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Evaluation of Moringa Oleifera (Lam) (Moringaceae) Seed Oil For Larval Control of Aedes Aegypti L. (Diptera: Culicidae)

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Abstract:
Comparative bioassays were carried out with 1^{st} and 4^{th} instar larvae of Aedes aegypti L. (Diptera: Culicidae) using Moringa oleifera seed oil. This was to investigate whether the oil extract would show lethal effects on the mosquito larvae and to further establish at which point during the larval growth the oil would be effective. The larvae were exposed at ambient laboratory temperatures of 28 ± 2^0C, 80 ± 5% r.h and photoperiod of 12:12 light and dark hours. Seven dosages of 200, 100, 50, 25, 12.5, 6.25 and 3.125 µl of oil dissolved in acetone per millilitre of water and control treatments with ordinary acetone were included in the trials. These were arranged on the laboratory bench in a Completely Randomised manner. Larval mortality was recorded at 3, 6, 9, and 12 hours post treatment. Mortality data were subjected to analysis of variance and log-probit regression, accordingly. Results showed that significant ($P<0.05$) dosage-related morality responses were noted for both instars. Thus, Moringa oil showed significant ($P<0.05$) toxic action against all larval instars of Ae. aegypti tested. Higher concentrations of the oil resulted in higher kill of the larvae within 12 hours, while sublethal dosages gave low mortality rates. The LD_{50} values determined were 208.35 µl/ml and 62.94 µl/ml for the 4^{th} and 1^{st} instar larvae, respectively. The present studies therefore, demonstrate that moringa seed oil exhibits significant toxic action against all larval instars of Ae. aegypti especially the 1^{st} instar. Higher levels of toxicity with increasing larval mortality were achieved with increasing dosage levels and longer exposure time.

Keywords*: Moringa oleifera oil, Aedes aegypti, larval control*

1. Introduction

Mosquitoes are vectors of many human diseases. According to Clements (1992) and Reinert, (2000), there are over 3000 different species of mosquitoes throughout the world. About 1,900 species occur in the humid tropics and subtropics, where the climatic conditions are favourable for rapid immature stages development and adult survival. Global efforts to reduce the number of mosquitoes usually are due to the deadly diseases they transmit to man and animals. The most important mosquito-transmitted

diseases with high public health concern include malaria, yellow fever, Dengue haemorrhagic fever and filariasis, which are transmitted by various species of Anopheles, Aedes and Culex mosquitoes.

Attempts to control these vectors have relied heavily on the use of synthetic insecticides resulting in several problems, including environmental pollution, insect resistance, unacceptable levels of residues, escalating cost of production among others (Otto, 1992). Hence, the use of plant extracts in public health and agro-ecosystem is now emerging as one of the prime methods of protecting the environment from residual toxic synthetic pesticides pollution (Prakash and Rao, 1997; Schmutterer, 1995). However, plants are by far the most efficient 'factory' for synthesis of chemical compounds, many of which they use in the defense against herbivores (Schoonhoven et al., 1998). The insecticidal metabolites from plants could therefore, be applied to other flora and fauna in the environment to protect them from injurious species.

For example, Amonkar and Reeves (1970) reported the action of garlic oil against the larval stages of five mosquito species. Badruddoza and Rahman (2008) reported the larvicidal action of moringa roots and other plants against Aedes albopictus and Culex quinquefasciatus, while Bassole et al. (2003), reported the ovicidal and larvicidal activities of leaves of three plants naturally growing in Burkina Faso. Botanical pesticides derived from members of Meliaceae family, particularly the Indian neem, Azadirachta indica A. Juss, are equally promising (Akou-Edi, 1984, Niber, 1994). The main active component of the neem extract is Azadirachtin, which has both deterrent, antifeedant, anti-ovipositional, growth regulatory and fecundity – reducing effects on various insects, including mosquitoes (Schmutterer, 1990). Betra et al. (1998) reported larvicidal effects of neem oil-water emulsion against Anopheles stephensi, Culex quinquefasciatus and Ae. egypti in mosquito breeding habitats.

