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Response of *Aedes aegypti* (L.) Larvae to Seed-Derived Essential Oil and Seed-Derived Extract of *Moringa oleifera* (Lam.)

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Abstract: Billions of people face severe problems throughout the world due to vector-borne diseases. Effective management of vectors can reduce the burden of vector-borne diseases. The present study was conducted to assess the efficiency of *Moringa oleifera* (Lam.) seed extract and seed essential oil against *Aedes aegypti* larvae. Log probit, regression, and ANOVA were carried out to find out the statistical significance. Probit analysis of mortality rates of crude extracts of seeds of *Moringa oleifera* showed LC_{50} and LC_{90} values as 56.53 ppm and 186.50 ppm and for essential oil it was 1414.34 ppm and 5423.48 ppm, respectively against 3rd instar larvae within 24 h of exposure. ANOVA indicated a significant relationship between exposure time and concentration of extracts in imposing mortality to the mosquito larvae. Since seed extract and essential oil were from natural origin so they are safe on non- targeted organisms. The outcomes of present study suggested that the extracts of *Moringa oleifera* was more effective against larvae than essential oil and can be further used as an eco-friendly mosquito larvicidal agent. The isolated compounds from this plant may also act as a potent larvicide and are worth further study.

Keywords: *Aedes aegypti*, *Moringa oleifera*, Seed extract, Essential oil, Larvicidal activity, LC_{50}

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Introduction

Mosquitoes are vectors of pathogens which are responsible for the spread of severe human diseases presenting a major burden to public health worldwide (David *et al*., 2014). *Aedes aegypti* L. (Diptera: Culicidae) has a wide geographic distribution with great epidemiological importance to spread disease. This is because females of this species can carry some arboviruses, such as Dengue, Yellow Fever and Chikungunya (Diniz *et al*., 2015). The species has peak activities for blood feeding in day time. *Ae. aegypti* prefer to feed and rest indoors in close proximity to their breeding sites (Koou *et al*., 2014). Due to the daytime biting behaviour the use of bed-nets as a protective barrier is not generally suggested, nor effective (Lenhart *et al*., 2008). Since Dengue becomes a global challenge to public health, insecticide-based vector control

interventions have been widely implemented across many countries (Deming *et al*., 2016). Emergence and development of insecticide resistance in dengue mosquitoes, especially *Ae. aegypti,* produces increasing challenges for vector control programs. Dengue has intensely spread throughout many areas in India which were free from this disease just 10 years ago. Thus, there is a need to determine the susceptibility status of the prevalent dengue vector, *Ae. aegypti*, to commonly used insecticides in the current control programme (Dhiman *et al*., 2014). Research on various natural plant products against mosquito suggests that some may serve as possible alternatives, or as rotational chemistries to the heavily used synthetic chemical insecticides (Lija-Escaline *et al*., 2015). Plant derived essential oils, or extracts, may provide alternative sources of mosquito control agents since they constitute a rich source of bioactive insecticidal compounds that are easily degradable (Cheng *et al*., 2013; Pavela, 2015).

Moringa oleifera (Lam.) is the most widely cultivated species of a Moringaceae family native to the sub-Himalayan tracts of India (Nasir and Ali, 1972). This rapidly-growing tree has tremendous source of highly digestible protein, Ca, Fe, Vitamin C, and carotenoids (Manzoor *et al*., 2007). Its essential oil and plant extract contains various phytochemicals which are very effective against mosquito.

In the present study an attempt has been made to evaluate the larvicidal activity of *Moringa oleifera* essential oil and seed extract on 3rd instar larvae of *Aedes aegypti*.

Materials and Methods

Plant collection:

The collection of *Moringa oleifera* (Lam.) (Moringaceae) seeds was done in the month of March and April when plant is loaded with pods. The pods were collected directly from the trees which were present in the college campus of University College of Science, Udaipur, Rajasthan. Then the seeds were taken out of the pods, brought to laboratory and shade dried for 5-7 days. The dried seeds were powdered in the grinder and stored in the bottle for extraction purposes.

Essential oil Extraction:

The essential oil was obtained from *Moringa oleifera* (Lam.) (Moringaceae) seeds by process called hydro-distillation using Clevenger apparatus (Jusoh *et al*., 2015). Heat was applied to the sample for 7-8 h. After 8 h, the collected oil in the Clevenger was allowed to cool at room temperature. The water present at the bottom of the oil was first drained to separate from the oil. The oily sample was then treated with anhydrous sodium sulphate (Na_2O_4) to remove the remaining trace water (Tsou and Mori, 2002). Stock solution was prepared by mixing essential oil in acetone. Different concentrations (500, 1000, 1500, 2000, 2500 ppm) were prepared from the stock solution.

