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Comparative Studies on the Larvicidal Action of Novaluron (Mosquiron® 100EC) and Moringa Oliefera (LAM) Seed Oil against Aedes Aegypti (Diptera: Culicidae) Larvae

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Abstract

Inhibition of emergence (IE) by Novaluron (Mosquiron 100EC) and Moringa oliefera Lam.(Moringaceae) seed oil (MOSO) was assayed with first and fourth instar larvae of Aedes aegypti (Diptera: Culicidae) at ambient laboratory conditions. graded concentration ranging from 3.125- 200µg/ml including a control were tested. Four replicates of each concentration in RCBD were variously challenged with cohorts of 20 active larvae of a given instar. Mortality resulting from eclosion inhibition was recorded at 3-hourly intervals for 12 hours, and data analysed using log/probit transformation. Results indicated dosage-related mortality differences. High concentration of Novaluron gave 95% mortality of both 1st and 4th instars, while sublethal dosages resulted in 45 and 35% mortality of 1st and 4th instars, respectively.

Exposure to Moringa oil resulted in 90 and 100% mortality of 1st and 4th instars, respectively, and sublethal dosages gave 30 and 25% mortality, respectively. Both toxicants showed significant (P<0.05) inhibition of emergence following post-treatment culturing. The IE_{50} values for 1st instar larvae were 4.546µg/ml and 15.44µg/ml of Novaluron and Moringa oil, respectively; and 8.028µg/ml and 9.977µg/ml, respectively, for 4th instar. Although the 1st instar larave were more susceptible, Novaluron was about twice more potent. Nevertheless, Moringa oil has shown promise as biopesticide for Ae. aegypti larvae control.

Key Words: Novaluron, *Moringa oliefera, Aedes aegypti,* Toxicity, Nigeria.

Introduction

Mosquitoes are vectors of many human diseases. According to Clements, 1992, 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 important mosquito transmitted disease with high public health importance include malaria, yellow fever, Dengue haemorrhagic fever and filariasis, these are transmitted by various species of Anopheles, *Aedes* and *Culex* mosquitoes.

Early attempts to control these vectors have relied heavily on the use of synthetic insecticides resulting in several problems, including environment pollution, insect resistance, unacceptable levels of residues, escalating cost of production among others (Otto, 1992).

Science and Technology have recognized the need to reduce synthetic pesticide usage in order to achieve a cleaner environment, even though this would certainly not result in reduced vector control. This conflict in the goal to reduce synthetic pesticide usage and elimination of vectors of diseases, provides a strong impetus for the development of cost-effective and ecologically more begnin alternatives.

Emphasis is on the use of botanical products, ecological controls and others such as insect growth regulators. Synthetic pesticides are used only when necessary and truly justifiable and when acceptable alternatives are lacking (Lim *et al*, 1997). Although, successful control of mosquito vectors with Malathion has been reported (Fradin *et al* 2002), many pyrethroid

insecticides and insect growth regulators (IGRS) are now being directed towards mosquito control. Prominent among the IGRS is Novaluron, a novel acylurea acting as a chitin synthesis inhibitor (Mulla *et al* 2003) affecting the moulting in insect development. It causes mortality in larvae on acdysis after ingestion or contact and causes abnormal endocuticlar deposition and abortive moulting (Stephen, 2005). Emergence prevention is the primary goal of IGR larvicidal treatment and inhibition of emergence (IE) results in death. Mulla *et al* (2003) reported that inhibition of larval emergence was 100% at 0.25 to 1.0g/l of Novaluron® under laboratory condition while in the field condition using clay jars, there was 86-96% inhition of emergence (IE) for about 190 days. Novaluron is registered as insecticide for both food crop and ornamentals in many countries. World Health Organization assessed its mosquito Larviciding activity for *Aedes species* and recommended a dosage application of 0.05mg/l (WHO, WHOPES, 2005).

On the other hand, plants are by far the most efficient "factory" of chemical compounds, synthesizing many products that are used in the defense against many pests and vectors (Schoonhoven *et al*, 1998). Thus, the use of plant extract in agro-ecosystem is now emerging as one of the primary means to protect the environment from toxic synthetic pesticides pollution (Schumuterer; 1995). Jany *et al.* (2002) reported the larvicidal activity of leguminous seeds and grains against *Aedes aegypti* and *Culex pipens pallens* while Bassole *et al* (2003), investigated the ovicidal and larvicidal activities of leaves of three plants naturally growing in Burkina Faso.