The seasonality of many plant products such as neem seeds makes it necessary to investigate as many other plants as possible. Thus many researchers are now investigating Moringa oleifera Lam (Horse radish) for use as pesticide. For instance, Donli and Dauda (2002) reported the use of aqueous Moringa seed extracts as seed dressing bio-fungicide for groundnut production in Nigeria. Adendonon et al. (2006) combined a biocontrol with M. oleifera seed extract for integrated control of Sclerotium damping-off of cowpea stem and root. Ajayi (2007) compared efficacy of M. oleifera seed oil with Sesamum indicum and Olea europaea against T. castaneum. Aqueous extracts of leaves of M. oleifera, Vernonia amygdalina and Annona muricata were evaluated for the control of Collectotrichum destructivum on seeds of cowpea (Vigna uniguculata) (Akinbode and Ikotun, 2008). Badruddoza and Rahman (2008) reported the larvicidal action of M. oleifera root extract together with other Indian plants against Ae. albopictus and C. quinquefasciatus.

The Horse radish, M. oleifera Lam. is an abundant invasive plant found growing throughout Nigeria and many other African countries. The many attributes of this plant have generated a lot of interests among Scientists in many African countries both for its medicinal and other uses (Ozumba, 2005). Thus its potentials as bio-pesticide need to be evaluated in different environments.

Hence, the present studies were aimed at evaluating the larvicidal action of the M. oleifera seed oil extract for larval control of Ae. aegypti mosquito.

2. Materials and Methods

2.1. Study Area

These investigations were carried out in the Research Laboratory of the Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Nigeria.

2.2. Rearing of Mosquito Larvae

Eggs of Aedes aegypti were obtained from the Federal Ministry of Health, Department of Public Health, National Arbovirus and Vectors Research Centre, Enugu, Nigeria. They were hatched and reared in the laboratory at 28 ± 2^0C and $80 \pm 5\%$ r.h. The eggs were placed in transparent plastic containers containing 500mls of distilled water and allowed to hatch into 1st instar larvae and further kept to reach the 4th instar. The larvae were fed on crumbs of Yale Fortune Cabin Sweetened Biscuit; the feed was supplied every other day for normal development of the larvae.

2.3. Moringa Seed Collection and Oil Extraction

The M. oleifera seeds were collected from a Moringa Plantation in Enugu, Nigeria. The seeds were dried in a shed for 7 days and dehusked to obtain the kernels which were subsequently pulverized in an electric blender to obtain fine powder. Thereafter, the oil extraction was carried out using petroleum ether (Analar grade) in Soxhlet Extractor by refluxing for 3 hours. About 40g of the pulverized sample was wrapped in a double layer of Whatman No. 1 filter paper and placed inside the Soxhlet thimble. Measured volume of 250 ml of petroleum ether was poured through a funnel, by-passing the thimble containing the sample, into the round bottom flask component of the Soxhlet apparatus. This was positioned about 5 cm above an electric-heated plate while cold water was allowed to reflux in the Extractor to cool the condenser compartment. After many refluxes, the mixture of oil and ether extract was allowed to evaporate at about 75^0C. The process was repeated several times at the end of which, the ether solvent distilled off and finally leaving the light yellowish oil.

2.4. Preparation of the Oil Extracts

Graded concentrations of the M. oleifera seed oil were prepared in acetone (Analar grade). The pure oil extract was regarded as 100% concentrate and subsequently diluted serially to give 20, 10, 5, 2.5, 0.625 and 0.3125% w/v in acetone.

2.5. Bioassay Tests

Aliquots of 1 ml of each of the above dilutions were separately placed in plastic cups containing 160 ml distilled water. Each replicate container was challenged with batches of 20 active fourth or first instar larvae of Ae. aegypti accordingly. Controls without oil extract were also included.

Each treatment or control was replicated five times. The bioassay tests were repeated two times at ambient laboratory temperature of 28 ± 2^0C, 80 ± 5% relative humidity and photoperiod 12:12 light and dark hours. Mortality / inhibition of emergence counts were made at 3 – hourly intervals for 12 hours. Dead and moribund larvae, that is, those unable to wriggle after gentle prodding were counted as dead and recorded. However, exuviae or shed cuticle started appearing in the control treatments about 2 hours post treatment but none matured into pupae or adult.

