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Ohia Chinenyenwa MD
Department of Environmental
Health Sciences, Faculty of
Public Health, College of
Medicine, University of Ibadan,
Ibadan

Ana Godson REE
Department of Environmental
Health Sciences, Faculty of
Public Health, College of
Medicine, University of Ibadan,
Ibadan

Bio-insecticidal efficacy of *Moringa oleifera* on the malaria vector, Anopheles and toxicity evaluation on fish behaviour

Ohia Chinenyenwa MD and Ana Godson REE

Abstract
Mosquito control efforts are being undermined due to development of insecticide resistance in mosquito vectors of public health importance to synthetic insecticides, hence leading to the search for more sustainable control inputs. There is an increasing interest in developing plant-based insecticides as sustainable alternatives to chemical insecticides. *Moringa oleifera* extract was evaluated on *Anopheles gambiae s.s.* larvae and to limit potential toxic pollution of aquatic ecosystems, acute and sub-chronic toxicity bioassay experiments were carried out on adult male *Poecilia reticulata* (guppies) to determine its eco-toxicological impact. Three independent larvicidal experiments were replicated three times; the pattern of effectiveness and the LC_{50} were determined for the mosquito larvicide; the toxicity experiments were carried out in three replicates by static method and behavioural changes in the guppies were determined for the different concentration levels used and in relation to exposure time. Data from the bioassays were evaluated using Probit analyses statistical method; Descriptive analyses were also used to describe the toxicological effects of the extract on the guppies. The larval bioassay gave a dose-dependent ascendency of larval mortality and mortality records over the exposure period were significantly different among the treatment groups ($p < 0.05$); while the guppies exhibited varying degrees of behavioural effects that were concentration- and exposure-time dependent with a 24-hour LC_{50} of 36.4 (34.2-39.4)mg/L. The study showed that *Moringa oleifera* extract had larvicidal activity on *Anopheles gambiae* with minimal adverse behavioural effects on *Poecilia reticulata*, a natural control agent of mosquito. Hence, production of Moringa-based larvicides can be stimulated through local sourcing to reduce cost and promote sustainable research to develop insecticides based on bioactive natural chemical compounds from indigenous plant sources.

Keywords: Malaria, toxicity, bio-insecticidal efficacy, *Moringa oleifera*, fish behaviour

Introduction

Mosquito transmittable diseases such as malaria are the leading causes of morbidity and mortalities globally: over 300 million clinical cases are attributable to mosquito-borne illnesses yearly [1]. Mosquitoes are common throughout the world; are usually nuisances as well as being primary hosts and or vectors of many diseases of public health importance; such as malaria, filariasis, dengue fever, yellow fever, mosquito-borne viral encephalitis among others. *Anopheles gambiae,* is among the most notorious mosquito vectors of malaria and is the principal vector of Lymphatic filariasis [2, 3].

Vector control remains the major means of combating these mosquito-borne diseases of medical importance: However, the attendant problems associated with the continued use of synthetic insecticides and the increased concern for the protection of the environment in its entirety has necessitated a reduction in the use of the synthetics and an increase in the search for better efficient, ecologically sound and environmentally safe insecticides. In recent times, more attention has been paid to studies of natural pesticides in pest and vector control because they are considered more environmentally safe inputs in pest and vector management [4, 5]. The insecticidal activities of several plant products on either larval or adult stages of mosquitoes and as repellents for mosquito biting have been reported; hence plant-based insecticides are recommended as better alternatives for mosquito control [6, 7]. Guppy fish (*Poecilia reticulata)* is an aquatic organism that predates on mosquito larvae in the ecosystem, thus it plays a key

Correspondence
Ohia Chinenyenwa MD
Department of Environmental
Health Sciences, Faculty of
Public Health, College of
Medicine, University of Ibadan,
Ibadan

role in the biological control mechanism of mosquito as it is readily found in the natural habitats of mosquitoes. *Moringa oleifera* (Lam)*,* a multi-purpose tree native to north western India [8, 9] has so many names depending on ethnic or local languages, including Zogalle (Hausa), Ewé ilé (Yoruba) and Okochi egbu (Ibo).; it is a widely cultivated, fast growing edible plant that is naturalized in the tropics; it is grown in settled areas as a backyard vegetable and oftentimes utilized as a border plant. The *Moringa* tree is a deciduous perennial tree that is regarded as one of the world's most useful trees since almost every part of it is useful; an alkaloid and triterpenoids have been reported in Moringa [10, 11] (Don Pedro, 1990; Isman, 1993). Also Pterrygospermin, a bactericidal and fungicidal compound has been isolated from *Moringa*. The present work's objective was to test the bioefficacy of seed extract of *Moringa oleifera* on larvae of *Anopheles gambiae* and to determine its effects on *Poecilia reticulata* (guppy fish) behaviour.

