductive disadvantage. With the percent effective oviposition deterrence ranging between 85 and 99% at 1000 ppm, the PSE of *A. mexicana* was found to be the most efficient deterrent agent against *A. aegypti* while the ASE showed the least effective oviposition deterrent potential. These results are in conformity with those of Warikoo and Kumar[4] who reported the 100% oviposition deterrence potential of 1000 ppm petroleum ether root extract of *A. mexicana* against an Indian strain of *A. aegypti*. Similarly, Kumar et al[12] reported the appreciable efficacy of 1000 ppm diethyl ether leaf extract of *Parthenium hysterophorus* causing the significantly diminished fecundity in *A. aegypti* with

Table 3. Oviposition activity index of the stem extracts of *Argemone mexicana* against females *Aedes aegypti.*

EXTRACT	OVIPOSITION ACTIVITY INDEX								
	40*	60	80	100	200	400	600	800	1000
PSE	(+) 0.03	(–) 0.08	(–) 0.15	(–) 0.24	(–) 0.39	(–) 0.47	(–) 0.57	(–) 0.86	(–) 0.99
HSE	(+) 0.03	(+) 0.008	(–) 0.10	(–) 0.15	(–) 0.22	(–) 0.41	(–) 0.69	(–) 0.77	(–) 0.90
BSE	(+) 0.04	(+) 0.01	(–) 0.07	(–) 0.11	(–) 0.52	(–) 0.67	(–) 0.83	(–) 0.89	(–) 0.93
ASE	(+) 0.03	(+) 0.02	(+) 0.01	(–) 0.01	(–) 0.05	(–) 0.24	(–) 0.43	(–) 0.55	(–) 0.74
ESE	(+) 0.09	(+) 0.02	(–) 0.03	(–) 0.05	(–) 0.09	(–) 0.29	(–) 0.49	(–) 0.71	(–) 0.89

Notes: Positive and negative signs in parentheses indicate decreased and increased oviposition deterrence, respectively. *Concentration of the extracts in ppm.

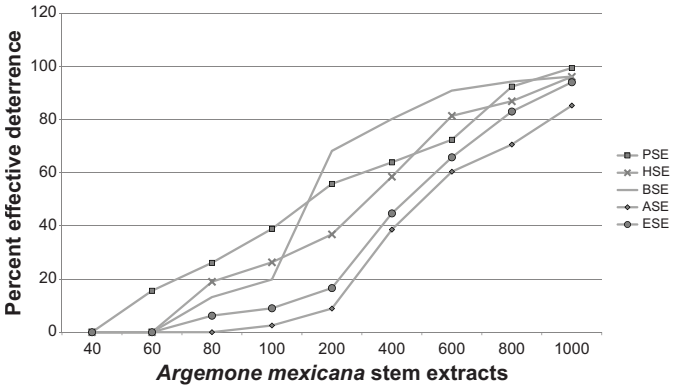

Figure 2. Percent effective deterrence of various stem extracts of *Argemone mexicana* against female *Aedes aegypti*.

99.7% effective repellency and considerably high deterrence potential of petroleum ether leaf extracts resulting in 94% effective repellency at 1000 ppm. The oviposition deterrence effects of ethanolic leaf extract of *Cassia obtusifolia* was studied by Rajkumar and Jebanesan[17] who revealed the 75.5% ED at 100 mg/L rising to 92.5% at 400 mg/L. Elango et al[18] had reported that 500 ppm of acetone, ethyl acetate, and methanol leaf extracts of some indigenous plants exhibited strong deterrent effect against *Anopheles subpictus*.

The investigation of the oviposition deterrent potential of *A. mexicana* stem extracts against *A. aegypti* expresses the OAI of extract, which signifies a global perspective of the relative preference of a substrate by gravid females.[19] Positive index values indicate more oviposition in the experimental bowls than in the control bowls proving the inefficacy of extracts affecting reproductive fitness of *A. aegypti*. On the contrary, more egg laying in the control medium as compared to the experimental media results in negative index values indicating the preference of distilled water for egg deposition over the water-extract mixture. Present investigation resulted in negative OAI values when mixture of petroleum ether stem extracts of *A. mexicana* and water was used as oviposition medium. It caused much reduced fecundity of *A. aegypti* affecting its reproductive fitness adversely. Chenniappan and Kadarkarai[20] evaluated the oviposition deterrent effects of ethanolic extract of *Andrographis paniculata* against females of *Anopheles stephensi*, and reported OAI values of (–)0.28, (–)0.45, (–)0.49, and (–)0.59 for extract concentrations of 29, 35, 41, and 46 ppm, respectively. Kweka et al[16] reported the significantly increased oviposition by gravid *Anopheles gambiae* in the control cups as compared to that in water containing essential oils from *Ocimum kilimandscharicum* and *Ocimum suave*. The OAI in water–oil mixture was found to be negative ranging from (–)0.19 to (–)1.0 indicating the potential of *Ocimum* sp. to deter oviposition in *A. gambiae* leading to reproductive disadvantage.