3. Data Analysis

Mortality data obtained were corrected using Abbot Formula (1925). Log_{10} versus probit regression analysis was carried out (Finney, 1972) for determining LD_{50} or level of inhibition of emergence (IE_{50}). Analysis of variance (ANOVA) was further carried out on the mortality data and means separated using least significant difference (LSD) for the different time intervals and concentration levels.

4. Results

The mortality values of 4th instar larvae of Ae. aegypti exposed to M. oleifera seed oil extract at 3 hourly intervals are presented in Table 1. The results show that moringa oil exhibited significant (P<0.05) levels of toxicity to Ae. aegypti larvae. Mortality increased with increasing concentrations of the oil and also with increasing period of exposure. However, there were much greater responses at higher concentrations of the oil while no mortality was observed in the control treatments. Generally, dose-related mortality responses were noted in all treatments with higher ranges of the oil resulting in about 90% mortality or inhibition of emergence in the larvae, while at lower concentrations, larval mortality was low.

	Mean of Mortality (%)			
Dose (µl)	**3 (hrs)**	**6 (hrs)**	**9 (hrs)**	**12 (hrs)**
200	5	20	20	90
100	5	10	55	85
50	10	20	55	80
25	5	15	35	65
12.5	5	15	30	60
6.25	5	10	20	45
3.125	5	10	15	25
Mean	5.7±1.2a	14.3±2.2a	32.1±6.5b	65.0±7.0c
Control	0	0	0	0

Table 1: Percentage mortality of 4th instar larvae of Aedes aegypti exposed to moringa seed oil at various time intervals.

LSD = 12.0

**Mean of five replicates (± s.e)*

***The means followed by the same letter in column are not significantly different from each other (P<0.05) by LSD.*

The probit regression analysis (Figure 1) showed that after 12 hours of exposure, the LD_{50} (IE_{50}) of the moringa oil – treated 4th instar larvae was 208.35 µl and 62.84 µl for 1st instar larvae.

Table 2 also shows that mortality of 1st instar larvae of the mosquito increased significantly (P<0.05) with increasing levels of oil concentration and also with increasing period of exposure, giving an LD_{50} (IE_{50}) value of the oil for this instar as 62.94 µl.

Mortality of both 4th and 1st instar larvae (Table 1) after the 6th hour was significantly (P<0.05) higher than at the first 3 – hours. This trend was observed in all the treatments irrespective of the dosage applied. However, at the highest concentration of 200 µl, the mean toxicity of the oil to the 4th instar was 49% while that of the 1st instar was 66.5% (Table 3). The LD_{50} values determined at the 3 hourly intervals are shown in Figure 2. This indicates that exposure of the 4th instar larvae for 3, 6, 9 and 12 hours would require 2.29×10^{38} (i.e. α), 2.76×10^{7}, 4.08×10^{2} and 1.35×10^{1} µl of moringa oil respectively, order to achieve the same level of 50 percent kill. Similarly, the 1st instar larvae would require exposure to 3.85×10^{3}, 7.11×10^{1}, 3.45×10^{1} and 1.22×10^{1} µl of the oil extract for 3, 6, 9 and 12 hrs, respectively, to kill 50 percent of the larvae (Figure 3). These are all indications that the early instars are moresusceptible and require less toxicant to kill than later instars, irrespective of the duration of exposure.