Materials and methods
Study area and design
A laboratory based experimental study design was employed and this was conducted at the Molecular Entomology and Vector Control unit, Public Health Division, Nigeria Institute of Medical Research, Yaba, Lagos. The study was carried out in two phases viz: A larval bioassay to test the insecticidal efficacy of the aqueous extract of *M. oleifera* (AEMOS) on 3rd instar larvae of *Anopheles* mosquito and a toxicity experiment to assess the acute and chronic toxicological effects of the AEMOS on *Poecilia reticulata* behaviour commonly referred to as Mosquito or Guppy fish. Complete Randomized Block Design was used for the study.

Plant collection and preparation
The plant, *M. oleifera* (Lam) commonly called *Moringa* or Drumstick tree was used in this study and the plant part used was the seed (Kernel). The method of seed collection and handling was according to Vyas and Mistry [12]. Purposive sampling was applied due to the plant's ubiquitous distribution in Nigeria and particularly Ibadan. The seeds used in this study were collected from Akobo area of Ibadan, Oyo State, Nigeria. Fully matured pods of *Moringa* were collected and left under shade to dry at ambient temperature before de-pulping. The de-pulped seeds were pulverised using an electric blender and the powder was stored in an air tight container until used for extraction.

Extraction procedures
The aqueous extract technique was chosen in this study due to the high polarity of water. Being a benign solvent, water is safe, self-preservative and evaporates faster. These features aligned with the primary goal of this study, which was to identify an eco-friendly bio-insecticide, hence the use of a green-solvent extraction technique *in vivo*.
Distilled water was added to the powdered seed in the proportion of 1 seed (approx. 200 mg) per 10 ml of distilled water [13, 14, 15]. The whole mixture was then stirred for 60 minutes at room temperature (25˚C) using a magnetic stirrer and then filtered through Whatman No. 1 paper. Soluble solids concentration of the aqueous extract of *Moringa oleifera* seed (AEMOS) was calculated for the mass present in the water extract to give the yield in weight of soluble solids per weight of powdered seeds.

Mosquito larvae breeding
3rd instar larvae of *A. gambiae s.s.* larvae were cultured and maintained in the insectary of the Molecular Entomology and Vector Control unit, Public Health Division, Nigeria Institute of Medical Research, Yaba, Lagos, under greenhouse conditions (25-30 ºC, Relative humidity 60-70%) following standard operating procedures for mosquito maintenance[16] and modified by Adebayo *et. al.* [17].
The female adult *A. gambiae s.s,*(Kisumu) from already established colony in the laboratory were fed with blood meal from exposed skin of experimental animals (Guinea pigs) in a netted cage (37x30x28 cm) at ambient temperature overnight in a dark room. Also moistened filter papers were gently placed on moistened cotton wool and mounted on petri dishes in the cage to facilitate oviposition of mosquito. After 24 hours, the moistened filter papers were filled with batches of brown-black coloured eggs laid singly on the papers. The filter papers containing the eggs were then carefully transferred into bowls of water. Within 48 hours, the eggs had hatched to larvae and were seen floating parallel to the water surface and examined to confirm that they were *Anopheles* species. The larvae obtained were fed *ad libtum* with baby fish meal (approximately 0.015g) which was evenly spread across the water surface daily in the bowls; the bowls were covered with plastic mosquito net to prevent intrusion of predators of the larvae and escape of emerged adult. The culture medium was maintained according to the standard maintenance procedure [16] until used for the bioassay i.e. third instar stages.

Sampling of *Poecilia reticulata* (GUPPY FISH)
Poecilia reticulata, also called Guppy or mosquito fish used for the toxicity test were obtained from open drains of the Nigeria institute of Medical Research, Yaba, Lagos. The fishes were left to acclimatize for 8 weeks and were kept in well aerated holding tanks under standard conditions of light (12h with alternate day and night cycles) and temperatures 27 ± 2ºC, with access to commercial fish feed diet. The investigational protocol was in accordance with international standard on the care and use of experimental animals [15, 18, 19].

