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Research Article

Study on larvicidal efficacy of *Moringa Oleifera* against the vector *Anopheles* Mosquitoes

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Abstract

The present study was conducted to evaluate the lethal action of aqueous *Moringa oleifera* seed and leaves extract against third instar larvae of *Anopheles* larvae. *Anopheles* larvae exposed to aqueous extracts of *Moringa oleifera* gave behavioral responses to various toxic reactions such as erratic movements; air gulping with prolonged exposure resulted in dullness, inability to swim and discoloration. For 24 hrs the LC50 values for seed and leaves were 381.219 and 904.270 mg/10 ml while LC90 values for seed and leaves were 414.299 and 4814.49 mg/10. Seed extract was more effective than leave extract. These results suggest that *Moringa oleifera* may be a potentially valuable source of insecticide and may well play a more prominent role in mosquito control programs in the future. Further research into its specific mode of action and its lethal concentration on non target animals are recommended.

INTRODUCTION

Malaria is amosquito-borne infectious disease affecting humans and other animals caused by parasitic protozoans belonging to the Plasmodium type. Malaria causes symptoms that typically include fever, tiredness, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma, ordeath. Symptoms usually begin ten to fifteen days after being bitten. If not properly treated, people may have recurrences of the disease months later. In those who have recently survived an infection, re-infection usually causes milder symptoms. This partial resistance disappears over months to years if the person has no continuing exposure to malaria (Caraballo, 2014). Vectors for plasmodium infection to human being are female Anopheles mosquitoes. About 460 species are recognized; while over 100 can transmit human malaria, only 30–40 commonly transmit parasites of the genus Plasmodium, which cause malaria in humans in endemic areas. *Anopheles gambiae* is one of the best known, because of its predominant role in the transmission of the most dangerous malaria parasite species to humans is *Plasmodium falciparum*. Vector control refers to methods used to decrease malaria by reducing the levels of transmission by mosquitoes. For individual protection, the most effective method is to use the insect repellents, Insecticide-treated mosquito nets (ITNs) and indoor residual spraying (IRS) including DDT and the pyrethroids cyfluthrin and deltamethrin (Enayati *et al.*, 2007; Tusting *et al.*, 2013). The use of these chemical to control the vectors can have adverse effects on human health. Acute health problems may occur in workers that handle insecticides, such as abdominal pain, dizziness, headaches, nausea, vomiting, as well as skin and eye problems.

Pyrethrins, insecticides commonly used in common bug killers, can cause a potentially deadly condition if breathed in. the long term effects may include the cancer, birth defect, loss of fertility, diabetes etc. (Gilden *et al*., 2010). The use herbal larvicidal can be one of the best practice in vector control. The moringa tree can be the best larvicide to control Anopheles mosquitoes. As the moringa tree is not affected by any serious diseases in its native or introduced ranges. In India, several insect pests are seen, including various caterpillars such as the bark-eating caterpillar, the hairy caterpillar or the green leaf caterpillar. The bud worms Noctuidae are known to cause serious defoliation. Damaging agents can also be aphids, stem borers, and fruit flies. In some regions, termites can also cause minor damage. If termites are numerous in soils, insect's management costs are not bearable (Iqbal and Bhanger, 2006). In this context the present research aims to evaluate the larvicidal potential of aqueous extract of *Moringa oleifera* seeds against Anopheles mosquito species.

MATERIALS AND METHODS

Collection of plant materials: The leaves and legumes of *Moringa oleifera* were collected from nearby area of Government Vidarbha Institute of Science and Humanities, Amravati.

Preparation of the extract: The seeds separated from legume and leaves of *Moringa oleifera* were dried at environmental temperature (25-37^0 C). Dried leaves and seed were powdered separately by pestle and mortar. The distilled water was added to the powder in the proportion of approximately 125 mg, 250 mg, 375 mg and 500 mg per 10 mL of water to from solution of 300 ml each. For preparation of solution, considered amount of powder keep packed in muslin cloth pouch and dip into required warm water for 24 hour. Then pouch removed and formed solution used for experiment.

Collection of larvae: The fourth instars larvae of *Anopheles sp.* was used in this study and was obtained from stagnant water nearby the Institute. Anopheline larvae tend to float horizontal at the surface of the stagnant water. These larvae were maintained at 25-30 ^{0}C, with relative humidity 60-70% under a photoperiod of (12h: 12h) following standard operating procedures for mosquito maintenance (WHO, 1975). The larvae were free of exposure to pathogens, insecticides or repellents and were maintained in beaker by providing dog biscuit or yeast powder.