Extract from seeds:

From the seeds the extraction was done with organic solvent methanol using Soxhlet Apparatus (Azmir *et al*., 2013). The extracts were filtered through a Buchner funnel with Whatman filter paper. The extract was evaporated to dryness using rotary evaporator. From the dried extract stock solution was prepared. From this stock different concentration (20, 40, 60, 80, 100 ppm) were prepared for the experiment.

Sources of Mosquito Larvae and Larvicidal assay:

Aedes aegypti (L.) mosquitoes were reared and a pure line culture was maintained in the insectory of Laboratory of Public Health Entomology, Department of Zoology, MLSU, Udaipur, Rajasthan. The eggs were hatched and larvae were reared in the laboratory at 28±2^{0}C temperature and 85±5% relative humidity. The eggs were placed in transparent air tight plastic containers containing distilled water and allowed to hatch to 1st instar larvae and kept to reach the 4th instar. The larvae were fed on dog biscuits and yeast powder in the ratio 3:1.

The larvicidal activity was monitored as per the

Table 1: Larvicidal mortality shown by seed extract

Concentration	N	Mean	SD	F	df	p-value	Result
20	3	5.33	0.58	784.20	1, 13	0.000	***
40	3	9.33	0.58				
60	3	13.33	1.15				
80	3	19.00	1.00				
100	3	24.33	0.58				

Table 2: Larvicidal mortality shown by essential oil

Concentration	N	Mean	SD	F	df	p-value	Result
500	3	6.67	1.15	213.80	1, 13	0.000	***
1000	3	9.67	0.58				
1500	3	11.67	0.58				
2000	3	19.00	1.00				
2500	3	24.33	0.58				

standard procedures recommended by the WHOPES, World Health Organisation (2005). The third and fourth instar larvae were tested against the discriminating dosage of 20, 40, 60, 80, 100 ppm of seed extract and 500, 1000, 1500, 2000, 2500 ppm of essential oil. Each dose was prepared and 1 ml of the prepared dose from each concentration was mixed with 100 ml of water in the beaker. 30 larvae of known age were released in the beaker and and three replicates were run simultaneously for each concentration. During this experiment, no food was provided to the larvae. The larval mortality was calculated post 24 h exposure period.

Data Analysis:

Mortality data obtained were corrected using Abbot Formula (1925). The lethal concentrations, LC_{50} and LC_{90} and their 95% confidence limits were calculated by Log probit regression analysis (Finney, 1971). Analysis of variance (ANOVA) was further carried out on the mortality data. The percentage mortality was calculated using the formula (1) and corrections for mortality when necessary were done using Abbott's.

$$\text{Percentage of mortality} = \frac{\text{Number of death larvae}}{\text{Number of larvae introduced}} \times 100 \quad \ldots(1)$$

$$\text{Corrected Percentage Mortality} = \frac{\text{Control Group} - \text{Treated Group}}{\text{Control Group}} \times 100$$

Results and Discussion

Probit analysis of mortality rates of crude extracts of seeds of *Moringa oleifera* showed LC_{50} and LC_{90} values as 56.53 ppm and 186.50 ppm whereas for essential oil these were 1414.34 ppm and 5423.48 ppm, respectively against 3rd instar larvae within 24 h of exposure (Tables 1, 2; Figs. 1, 2). Analysis of variance (ANOVA) revealed that the mortality rates of larvae were significantly influenced by the time and concentrations in both the crude extract (Table 1) and essential oil (Table 2) tested. When interactions of the two factors (concentration and time) were taken into the account, no significant difference was noticed.