Data collection
Larval bioassay
Treatment doses were determined from a preliminary study [15]; five aliquots from the stock solution were prepared by serial dilution method (1160, 1450, 2900, 5800 and 8700 µg/mL). Three independent experiments were carried out in triplicates each. Distilled water was used as control and 20 larvae per treatment were used [20, 21]. The mosquito larvae were treated with the extracts according to the methodology described by WHO [22]. Larvae behaviour and mortality were verified and recorded after 24 hours of treatment at room temperature (25 ºC) and onwards for 5 days. Larvae were considered dead if they were immobile and unable to reach the water surface after removal into clean water and a further observation for 24 hours.

Toxicity assay
Acute toxicity bioassay on *p. reticulata*
Twenty guppies per treatment were exposed to graded

concentrations (10, 20 and 30 mg/L) of the aqueous extract of *Moringa oleifera* seed (AEMOS); the dosages used in the toxicity study of the guppies are way higher than the dosages found to be larvicidal to the *Anopheles* larvae in the larval bioassay. The highest concentration found to be effectively larvicidal to the *A. gambiae s.s.* was 8700ug/ml and this served as the basis for the determination of doses for the guppy toxicity tests in accordance with toxicity test regulation (i.e. 10-fold of the doses found larvicidal to larvae). These were prepared from the stock solution by serial dilution method; the control contained distilled water without any test solution. Two independent tests in triplicates was setup for the experiment.

Parameters such as effect of AEMOS on loss of reflex, swimming activity and effect of exposure time on loss of reflex and acute toxicity (mortality) were monitored and recorded hourly for the first six hours and thereafter at three hour intervals for the rest 24 hour period [15, 23, 24, 25]. The investigational protocol was carried out in controlled environmental conditions in well aerated holding tanks under standard conditions of light (12h with alternate day and night cycles) and temperatures $27 \pm 2\,^{\circ}C$, with access to commercial fish feed diet and it was in accordance with international standard on the care and use of experimental animals [18, 19, 26].

Statistical analyses
Data were analysed using both descriptive and inferential statistics, percentage mortality observed and the control larval mortalities were corrected using Abbott's formula [27] during observation of effectiveness of the extract. Means were analysed using the Statistica 7 Program and MS EXCEL 2007 to determine the pattern of effectiveness of the extract. Log-Probit analysis was carried out to determine the median (LC_{50}) and 90% lethal concentrations (LC_{90}) values, their 95% confidence intervals were obtained [28]. Regression analysis was also carried out to compare and determine the strength of relation between the doses administered and mortality observed in the mosquito population used in the study. Statistica 7 program and SPSS Software version 15 were used for the analyses at p=0.05.

Results
### Physical characteristics of the aqueous extract of *m. Oleifera* seed powder
M. oleifera seed has important medicinal properties and value and the physical characteristics of the aqueous extract of *M. oleifera* seed powder with respect to weight, colour, and smell are presented in Table 1. Soluble solids concentration of the aqueous extract of *M. oleifera* was taken into consideration in the experiment and was calculated for the mass present in the extract.

Table 1: Physical characteristics of the aqueous extract of *M. oleifera* seed powder

Parameters	Seed Powder	Aqueous Extract
Soluble solids (w/w)	100mg	37mg
Colour	White	Translucent
Smell	Nutty smell	Mild
Taste	Aspartame sweetness	Bland
Appearance	Fine Powder	Clear