Dose response assay: During preliminary screening with the laboratory trial, the larvae were identifies with available morphological keys. The four batches of 30 larvae were use in each bioassay to test the different concentration of aqueous extracts. The mosquito larvae were treated with extract according to the methodology described by WHO (2005). Larvae were kept in 500 ml beaker containing 300 ml solution of aqueous extract. The numbers of dead larvae were counted at different intervals of 0, 6, 12, 18 and 24 Hours.

Behavioral response assay: Behavioral changes are physiological responses shown by the animal, which are often used as the sensitive measure of stress syndrome in the organism experiencing it, consequently the behavioral changes were also observed in control and exposed organism. During the course of time, the behavioral responses of the larvae exposed to extracts were also observed. These observations were mainly related to their erratic behavior, air gulping, dullness, loss of reflex and discoloration

Data analysis: The Log-probit analysis was carried out to determine the median lethal concentrations (LC_{50}) and higher lethal concentrations (LC_{90}) values at their 95% confidence intervals were obtained by the method of Finney (1979). Data were collected, organized and analyzed using one-way analysis of variance (ANOVA). The value of $P<0.05$ was used to indicate statistical significance.The computer statistical program BioStat Pro Verson 5 (AnalystSoft Inc.) was used for analysis.

RESULTS AND DISCUSSION

The study was conducted to evaluate the lethal action of aqueous *Moringa oleifera* seed and leaves extracts against *Anopheles* larvae. The four extracts 125 mg/10ml, 250 mg/10ml, 375 mg/10ml and 500 mg/10ml respectively. The four batches of 30 larvae each were kept in 500 ml beaker containing 300 ml solution of aqueous extract. The observations made by the intervals of 6 hrs, 12 hrs, 18 hrs and 24 hrs. The observations are quoted in Figure 1 and 2.

From the tested extracts, maximum mortality of fourth instar *Anopheles* larvae was observed in 500 mg/10ml. The obtained results were significantly different at *P<0.05.* Obtained results clearly demonstrated that aqueous extract of *Moringa oleifera* seeds and leaves have lethal action against *Anopheles* Larvae. These findings are

in well agreement with results quoted by some authors (Kamraj and Rahuman, 2010; Prabhu *et al*., 2011; Ghosh *et al*., 2012; Ohia *et al*., 2013; Prasad and Parveen, 2016). But these finding are controversial with observations of Kumar *et al*. (2016) who did not observed any larvicidal activity of aqueous extract of *Moringa oleifera* seeds.

The behavioral responses observed in the toxicity assay show variationsin their tolerance to aqueous extracts of *Moringa oleifera* by *Anopheles* larvae (Table 1) On the addition of the toxicant,various toxic reactions such as erratic movements, air gulping with prolonged exposure resulted in dullness and discoloration in higher concentrations (Table 1). This report is in well agreement with Ayotunde *et al.* (2011) and Ohia *et al.* (2013).

The result of the experiment conducted to evaluate the larvicidal effectiveness of the aqueous extract showed that it was toxic to the larvae of *Anopheles* mosquito exposed to the different concentrations showed significant behavioral effects such as inability to swim to the top within 1 minutes of exposure. All the concentrations were lethal to the larvae but with different degree of effectiveness as were observed and discussed earlier. In addition to above, the probit assay was carried out to estimate the LC_{50} values of aqueous extract lethality against the *Anopheles* mosquito larvae (Figure 3 and 4).The probit assay was carried out to estimate the LC50 and LC90 values for 24 hrs of aqueous extract lethality against the *Anopheles* mosquito larvae. For 24 hrs the LC50 values for seed and leaves were 381.219 and 904.270 mg/10 ml while LC90 values for seed and leaves were 414.299 and 4814.49 mg/10 ml. The present observations support the previous findings of Ohia *et al.* (2013).

The observations proved that the aqueous extracts of seed and leaf of *Moringa oleifera* have larvicidal potential to control the mosquitoes which are vector for many diseases. Sharma and Paliwal (2013) cleared that the *Moringa oleifera* is rich with saponin content. Bagavan *et al.* (2008) and Ferreira *et al.* (2009) had observed that saponin is most effective mosquito larvicidal compound against *Aedes aegypti* and *Culex quinque fasciatus.* Hence the presence of saponin in the *Moringa oleifera* can be the prominent reasons for obtained result.

Conclusion

In brief summarizing the obtained results, it was cleared that the valuation of the aqueous extract of *Moringa oleifera* seed and leaves against third instar larvae of *Anopheles* sp. in the laboratory showed that it was larvicidal to the mosquito species. Seed extract is more effective than leave extract. These results suggest that *Moringa oleifera* may be a potentially valuable source of insecticide and may well play a more prominent role in mosquito control programs in the future. Further research into its specific mode of action and its lethal concentration on non target animals are recommended.