Many researchers are now investigating Moringa plant for its use as pesticides. Donli and Dauda (2002) reported the use of aqueous Moringa seed extracts as seed dressing bio-fungicide for groundnuts in Nigeria. Adandonon *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 *Moringa oleifera* seed oil with *Sesamum indicum* and *Olea europaea* against *T. castaneum.* Aqueous extracts of leaves of *Moringa oleifera* Lam, *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 together with other nineteen Indian plants against *Ae. albopictus* and *C. quinquefasciatus*.

Horse radish, *Moringa oleifera* Lam. is an abundant invasive plant found throughout Nigeria and many other African countries which has generated a

lot of interests among Scientists in many African countries for its medicinal and other uses (Ozumba, 2005). It potentials as bio-pesticide need to be further evaluated. In the present study investigations were aimed at evaluating the larvicidal action/ inhibition of emergence by an insect growth regular, Novaluron (Mosquirom® 100EC) and botanical plant *Moringa oleifera* seed oil extract for the control of mosquito, *A. aegypti* larvae.

Materials and Methods

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

Sources of Mosquito Larvae and Rearing

Eggs of *Aedes aegypti* were obtained from at the Federal Ministry of Health, Department of Public Health National Arbovirus and Vector Research Center, Enugu, Nigeria. They were hatched and reared in the laboratory at $26\pm2^{o}C$, $80\pm5\%$ r.h. The eggs were placed in transparent plastic containers containing 500mls of distilled water and allowed to hatch to 1^{st} instar larvae and kept to reach the 4^{th} instar. The larvae were fed on crumbs of biscuit brand, Yale Fortune Cabin Sweetened Biscuit; the feed was applied on alternate days for normal development.

Moringa Seed Collection and Oil Extraction

The *Moringa oliefera* seed was collected from the moringa plantation in Enugu, Nigeria owned by the third author. The seeds were dried under shed for 7 days and decorticated to obtain the white kernel. The dried seeds were pulverized using an electric blender to obtain fine powder. Thereafter, the oil extraction was carried out using petroleum ether in Soxhlet Extractor 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 250ml of petroleum ether was poured through a funnel, by-passing the thimble, containing the sample into the round bottom flask system of the Soxhlet apparatus. This was heated about 5cm above a hot electric plate while cold water was allowed to reflux in and out of the Extractor to cool the condenser compartment. After many refluxes, the petroleum ether was gradually evaporated from the oil extract. The process was repeated six times to allow the solvent distill off at about $75^{o}C$, leaving the light yellow oil.

Prepaartion of the Oil Extracts

Serial dilutions of the *M. oliefera* seed oil (MOSO) were prepared in acetone. The pure oil extract was regarded as 100% concentrate and subsequently

diluted serially to 20%, 10%, 5%, 2.5%, 1.25%, 0.625% and 0.3125% in acetone.

Novaluron (Mosquiron® 100EC) Preparation

The Novaluron (Mosquiron®100EC) was obtained from Dizengoff West Africa Ltd, Nigeria. Based on the WHO (2005), recommended dosage of 10% ai, a measured volume of Mosquiron® 100EC was first diluted down 10% ai equivalent to (10EC) subsequently serial dilutions were made in acetone to obtain 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml, and 3.125µg/ml.

Bioassay Tests

The method and standard procedures used were those of Mulla *et al.* (1986) and WHO (1981). The different concentrations of the above mentioned preparation obtained from diluting in acetone were used. Aliquots of 1ml of each dilution were separately placed in plastic cups containing 160ml distilled water. Each container was challenged with batches of 20 active fourth or first instar larvae of *Aedes aegypti* accordingly. Controls were also included.

Each treatment and control was replicated five times. The bioassay was repeated two times. The tests were carried out at ambient laboratory temperature of 26$\pm$ 2^{o}C, 80$\pm$5% relative humidity and photoperiod 12:12 light and dark hours. Mortality/ inhibition of emergence assessments were made at 3-hourly intervals for 24 hrs. Dead larvae were counted and recorded. However, exuviae or shed cuticle started appearing in control treatments after about 2 hrs but none became pupae or adult. Both moribund larvae, that is, those unable to wriggle were counted as dead.