Pattern of effectiveness of aemos on *a. Gambiae s.s.* and prolongation of developmental period
The trend of effectiveness of the AEMOS as a larvicide at different concentrations shows that at 24 hours the rate of larval mortality increased as the dose increased across the different treatment levels with the lowest mortality (12.7%) observed for the least concentration (1160 µg/ml) while at the highest concentration it was over 94% mortality. In relation to exposure time, the effect of the larvicide at higher concentrations (2900 µg/ml) at 48 hours was more than twice that recorded at lower concentrations ((1450,1160 µg/ml).(Fig. 1). The optimum effect was observed at 24 hours in the highest concentration(8700 µg/ml) whereas lower concentrations(5800, 2900 µg/ml) attained this only after 48 hours of exposure and the lowest concentration (1160 µg/ml) was only able to produce very minimal larvicidal effect at the end of the experiment. The result of the ANOVA for comparing mortality across the different treatments indicated that mortality records over the exposure period were significantly different among the treatment groups ($p < 0.05$). The larvicidal effect due to the action of the AEMOS increased with increase in concentration whereas there was no mortality in the control (Fig 1). At 2900ug/ml, 59% mortality was observed within 24 hours and at concentrations above this, over 80% of the mortality occurred within 24hours. At lower concentrations, the rate of mortality was very slow and did not reach 40% mortality. In terms of prolongation of larval development, it was also observed that at higher concentrations there was complete inhibition of pupation during the periods of exposure while at lower concentrations some of the larvae in spite of being 3rd instar larvae lived as long as 7 days before they either pupated or died.
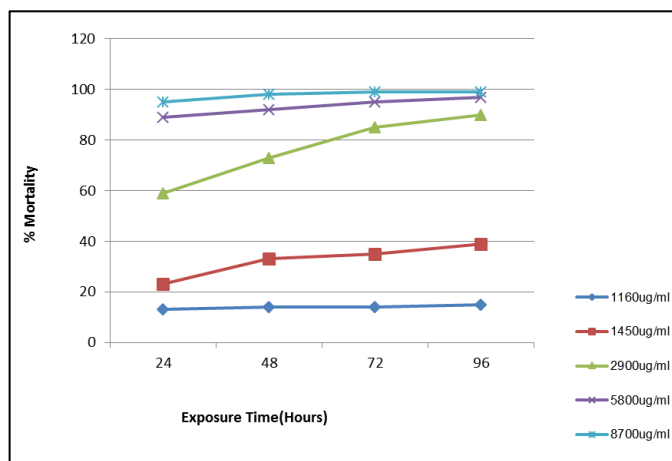
Fig 1: Pattern of Dose-Response relationship at different Exposure periods

Dose-response relationship of aemos and *A. gambiae*
All the concentrations were lethal to the larvae, but with different degree of effectiveness; Fig.2 shows that the exposed larvae responded to the treatments in a dose dependent manner. The effect of the AEMOS on larval activity was evident on introduction of the extract and even hours after exposure. Moribund larvae sank to the bottom of the test solution but when touched with a pin or dropping pipette, they responded with little body movement to move away from the area of disturbance and larval mortality increased across the treatment levels as the concentration increased giving a

sigmoid-like curve upon analysis. This pointed to the fact that mortality was caused by the introduction of the extract while zero percent mortality was observed in the control. All dead and moribund larvae were removed from the solution as soon as sighted using picking pins.
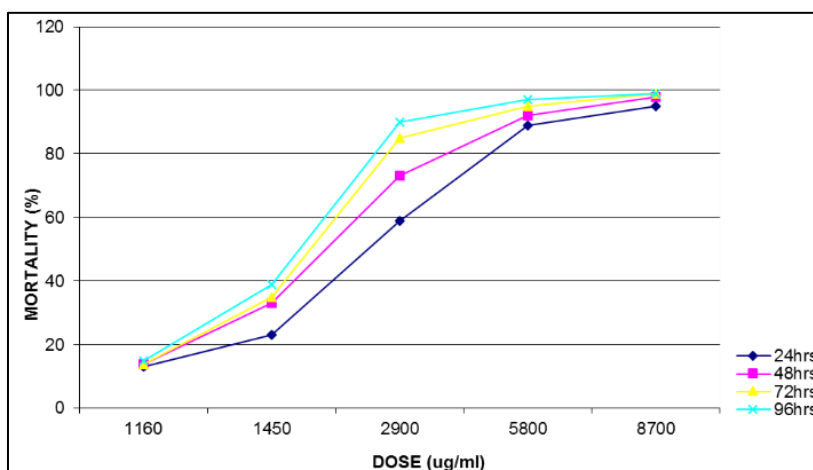

Fig. 2: The larvicidal effectiveness of aqueous extract of *M. oleifera* seed at different Concentrations within 96 hours of exposure

Lethal concentrations determination of the aemos.
The result of the Regression analysis (Fig.3) shows that there was a high linear relationship between mortality of the mosquito larvae and the concentrations of the AEMOS based on the coefficient of Determination values (r^2=0.87) with a linear equation of (Y=0.01x+12.41). The median anti-larval potency (LC_{50}) of the extract at 24 hours was 2505.8µg/ml while the lethal concentration that results in 90% mortality of the population (LC_{90}) was 6293.4µg/ml, as projected by logarithm of the concentration in base 2 (Table 2). The median anti-larval potency (LC_{50}) of the extract at 96 hours was 1754.7µg/ml while the corresponding LC_{90} was 3396.7µg/ml, as projected by logarithm of the concentration in base 2.