Present investigations have also tried to assess the ovicidal potential of *A. mexicana* stem extracts, which could then allow

Table 4. Ovicidal effects of the stem extracts of *Argemone mexicana* on freshly laid eggs of *Aedes aegypti*.

EXTRACT	CONCENTRATION (ppm)									
	CONTROL	40	60	80	100	200	400	600	800	1000
PSE	100 ± 0.0*a	99.95 ± 3.9a	99.91 ± 2.1a	99.77 ± 8.9a	99.74 ± 7.0a	98.72 ± 0.4a	98.65 ± 2.4a	84.89 ± 2.2ab	68.69 ± 12.6bc	53.97 ± 3.8c
HSE	100 ± 0.0a	99.79 ± 12.1a	99.49 ± 5.7a	99.41 ± 8.6a	99.18 ± 1.7a	99.13 ± 1.2a	99.08 ± 1.6a	98.99 ± 5.3a	80.55 ± 6.7ab	57.35 ± 0.2b
BSE	100 ± 0.0a	99.65 ± 4.7a	98.83 ± 0.3a	98.64 ± 1.0a	98.33 ± 0.4a	97.59 ± 3.5a	96.65 ± 1.3a	79.75 ± 6.1b	53.15 ± 5.2c	32.15 ± 2.9d
ASE	100 ± 0.0a	99.46 ± 2.6a	99.20 ± 4.3a	98.8 ± 6.4a	98.69 ± 1.9a	98.38 ± 11.4a	98.18 ± 3.2a	97.94 ± 7.1a	85.78 ± 2.2a	51.60 ± 10.6b
ESE	100 ± 0.0a	99.79 ± 3.5a	99.79 ± 2.8a	99.59 ± 5.0a	99.30 ± 4.0a	98.88 ± 7.6a	98.78 ± 0.3a	98.08 ± 0.5a	79.34 ± 1.8a	55.74 ± 7.0b

Notes: *Percent hatch ± S.E.M., calculated for 250 freshly laid eggs in each replicate. Figures in each row followed by different letters are significantly different ($P < 0.05$, one way ANOVA followed by Tukey's all pair wise multiple comparison test).

Table 5. Statistical analysis (ANOVA) for ovicidal activity of the stem extracts of *Argemone mexicana* against female *Aedes aegypti*.

S.NO.	EXTRACT	SUM OF SQUARES (SS)	MEAN OF SQUARES (MS)	*F* VALUE	PROBABILITY (*P* VALUE)
1.	PSE	7244.822	804.980	8.087	<0.001
2.	HSE	5255.913	583.990	5.850	<0.001
3.	BSE	15365.164	1707.240	51.763	<0.001
4.	ESE	5675.222	630.580	12.261	<0.001
5.	ASE	6257.490	695.277	6.137	<0.001

Notes: *Df (numerator) = 8. Df (denominator) = 741.

formulation of effective ovicides for reduction of *A. aegypti* population in future. Our results revealed that the stem extracts of *A. mexicana* could result in only moderate ovicidal effect against the freshly laid eggs of *A. aegypti*. Among the five extracts tested, the BSE resulted in the lowest percent egg hatch of 32.15% at 1000 ppm, whereas the percent hatch caused by other stem extracts ranged between 51 and 58% only. Similar studies by Kumar et al (2011) revealed the moderate ovicidal effect of 41% shown by petroleum ether leaf extract of *P. hysterophorus* against eggs of *A. aegypti* resulting in 59% hatch at 1000 ppm. On the contrary, Govindarajan[21] reported that at 200 ppm, the methanol leaf extract of *Coccinia indica* exerted zero egg hatchability in *A. aegypti* eggs. Krishnappa and Elumalai[6] also reported 100% mortality of *A. aegypti* eggs at 300 ppm extracts of *Basella rubra* and at 420 ppm extracts of *Cleome viscosa*.

Our results clearly indicate the significant and variable efficacy of various extracts prepared from the stems of *A. mexicana* leading to reproductive disadvantage in *A. aegypti*. The extracts have established their potential as oviposition deterrents though they resulted in only moderate ovicidal efficacy. However, the mechanism causing these variable impacts of stem extracts of *A. mexicana* is still unknown and needs to be investigated further. However, it seems probable that variety of types and levels of bioactive constituents in each extract may be responsible for their variable potential against reproductive behavior of *A. aegypti*. Further investigations are thus recommended to establish *A. mexicana* as eco-friendly botanical to be used in mosquito control program replacing the harmful conventional insecticides.

Acknowledgments

The authors are highly grateful to University Grants Commission, New Delhi, for providing a Senior Research Fellowship to carry out the present investigations. Thanks are extended to Dr Savithri Singh, Principal, Acharya Narendra Dev College, for providing infrastructure and research facilities.