Data Analysis

Mortality data obtained were corrected using Abbot Formula (1925). Log-probit regression analysis was carried out (Finney, 1971) for determining LD_{50} or level of inhibition of emergence ($1E_{50}$). Analysis of variance (ANOVA) was further carried out also performed on the mortality data and means separated using least significant difference (LSD) effectiveness at the different time intervals and concentration levels.

Results

The mortality values of 4th instar larvae of *Ae aegypti* exposed to Novaluron (Mosquiron 100EC), an IGR and *Moringa oleifera* seed oil extract at 3 hourly intervals are presented in Table 1 and 2 respectively. The results show

that novaluron and moringa oil exhibited significant (P< 0.05) levels of toxicity to *Ae Aegypti* larvae. Mortality increased with increasing concentration of the IGR and oil and also with increasing period of exposure. However, there were much greater responses at higher concentrations of toxicant (no mortality was observed in the control). Generally, dose-time related mortality responses were noted in all treatments with higher ranges of the toxicant causing almost 100% death or inhibition of emergence in the larvae, while in lower concentrations, larval mortality was incomplete. However the time of exposure had more significant impact than dosage. The log- probit regression analysis revealed that after 12 hours of exposure, the LD_{50} (IE_{50}) of the novaluron treated 4th instar larvae was 8.028µg/ml and moringa is 9.977µg/ml (Fig 1).

Tables 3 and 4 respectively showed that mortality of 1st instar larvae of *Ae aegypti* exposed to novaluron and moringa oil increased significantly (P<0.05) with increasing levels of concentration and also with increasing period of exposure to the toxicant. The LD_{50} (IE_{50}) value of novaluron on 1st instar larvae after 12 hours was 4.546µg/ml and that moringa was 15.44µg/ml (Fig 2). Mortality in the second 3- hours for both 4th and 1st instar larvae was significantly (P< 0.05) higher than that of the first 3- hours. This trend was observed in all the treatment irrespective of the dosage. However, at highest concentration, 200µg/ml, the percentage mortality caused by the IGR on the 4th and 1st instar were 95% respectively (Tables 1 and 3) . A similar trend was observed in the moringa treated larvae (Tables 2 and 4). The 1st instar was significantly (P< 0.05) more susceptible to the two toxicant than the 4th instar larvae.

Discussion

The larvicidal action of Novaluron (Mosquiron® 100EC) and *Moringa oliefera* seed oil (MOSO) on both 1st and 4th instar larvae of *Ae. aegypti* was studied in the laboratory. The studies are important to understand how these two insecticides of both synthetic and plant origin can be used as independent control method or as adjunct to other control measures in reducing the impact of mosquitoes. In the present study the general performance of Novaluron in killing the larvae of *Ae. aegypti* was observed in the relatively high percentage larval mortality recorded in both 1st and 4th instar larvae, high percentage inhibition of emergence and LD_{50} values. This may explain why WHO recommended its use as a mosquito larvicide (WHO, 2005).

There was increase in the percentage mortality with increased concentration, as the time progressed. The 100% inhibition of emergence (IE %) in both 1st and 4th instar larvae observed in the present studies is in accordance with the studies by Mulla *et al.* (2003), who reported that percentage inhibition of emergence was 100% at concentrations of 0.25 to 1.0g/L on both 2nd and 4th instar larvae of *Ae. aegypti*. In addition, Kostyukovsky and Trostanetsky (2004) reported that novaluron reduced hatching of 3rd instar larvae of *Tribolium castaenum* by 100%. These suggest that novaluron selectively target immature insect stages by inhibiting chitin formation and so causing abnormal endocuticular deposition and abortive molting. The benzoylureas act on larval stages of most insects by inhibiting or blocking the synthesis of chitin (Soltani et al., 1995), a vital and almost indestructible part of the insect exoskeleton (Chebira et al., 2000). More than being the typical poisons that attack the insect nervous system, they interfere with chitin synthesis and they are more taken up by ingestion than by contact (Soltani et al., 1995). Typical effects on developing larvae are the cleaving of malformed cuticle or death by starvation (Chebira et al., 2000). Su et al. (2003) reported that Novaluron exhibited a high level of activity against Culex mosquitoes.