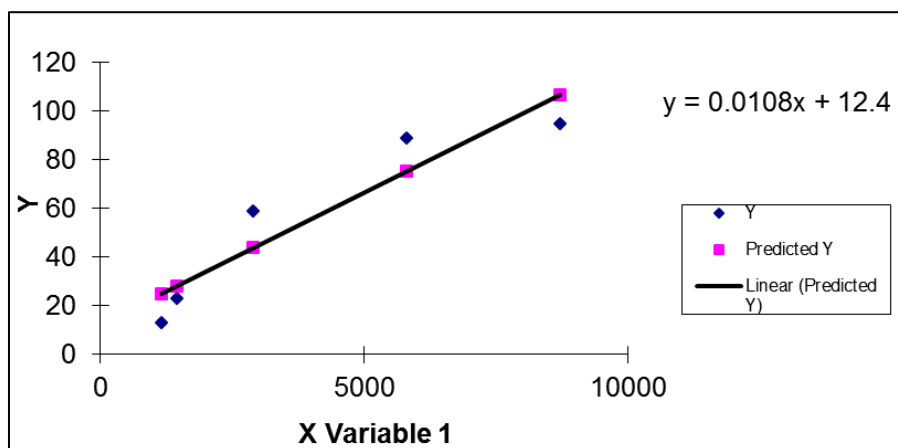**Fig 3:** Regression Analysis showing the line fit plot of larval mortality on concentration

Table 2: Lethal concentrations of AEMOS on larvae of *A. gambiae* s.s. hours after exposure

Time (Hrs)	96[1]	24[1]
LC_{50}[2]	1754.7 (1248.3-2427.4)	2505.8 (2271.6-2760.7)
LC_{90}	3396.7(2448.8-8634.7)	6293.4(5455.2-7528.6)

[1] Hours after exposure
[2] LC values were determined by Probit Analysis (Finney, 1971)
All concentrations were in µg/ml with 95% confidence intervals in brackets.

Toxicity results
**Effect of aqueous extract of *m. Oleifera* seed on fish behaviour**
The effect of the doses on the swimming ability of the fishes within the 24 hour exposure period showed that erratic swimming increased as the concentration increased, (Fig. 4) and at the highest concentration (30mg/L) this was observed to be highest at 12 hours of exposure to the treatment. The effect of exposure time on the guppies in terms of loss of reflex was directly proportional, (Fig.5). In essence, loss of reflex increased as the exposure time increased with the highest observed at 18 hours of exposure to the treatment. The