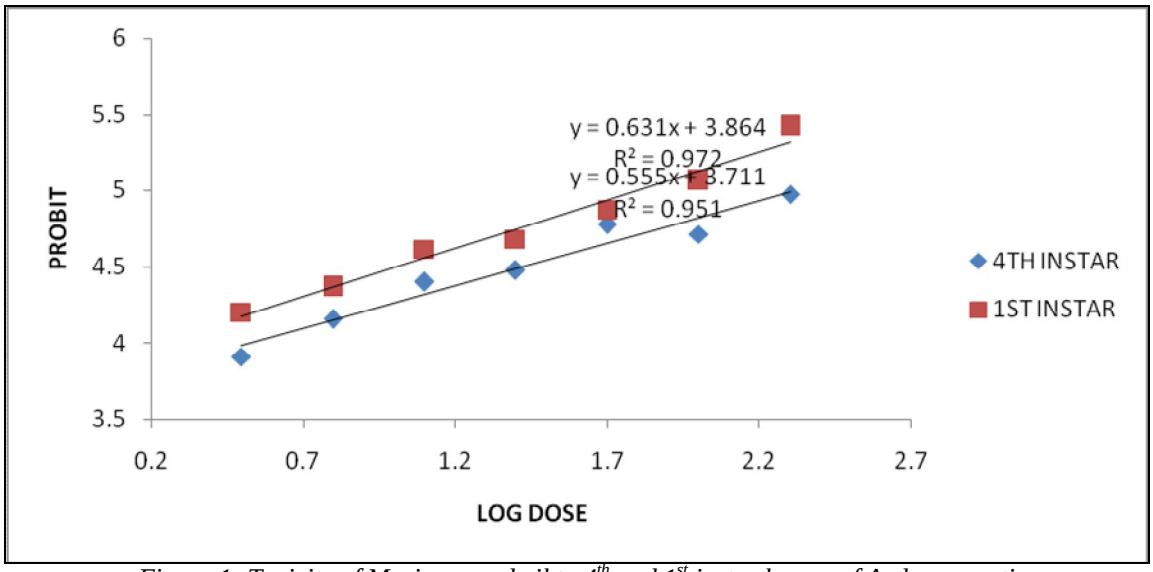

Figure 1: Toxicity of Moringa seed oil to 4th and 1st instar larvae of Aedes aegypti

◆ **4TH INSTAR**…….y = 0.5558 x + 3.7112
R^2 = 0.9517, LD_{50} = 208.35 µl

■ **1ST INSTAR**……..y = 0.6311 x + 3.8647
R^2 = 0.9727, LD_{50} = 62.94 µl.

	Mean of Mortality (%)			
Dose (µl)	**3 (hrs)**	**6 (hrs)**	**9 (hrs)**	**12 (hrs)**
200	25	70	80	100
100	25	50	60	75
50	30	40	45	65
25	25	35	40	50
12.5	25	30	40	45
6.25	10	25	30	40
3.125	10	20	25	30
Mean	21.43±1.5a	38.57±2.5b	45.71±5.4c	57.9±7.0d
Control	0	0	0	0

Table 2: Percentage mortality of 1st instar larvae of Aedes aegypti exposed to moringa seed oil at various time intervals.
LSD = 8.66
**Mean of five replicates (± s.e)*
**The means followed by the same letter in column are not significantly different from each other (P<0.05) by LSD.*

However, lower concentrations for longer periods of contact with the larvae would achieve similar level of kill compared with higher concentrations applied for shorter periods of time.

	Mean of Mortality (%)	
Dose (µl)	**4th Instar**	**1st Instar**
200	49.0	66.5
100	38.8	52.5
50	41.3	45.0
25	30.0	37.5
12.5	27.5	35.0
6.25	20.0	26.3
3.125	13.8	21.3
Control	0	0
Mean (±s.e.)	28.63 ± 5.6a	40.59 ± 5.4 b
LSD = 8.64		

Table 3: Cummulative Toxicity of Moringa seed oil to 4th and 1st instar larvae of Aedes aegypti, after 12 hours

Mean of five replicates (± s.e).
Means followed by the same letter(s) in column are not significantly different from each other ($P < 0.05$).