On the other hand, Moringa seed oil was also found to have promising larvicidal properties. The toxicity effect of the oil was high, as the mortality and the rate of inhibition of emergence increased with increased concentration and time. The effectiveness of plant oil in regulating growth and inhibition of many insect and fungal development has been reported previously (Jilani and Su, 1983, Mulla et al., 2003, Kostyukovsky and Trostanetsky, 2004). Manas et al. (2005) reported that *Moringa oleifera* extracts inhibited 97.32% germination of fungal pathogen. Shema and Sexena (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. This was observed in the present study as only few surviving larvae were seen in the treated sample, which were unable to develop to the next stage. Pushpalatha and Muthukrishnam (1995) reported that leaf extracts of *Vitex negundo* at very low concentration had larvicidal activity against *Culex quinquefasciatus* and *Anopheles stepensi* and also extended the duration of larval instar pupation.

These insecticides, Novaluron and Moringa seed oil have shown to be very effective in mosquito control as seen in the present study. Novaluron action is by chitin inhibition according to Soltani *et al*. (1995), Mulla *et al*. (1974, 2003) and Kostyukovsky and Trostanetsky (2004) for novaluron and MOSO action as growth regulator and by blocking the respiratory surfaces. According to Hirashima et al. 1998, Enan, 2001) some plant essential oil block the octopamine neuroreceptors that regulate the movement, heart rate, behaviour, metabolism, and pupation of insects. This regulatory activity of these two insecticides was evidenced by the death recorded in both 1st and 4th instar larvae and inhibition of their emergence to pupae. In both insecticide treated water, it was obviously noticed that the larvae were weakened before death occurs.

In the present studies, the 1st instar larvae of *Ae. aegypti* were more susceptible than the 4th instar. This observation was confirmed by the previous studies that early instars of insects are more susceptible to the inhibitory and growth regulatory effects of these insecticides (Mulla et al., 2003). This is however suggested to be due to the size and the tender nature of the chitin in the early instar of mosquitoes. The observation by Mulla *et al*. (2003) that 2nd instar of *A. aegypti* was more susceptible than 4th instar using novaluron and Akpa *et al.* (2003) that 3rd instar was more susceptible than 4th instar when a plant, *Phytolacca dodecandra* oil was used against *Ae. Egypti* larvae verify these findings.

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Table 1: The mean mortality effects of different concentrations of Novaluron on 4th instar larvae *Aedes aegypti* after 12 hours interval

Mean of Mortality (%)

Dose (µg/ml)	*3(hrs)*	*6(hrs)*	*9(hrs)*	*12(hrs)*	*Mean*	*% Mortality*
200	1	11	12	19	4.75±1.9	95
100	3	7	12	17	4.25±0.8	85
50	1	5	10	15	3.75±1.1	75
20	1	4	10	14	3.5±1.2	70
12.5	1	3	7	12	3.0±1.3	60
6.25	1	3	5	9.0	2.25±0.9	45
3.125	1	2	4	7.0	1.75±0.05	35
Total	9.0	35.0	60.0	93		
Mean	1.29± 0.2	5.00± 1.5	8.57±1.8	13.29±2.0		
Control	0	0	0	0		
LSD= 2.214						

Means of five replicates (± s.e)

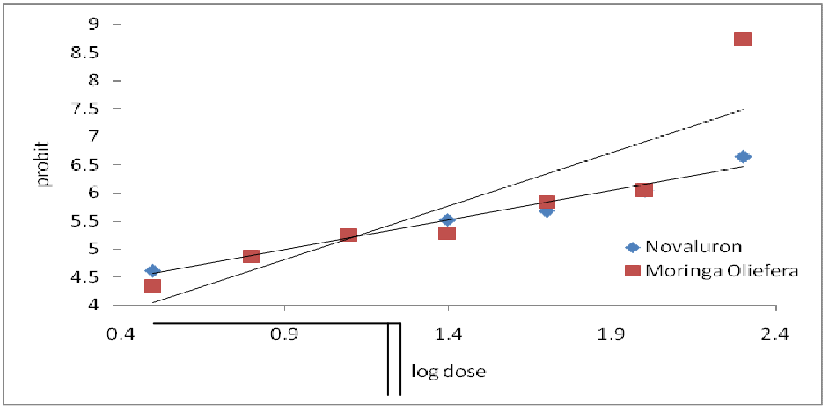
Table 2: The mean mortality effects of different concentrations of MOSO on 4th instar larvae of *Aedes aegypti* mosquito.