effect of the different concentrations on loss of reflex was also described (Fig. 6) and it shows that as the concentration increased, the propensity to loss in reflex increased in the fishes, hence, the fishes exposed to higher concentrations exhibited more reflex loss tendencies within the exposure time. The lethal concentration (LC_{50}) of the AEMOS on *P. reticulata* after 24 hour exposure using Probit analysis was, 36.4(34.2-39.4) mg/L, as projected by logarithm of the concentration in base 2.
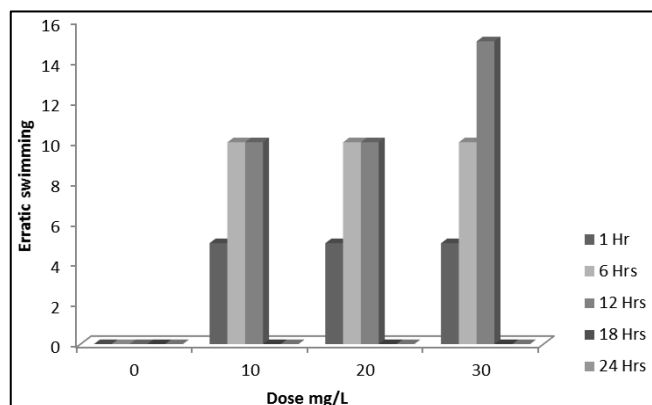
Fig. 4: Effect of treatment doses on fish swimming ability within 24-hours exposure time
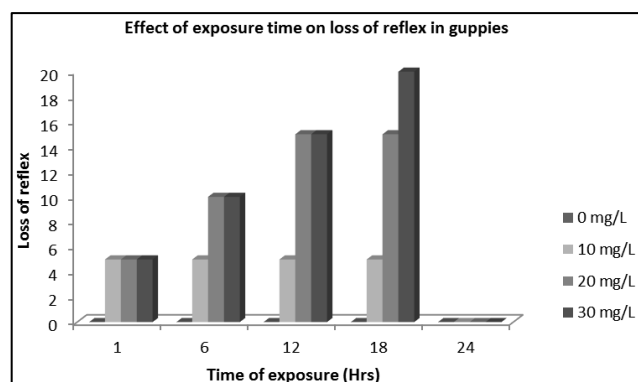
Fig 5: Effect of exposure time on loss of reflex in guppies
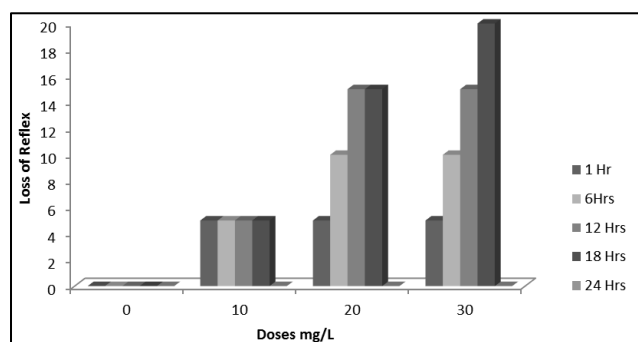
Fig. 6: Effect of treatment doses on loss of reflex in mosquito fishes

Discussion

Control of *Anopheles* mosquito is essential as it is the major and primary vector of malaria, filariasis, and many other arthropod-vector related diseases in sub-Saharan Africa; and they also constitute an intolerable biting nuisance [2, 29]. Recently, there have been concerted efforts at promoting botanicals as environment friendly pesticides, microbial sprays, and insect growth regulators amidst other control measures [30, 31]. A survey of literature on control of different species of mosquitoes reveal that assessment of the efficacy of different phyto-chemicals obtained from various plants have been carried out by a number of researchers in the field of vector control [4, 32, 33].

Efficiency of any mosquito control intervention should be measured by its selectivity for the target organism (specificity). Medicinal plant extracts can be effective as mosquito larvicides and may also greatly reduce the risk of adverse ecological effects as they do not induce any known or recorded insecticide resistance in mosquito; they are also expected to have low human toxicity and a high level of biodegradation [34]. The *Moringa* extract used in this study evinced high larvicidal activity against the third instar larvae of *A. gambiae* s.s. and was observed to increase as the dose increased resulting to an increasing progression in larval death over the exposure period in a dose dependent manner. Early reports on the use of plant extracts against mosquito larvae shows that chemicals from plant extracts have effective larvicidal, pupicidal or adulticidal activities on various species of mosquitoes and also at different stages of their life cycles [2, 32, 35]. Campbell *et.al.*, [36] reported that extract from the Russian weed, *Anabasis aphylla* was larvicidal to *Culex sp* larvae; Ajayi [7] screened 48 medicinal plants in Nigeria for their antimicrobial activity and 23 of these plants (47.9 %) caused over 70% mortality of the test organism including Anopheline and Culicine larvae. Similarly, Nath *et. al.*, [35] indicated that root extract of *M. oleifera* showed larvicidal activity against *Aedes albopictus* and *Culex quinquefasciatus* at higher doses.

In this study, the aqueous extract of *M. oleifera* seed (AEMOS) was observed to have slow action on the mosquito larvae, especially in the lower concentrations; the AEMOS is a natural product and little quantity of it will still yield the desired result over time. In previous studies 32.1ppm of de-oiled neem seed extract gave 85% mortality of *Culex quinquefaciatus* after 12 days of exposure [37]. Okumu *et.al.* [2] also observed that the action of neem oil formulation was slow and increased the mosquito larval period. The implication then is that if at lower concentrations the effect will still be produced, then it makes it a potentially cost-effective larvicide as little of it can achieve much in terms of mosquito control. As can be deduced from the response pattern, an increase in concentration of the AEMOS is directly proportional to larvicidal effectiveness over the exposure period (Fig 1). With respect to time, several factors may be responsible for the *Anopheles* mortality observed in the different concentrations. The main effect is possibly due to tracheal flooding and chemical toxicity. It is also pertinent to suggest that the histopathological effect on the larvae may differ qualitatively according to concentrations assayed and the duration of the treatment on the *A. gambiae s.s.* complex. Based on this findings, it is likely that treating of the 3rd instars would result in more efficient control while giving enough time in days or hours to act on the mosquitoes' breeding medium, thus producing positive outcomes unlike most synthetic chemicals which though may have quick knockdown effects on the mosquito population but with great environmental consequences including environmental imbalance and resistance in the long run. The aqueous extract of *M. oleifera* seed was also very effective on *A. gambiae* s.s.