Plants generate various secondary metabolites in their body in order to defend against predators which could be used as mosquito larvicidal agents. These bioactive compounds can be used as a substitute for synthetic insecticides as they are biodegradable, comparatively safe, low-cost, and often readily available all over the globe. The larvicidal action of the plant extracts may be due

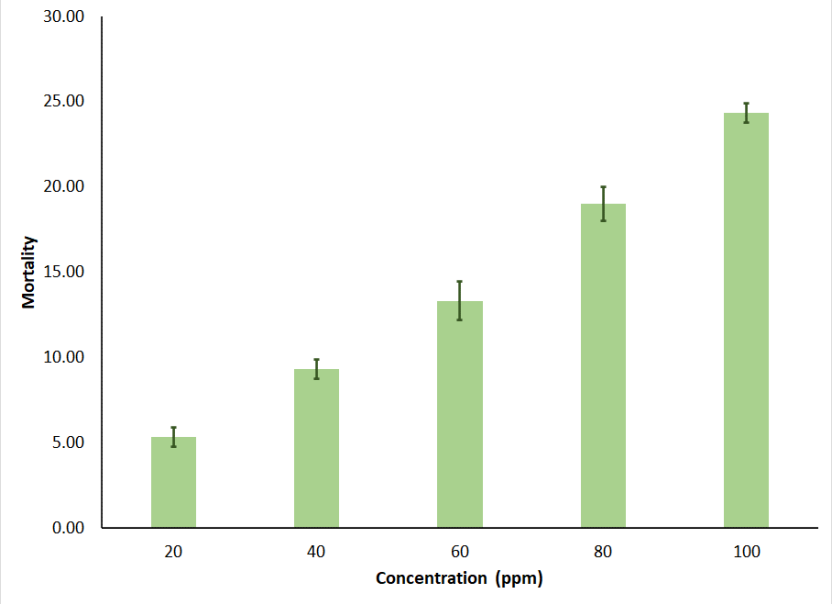

Fig. 1: Larvicidal activity shown by seed extract.

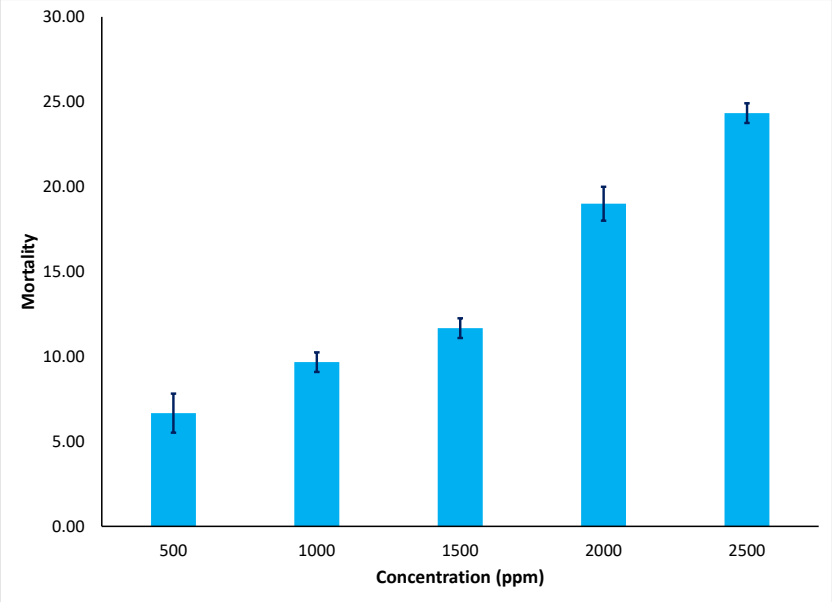

Fig. 2: Larvicidal mortality by essential oil.

to alkaloids, flavonoids, polyphenols, steroids, terpenoids, etc. synthesized to a variable extent in different parts of plants. The strategy of using phytochemicals for mosquito control has opened up novel prospects for large-scale extraction of phytochemicals. Screening of available plants for mosquitocidal efficacy is the first step in this regard. The present study revealed that crude extract was more effective against larvae of *Aedes aegypti*. Paule *et al*. (2009) found LC_{50} value of water extract of *Moringa* seeds as 1260 ug/ml. Juliene *et al*. (2009) reported that the LC_{50} value of *Moringa* seed extract was 0.197 mg/ml. Crude extract from seeds was much more effective than the essential oil.

Conclusion

Reduction and elimination of the problem of vector-borne diseases is necessary at the present time. Currently, WHO (2017) emphasizes vector

management approach that may be developed with locally available resources beyond the use of only available control interventions. The plant *Moringa oleifera* (Lam.) is grown all over the world, readily available, easy to cultivate, traditionally used for various purposes, and even known to be eaten raw by humans and other animals and hence should be safe to use. Present trial with seed extract showed a good prospect of using the plant as larvicide and may be helpful in the mosquito control program in the future. However, further studies to elucidate the effectivity against other species of mosquitoes, identify the active compounds, assess the effectiveness in field trials, repellent product development are essential and it is undergoing.

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