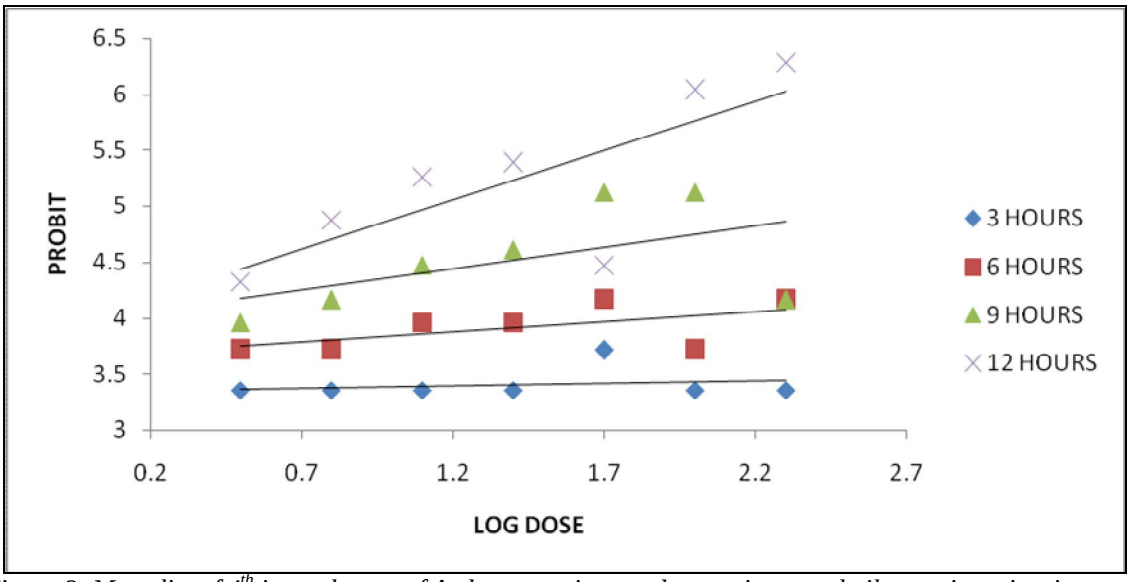

Figure 2: Mortality of 4th instar larvae of Aedes aegyptiexposed to moringa seed oil at various time intervals

3 HOURS …….y = 0.0431 x + 3.3467
R^2 = 0.0417, LD_{50} = (2.29 x 10^{38}) µl

6 HOURS…….y = 0.1797 x + 3.6629
R^2 = 0.3427, LD_{50} = (2.76 x 10^{7}) µl

9 HOURS…….y = 0.376 x + 3.9918
R^2 = 0.2726, LD_{50} = (4.08 x 10^{2}) µl

12 HOURS…..y = 0.8785 x + 4.0066
R^2 = 0.5942, LD_{50} = (1.35 x 10^{1}) µl

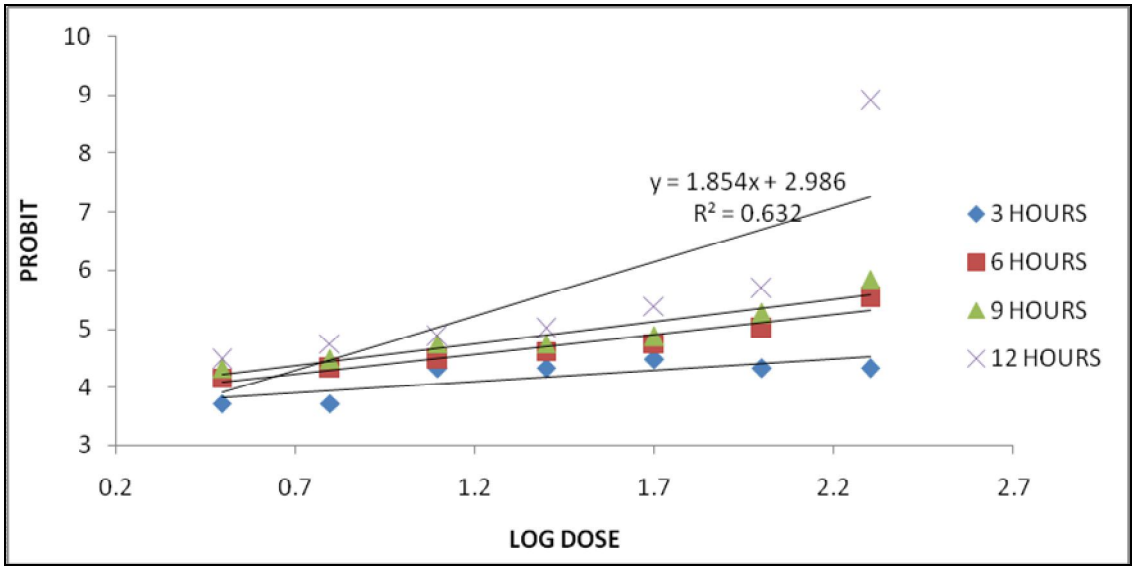

Figure 3: Mortality of 1STinstar larvae of Aedes aegypti exposed to moringa seed oil at various time intervals