to minimize its role in malaria transmission as larval mortalities were observed with the use of the respective concentration doses within the exposure periods. Studies with water extract of *M. oleifera* seeds showed a 24hour-LC_{50} value of 1260 ug/ml against 3rd instar larvae of *Aedes aegypti* [14], also methanolic extract of *M. oleifera* seeds were found larvicidal against 3rd instar larvae of *A. stephensis* with LC_{50} and LC_{90}values of 72.5ppm and 139.8ppm respectively[38]; Nath *et. al.*, [35], indicated that root extract of *M. oleifera* showed an LC_{90} of 498.2 and 486.60 ppm respectively against *Aedes albopictus* and *Culex quinquefasciatus.* In contrast, the present study showed 96 hour-LC_{50} value of AEMOS at 1754.7µg/ml and a 24 hour- LC_{50} value of 2505.8µg/ml on *A. gambiae s.s.* Ohia *et. al.*, [15] have also found that aqueous extract of *Moringa oleifera* seed gave a 72-hour LC_{50} value of 1885µg/ml against *Anopheles* larvae in the laboratory. Larvicidal activity may vary depending on the mosquito species and geographical location where plant was sourced [15]. This invariably will determine the level of susceptibility of the mosquito species to the extract and also the weight of soluble solids content present in the plant extract respectively.

The study confirmed that the Moringa extract is an effective larvicide since the control had minimal effect (i.e. less than 20% mortality) on mosquitoes according to WHO standard for testing potential larvicide effectiveness [27] and it is certain that the larvicidal effects observed were due to the AEMOS. Ferreira *et. al.* [14] reported that Water extracts of *Moringa oleifera* seeds (WEMOS) were larvicidal against 3rd instar larvae of *Aedes aegypti,* while Ohia *et. al.* [15] found that aqueous extract of *Moringa oleifera* seeds were larvicidal against 3rd instar larvae of *A. gambiae s.s.* The present study showed that extract of *Moringa oleifera* seed was highly effective as a larvicidal against *A. gambiae s.s.*, this is encouraging and the effect may be due to the active chemical compounds present in the seeds. Phytochemicals derived from Moringa seeds have been suggested as effective for mosquito vector control agents and plant extracts maybe used for future integrated pest management programs [38]. Appropriate bio-technology measures could be adapted to improve its yield and activity in future because according to Essien *et al.* [39], higher plants constitute a major area of resources for mankind especially in the third world; hence the need to fully harness these resources. Relatively, this study shows that the aqueous extract of *Moringa* could be useful for larvicidal purposes in Anopheline control. Burkhill [40], emphasized that the efficient use of plant resources for larviciding purposes serves as alternative to synthetic chemicals in case of insecticide resistance which could naturally occur in addition to higher cost and environmental pollution.

The mode of action of the extract as it impacted on the development of the larvae was studied and it was found that there was delay in the development of the larvae to the pupal stage after exposure of the third instar larvae to all the concentrations of the extract and this was especially noted in the lower concentrations. The benefits of larval prolongation is that mosquito larvae numbers are reduced due to the longer periods needed for new generations to complete the mosquito life cycle [41] and many studies have drawn attention to the effects of plant extracts on growth retardation and elongation of developmental periods on mosquito species. Okumu *et. al.,* [2] found that exposure of *A. gambiae* larvae to *Azadirachta*