Figure 1: Mortality of *Anopheles* larvae exposed to aqueous extract of *Moringa oleifera* seed for different interval of time

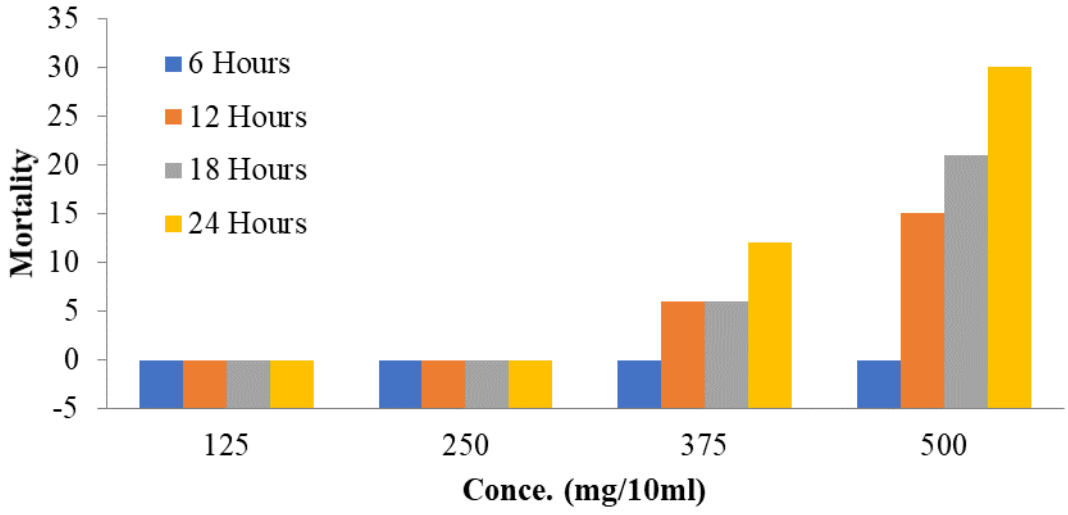


Figure 2: Mortality of *Anopheles* larvae exposed to aqueous extract of *Moringa oleifera* leaves for different interval of time

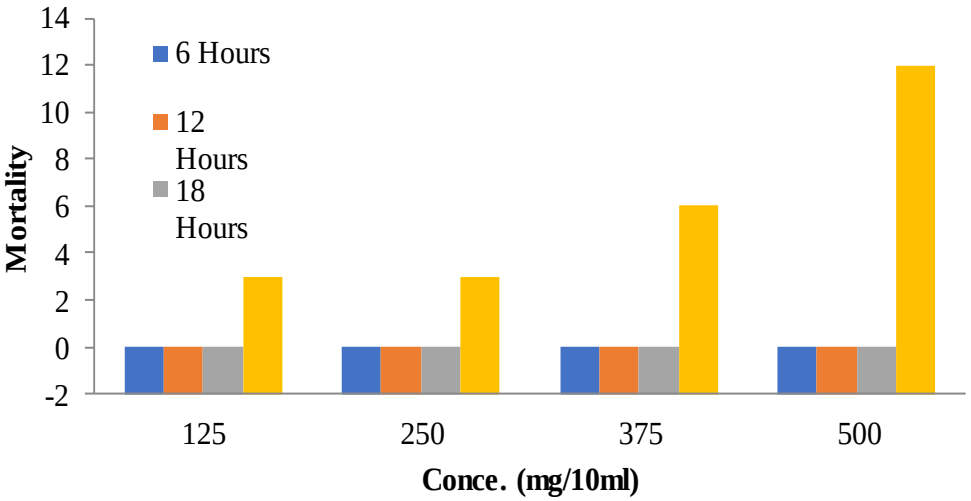


Table 1: Behavioural responses by *Anopheles* larvae exposed to Seed extracts

Behaviors	Exposure Time																				
	1 hr					6 hrs				12 hrs				18 hrs				24 hrs			
	Concentrations																				
	0	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Erratic	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-
Air Gulping	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dullness	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+
Loss of reflex	-	-	-	+	+	-	+	+	+	-	+	+	+	-	-	-	+	-	-	-	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
# : + Present/ - Absent *: 0 Control; A 125 mg/10ml; B 250 mg/10ml; C 375 mg/10ml; D 500 mg/10ml																					