3 HOURS……y = 0.3779 x + 3.6451
R^2 = 0.6062, LD_{50} = (3.85 x 10^3) µl

6 HOURS…….y = 0.6784 x + 3.7438
R^2 = 0.9249, LD_{50} = (7.11 x 10^1) µl

9 HOURS……..y = 0.7416 x + 3.8595
R^2 = 0.8854, LD_{50} = (3.45 x 10^1) µl

12 HOURS…….y = 1.8549 x + 2.9863
R^2 = 0.6324, LD_{50} = (1.22 x 10^1) µl

5. Discussion

The larvicidal toxicity of M. oleifera seed oil to both 1st and 4th instar larvae of Ae. aegypti was studied in the laboratory. The studies have shown that this insecticide of plant origin can be used as independent control method in reducing the impact of mosquitoes. Considering the high cost of conventional insecticides and other environmental concerns, moringa seed oil could provide a cheap alternative means of mosquito vector control. Moringa has added advantage of being comparatively harmless to man and other non-target organisms. It could, therefore, with little education, be cultivated and applied to major vector breeding sites by communities.

In the present studies, the general performance of moringa oil in killing the larvae of Ae. aegypti was observed with relatively high percentage larval mortality recorded in both 1st and 4th instar larvae, high percentage inhibition of emergence and LD_{50} values. The toxic effect of the oil was high, as the mortality and the rate of inhibition of emergence increased with increasing concentration and time. The effectiveness of plant oils in regulating growth and inhibition of many insects and fungal development has been reported previously (Jilani and Su, 1983, Mulla et al., 2003). For instance, Manas et al. (2005) reported that M. oleifera extracts inhibited 97.32% germination of fungal pathogen. Sharma and Saxena (1994) also found that the petroleum ether extract of Tagetes erectes had toxic effect on larvae of Anopheles stephensi and on its significant growth index. Mwangi and Mukiama (1988) observed that fraction of Melia volkensi fruit kernel extract had growth inhibition activity at low concentration, whereas two other fractions had acute toxic effects on the mosquito larvae. Thus, in the present studies only a few surviving larvae were left in some treated samples, and they were unable to mature into the next stage of development. Pushpalatha and Muthukrishnam (1995) reported that leaf extracts of Vitex negundo at very low concentrations had larvicidal activity against Culex quiquefasciatus and A. stephensi and also extended the duration of larval pupation.

The botanical pesticide, Moringa seed oil, has proved to be very effective in mosquito larval control as demonstrated in the present studies. The activity tended to extend over a longer period, and its effectiveness increased with increasing exposure time. The oil probably exhibited its effects on the mosquito larvae as growth regulator, as well as blocking the respiratory organs. According to Hirashima et al. (1998) and Enan (2001) some plant essential oils block the octopamine neuroreceptors that regulate the movement, heart rate, behavior, metabolism, and pupation of insects. Thus, the regulatory activity of Moringa seed oil was exhibited in the mortality of the larvae and inhibition of their eclosion into pupal stage.

The present studies have further demonstrated that the 1st instar larvae of Ae. aegypti were more susceptible than the 4th instar. This observation agrees with reports that early instars of insects are more susceptible to inhibitory and growth regulatory effects of most insecticides (Mulla et al., 2003). This, the authors however, suggested was related to the size and tender nature of the chitin in the early instars of the mosquitoes. Furthermore, Akpa et al. (2003) observed that 3rd instar was more susceptible than 4th instar when a plant oil of Phytolacca dodecandra was used against Ae. aegypti.

The present studies therefore, demonstrate that M. oliefera could be used as mosquito larvicide for the control of Ae. aegypti. This would help to contain any outbreak of the yellow fever often associated with the mosquito (Lee and Moore, 1972, Einterz, 1971). It would therefore, be necessary to increase campaign on the growing of the crop in all ecological zones of Nigeria, as an invaluable tool for the control of mosquito vectors and hence the diseases they transmit.

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