Author Contributions

Conceived and designed the experiments: SK, RW. Analyzed the data: RW. Wrote the first draft of the manuscript: RW. Contributed to the writing of the manuscript: SK. Agree with manuscript results and conclusions: SK. Jointly developed the structure and arguments for the paper: SK, RW. Made critical revisions and approved final version: SK. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Morisson AC, Zielinsky-guitierrez E, Scott TW, Rosenberg R. Defining challenges and proposing solution for control of the virus vector *Aedes aegypti*. *PLoS Med*. 2008;5:362–366.
2. Benedict MQ, Levine RS, Hawley WA, Lounibos LP. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis*. 2007;7:76–85.
3. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicides. *Indian J Med Res*. 2012;135:581–598.
4. Warikoo R, Kumar S. Diminished reproductive fitness caused by root extracts of *Argemone mexicana* in an Indian strain of *Aedes aegypti* (Diptera: Culicidae). *J Entomol Zool*. 2014;2:11–17.
5. Ali NOM, El-Rabaa FMA. Larvicidal activity of some plant extracts to larvae of the mosquito *Culex quinquefasciatus* (Say 1823). *Eur Rev Med Pharmacol Sci*. 2010;14:925–933.
6. Krishnappa K, Elumalai K. Mosquitocidal properties of *Basella rubra* and *Cleome viscosa* against *Aedes aegypti* (Linn.) (Diptera: Culicidae). *Eur Rev Med Pharmacol Sci*. 2013;17:1273–1277.
7. Govindarajan M, Rajeswary M, Sivakumar R. Larvicidal and ovicidal efficacy of *Pithecellobium dulce* (Roxb.) Benth. (Fabaceae) against *Anopheles stephensi* Liston & *Aedes aegypti* Linn. (Diptera: Culicidae). *Indian J Med Res*. 2013;138:128–134.
8. Jeeshna MV, Mallikadevi T, Paulsamy S. Screening of weed plant species, *Croton bonplandianum* Baill. for larvicidal activity of *Aedes aegypti* L. *J Biopestic*. 2010;3:192–194.
9. Kumar S, Nair G, Singh AP, Batra S, Wahab N, Warikoo R. Evaluation of the larvicidal efficiency of stem, roots and leaves of the weed, *Parthenium hysterophorus* (Family: Asteraceae) against *Aedes aegypti* L. *Asian Pac J Trop Dis*. 2012;2:395–400.
10. Warikoo R, Kumar S. Impact of *Argemone mexicana* extracts on the cidal, morphological and behavioural response of dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol Res*. 2013;112:3477–3484.
11. Kumar S, Warikoo R, Wahab N. Larvicidal potential of ethanolic extracts of dried fruits of three species of peppercorns against different instars of an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Parasitol Res*. 2010;107:901–907.
12. Kumar S, Singh AP, Nair G, et al. Impact of *Parthenium hysterophorus* leaf extracts on the fecundity, fertility and behavioural response of *Aedes aegypti* L. *Parasitol Res*. 2011;108:853–859.
13. Sakthivadivel M, Thilagavathy D. Larvicidal and chemosterilant activity of the acetone fraction of petroleum ether extract from *Argemone mexicana* L. seed. *Bioresour Technol*. 2003;89:213–216.
14. Warikoo R, Wahab N, Kumar S. Larvicidal potential of commercially available pine (*Pinus longifolia*) and cinnamon (*Cinnamomum zeylanicum*) oils against an Indian strain of dengue fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae). *Acta Entomol Sin*. 2011;54:793–798.
15. Tawatsin A, Asavadachanukorn P, Thavara U, et al. Repellency of essential oils extracted from plants in Thailand against four mosquito vectors (Diptera: Culicidae) and oviposition deterrent effects against *Aedes aegypti* (Diptera: Culicidae). *Southeast Asian J Trop Med Public Health*. 2006;37:915–931.
16. Kweka EJ, Lyatuu LL, Mboya MA, Mwang'onde BJ, Mahande AM. Oviposition deterrence induced by *Ocimum kilimandscharicum* and *Ocimum suave* extracts to gravid *Anopheles gambiae* s.s. (Diptera: Culicidae) in laboratory. *J Glob Infect Dis*. 2010;2:242–245.

17. Rajkumar S, Jebanesan A. Larvicidal and oviposition activity of *Cassia obtusifolia* Linn (Family: Leguminosae) leaf extract against malarial vector, *Anopheles stephensi* Liston (Diptera: Culicidae). *Parasitol Res*. 2009;104:337–340.
18. Elango G, Bagavan A, Kamaraj C, Zahir AA, Rahuman AA. Oviposition-deterrent, ovicidal, and repellent activities of indigenous plant extracts against *Anopheles subpictus* Grassi (Diptera: Culicidae). *Parasitol Res*. 2009;105:1567–1576.
19. Waliwitiya R, Kennedy CJ, Lowenberger CA. Larvicidal and oviposition-altering activity of monoterpenoids, *trans*-anithole and rosemary oil to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). *Pest Manag Sci*. 2009;65:241–248.
20. Chenniappan K, Kadarkarai M. Oviposition deterrent, ovicidal and gravid mortality effects of ethanolic extract of *Andrographis paniculata* Nees against the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae). *Entomol Res*. 2008; 38:119–125.
21. Govindarajan M. Ovicidal and repellent properties of *Coccinia indica* Wight and Arn. (Family: Cucurbitaceae) against three important vector mosquitoes. *Eur Rev Med Pharmacol Sci*. 2011;15:1010–1019.