Mean of Mortality (%)

Dose (µg/ml)	*3(hrs)*	*6(hrs)*	*9(hrs)*	*12(hrs)*	*Mean*	*% Mortality*
200	1	4	4	20	4.75±1.5	100
100	1	2	11	17	4.25±1.8	85
50	2	4	11	16	4.00±1.4	80
20	1	3	7	13	3.25±1.3	65
12.5	1	3	6	12	3.0±1.2	60
6.25	1	2	4	9	2.25±0.1	45
3.125	1	2	3	5	2.25±0.1	25
Total	8.0	20.0	64.0	91		
Mean	1.14± 0.1	2.85± 1.1	8.00±1.5	13.00±2.1		
Control	0	0	0	0		
LSD= 2.44						

Means of five replicates (± s.e)

Figure 1: Probit vs log_{10}dose of Novaluron and MOSO on 4th instar larvae of *Ae. aegypti*



The regression equations are given as: Probit (Y) = 0.912X + 3.916 where X = log dose value

From the graph, for Novaluron, log LD_{50} = 0.9046. So, LD_{50} = 8.0280µg/ml for *Moringa Oliefera* log LD_{50} = 0.999. So, LD_{50} = 9.977µg/ml

Table 3: The mean mortality effects of different concentrations of Novaluron on 1st *Aedes aegypti* mosquito after 12 hours interval

	Mean of Mortality (%)					
Dose (µg/ml)	*3(hrs)*	*6(hrs)*	*9(hrs)*	*12(hrs)*	*Mean*	*% Mortality*
200	10	13	17	19	4.75±1.6	95
100	8	11	16	18	4.5±1.4	90
50	6	8	11	17	4.25±1.3	85
20	9	12	14	15	3.75±1.2	75
12.5	8	9	11	14	3.5±1.2	70
6.25	6	8	10	11	2.75±1.1	60
3.125	4	5	7	9	2.25±0.09	45
Total	51	66	86	103		
Mean	7.29± 2.1	16.5± 4.2	12.29±2.5	14.71±3.3		
Control	0	0	0	0		
LSD= 1.59						

Means of five replicates (± s.e)

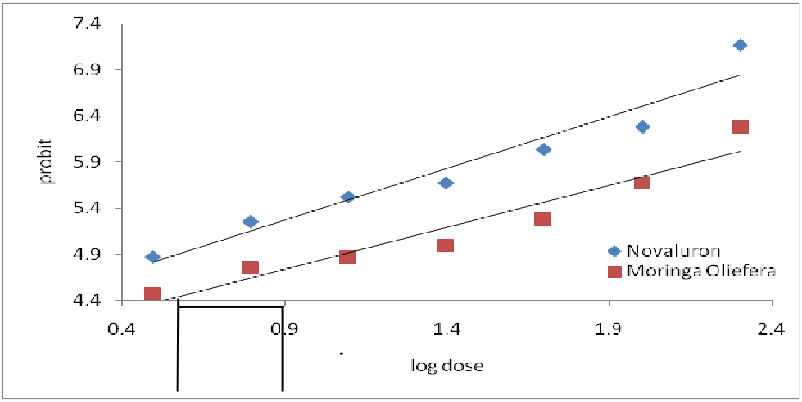
Table 4: The mean mortality effect of different concentrations of MOSO on 1st instar larvae of *Aedes aegypti* mosquito

	Mean of Mortality (%)					
Dose (µg/ml)	*3(hrs)*	*6(hrs)*	*9(hrs)*	*12(hrs)*	*Mean*	*% Mortality*
200	5	14	16	18	4.5±1.5	90
100	5	10	12	15	3.75±1.1	75
50	6	8	9	13	3.25±1.3	65
20	5	7	8	10	2.5±1.1	50
12.5	5	6	8	9	2.25±1.1	45
6.25	2	5	6	8	2.0±0.07	40
3.125	2	4	5	6	1.5±0.07	30
Total	30	54	58	79		
Mean	4.28± 0.8	7.71± 2.1	9.14±2.2	11.28±3.3		
Control	0	0	0	0		

LSD= 1.73

Means of five replicates (± s.e)

Figure 2: graph of probit against logdose on effect of Novaluron and MOSO on 1st instar larvae of *A. aegypti*.



The regression equation is given as
Probit (Y) = 0.912X + 3.916 where X = log dose value
From the graph, for Novaluron, log LD_{50} = 0.6578. So, LD_{50} = 4.546µg/ml
for *Moringa oliefera* log LD_{50} = 1.1886. So, LD_{50} = 15.44µg/ml