Table.2: Behavioural responses by *Anopheles* larvae exposed to Leaves extracts

Behaviors	Exposure Time																				
	1 hr					6 hrs				12 hrs				18 hrs				24 hrs			
	Concentrations																				
	0	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Erratic	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Air Gulping	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Dullness	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	-	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
# : + Present/ - Absent *: 0 Control; A 125 mg/10ml; B 250 mg/10ml; C 375 mg/10ml; D 500 mg/10ml																					

Figure 3: Probit Analysis for LC50 for 24 hours (Seed Extract)

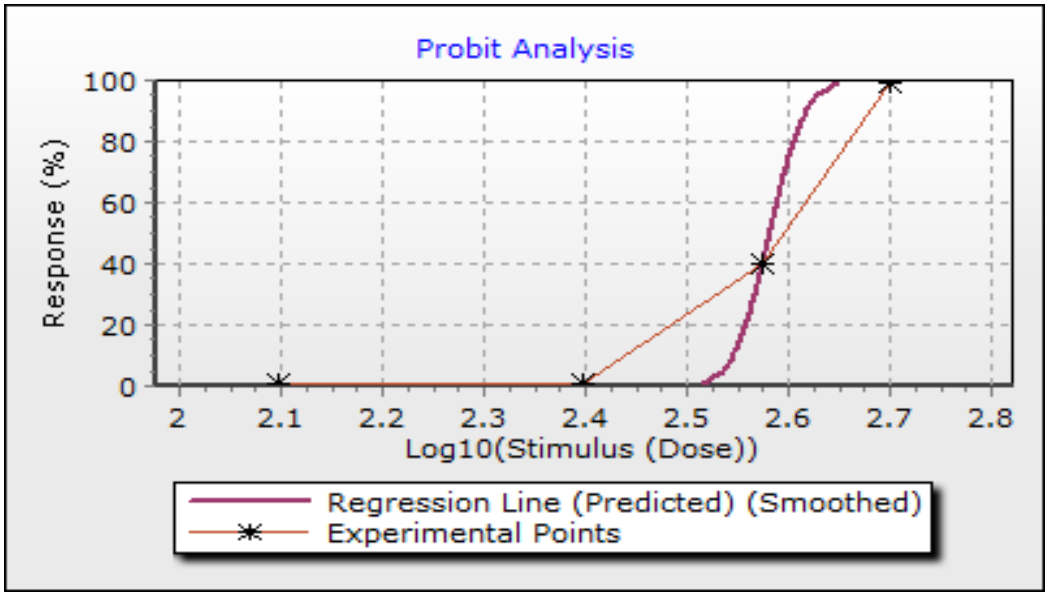
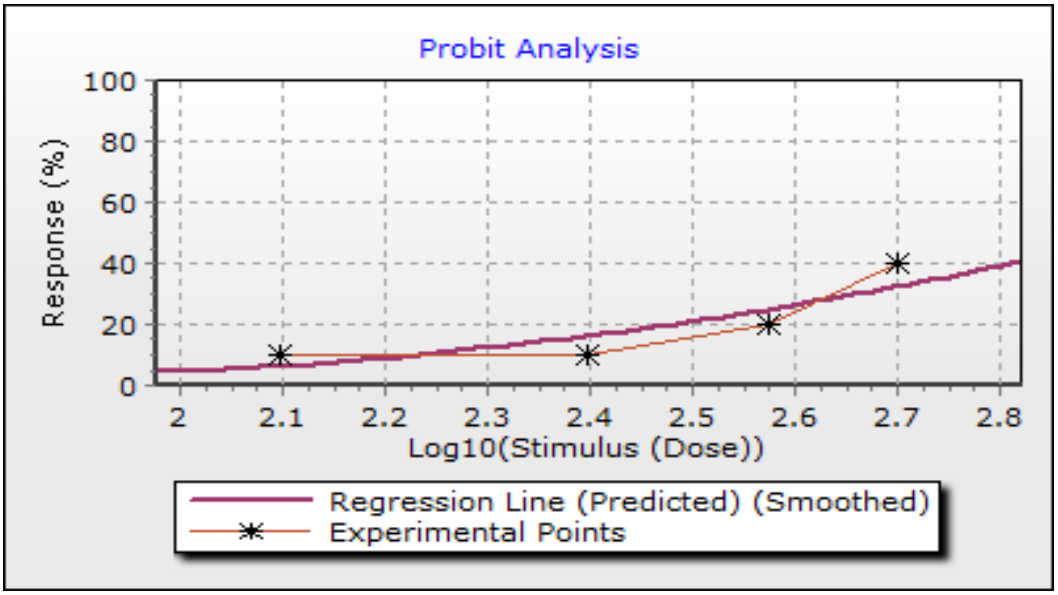


Figure 4: Probit Analysis for LC50 for 24 hours (Leaf Extract)



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