indica oil formulation resulted in prolonged larval periods, significant reductions in growth indices and pupation. Mohtar *et. al.,* [42] reported that a methanol-aqueous extract of *Nerium indicum* leaf at 100mg/L had elongation effect on the preimagio period for all the larval instars of *Aedes aegypti* treated compared to the control. Promisiri *et. al.,* [43] posited that there was delay in the development of *Aedes aegypti* larvae to the pupal stage after exposure to three medicinal plants, *Mammea siamensis, Anethum graveolens and Annona muricata.* Mwangi and Mukiama, [44] also observed that a fraction of *Melia volkensi* fruit kernel extract had growth inhibition activity at low concentration on mosquito larvae. However Ferreira *et. al.,* [14] found that water extract of *M. oleifera* seeds did not demonstrate capacity to prevent egg hatching on *Aedes aegypti.* The effect on prolongation of developmental period reported in this study may be due to the presence of low juvenile hormone levels in the larvae or else it may be due to chemical compounds in the plant extract suppressing the presence of ecdysone; preventing normal pupation and preventing movement to the next developmental stage thus preventing adult emergence from occurring with the resultant effect of reducing the mosquito population

With respect to the fish behavioural studies, the induction of erratic swimming on the fishes upon addition of the extract shows that the extract is a toxicant but this behavioural effect reduced with exposure time and this is so possibly because of the biodegradable component of the extract which enabled the fishes to overcome the effect within hours of exposure to the product. The guppies exposed to higher doses experienced continued loss of reflex till 24 hours after exposure unlike in the lower doses where loss of reflex was transient. This effect may be due to the higher dosage of extract in the medium which resulted in prolongation of the effect on the exposed guppies in terms of locomotive ability. This effect was also reduced with exposure time, showing that the effect was transitional in nature as it was not observed again throughout the duration of the study and the behaviour of the fishes in the treatment medium were comparable to what was obtainable in the control medium all through the study. The effect of exposure time on loss of reflex in the mosquito fishes revealed that there was an increasing propensity to reflex loss as exposure time increased especially in the higher doses; this effect climaxed at 18 hours of exposure. Summarily, there was a relationship between the dosage and exposure time on the behaviour of the guppy fishes in the medium throughout the study, these behavioural effects are good pointers and indicators of the effect of this aqueous extract on the non-target aquatic organisms in the natural environment.

The toxic effects on fish behaviour observed in this experiment showed that the extract is rich in toxic ingredients and higher concentrations of the extract of *Moringa* used in this study exerted corresponding toxic effects and death of guppies (*P. reticulata*) tested for the purpose of assessing the selective and acute toxicological impact of the plant extract on ecology. This finding is in tandem with earlier works; *M. oleifera* seed extract at 200mg/L were found to have toxicity and mutagenic effects on guppies, protozoan and bacteria[45] and this is far higher than the dose found to be toxic to mosquitoes in this study.

The acute toxicity assay in the present study showed that the aqueous extract had lethal effect on the guppies at higher concentrations. This report is in agreement with earlier

researches on the toxicity of different chemicals to freshwater fishes; some plant extracts with potential as larvicides have also been found to be lethal to guppies and non-target animals, examples include *Kaemferia galanga and Stemona tuberosa* extracts found to be toxic at concentrations of 50µg/ml [43].

Botanical insecticides possess a comparative advantage over synthetic insecticides due to their mode of action which is usually broad spectrum and also since the bio-insecticides usually have to be ingested by the insect [46]. The implication of this is that bio-insectides are target-specific, primarily harmful to the target pest with little or no deleterious effects on non-target organisms in the ecosystem [46]. Also, in line with the objectives of this study and from the perspective of environmental health, the *A. gambiae* were effectively controlled *in vivo* by direct mortality, thus could force a considerable reduction in their population in the natural environment without causing any adverse effect on the environment as deducible from the non-target study thus directly or indirectly safeguarding the health of the public.

This study showed the AEMOS as a promising and effective larvicide against *A. gambiae* larvae; the use of Moringa seed extract is a low cost appropriate technology that will promote sourcing of locally available natural resources to improve public health status and environmental safety especially in poor settings that are also the communities most vulnerable to mosquito-borne diseases. The report from this study provides clue(s) to what could be expected from a more in-depth investigation of *Moringa*-based extracts on the malarial vector *A. gambiae.* Based on its activity the *Moringa* seed extract may be used to control the malaria vector, *A. gambiae* s.s. and will not be toxic to non-target organisms if used within the dosages lethal to the mosquito larvae. Further study on the mechanism of action, possible synergism with biocides and toxicological impact on non-target organisms are recommended.

Acknowledgement

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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