TOXICITY AND LIFE EXPECTANCY EFFECTS OF *Moringa oleifera* SEED EXTRACTS ON THE LARVAE OF *Anopheles gambiae*

NJOM, Victor Stephen and EZE, Chukwuma Simon
Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu.

Corresponding Author: Njom, V. S. Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology, Enugu. **Email:** njomvic@yahoo.co.uk **Phone:** +234 8033898156

ABSTRACT

The toxicity and growth regulatory effects of Moringa oleifera seed extracts were assessed on third larval instars of Anopheles gambiae, under laboratory conditions. Mortality at different concentrations varied but increased as concentration level increased. Mortality progressed on daily bases throughout the period of experimentation. At highest and lowest concentrations of 5%w/v and 1%w/v, larval mortality was 80% and 10% respectively in a day. Molting to next larval instar was prolonged in all concentrations. No adult emerged in higher concentrations, though few adults emerged in lower concentrations, they were weak to fly. This study clearly showed that Moringa oleifera extract has larvicidal and growth regulatory properties on larvae of Anopheles gambiae.

Keywords: Toxicity, Growth regulatory, *Moringa oleifera* seed extract, *Anopheles gambiae* larvae

INTRODUCTION

Insects are the most important single group of the phylum arthropoda in terms of public health and agricultural importance. Many transmit diseases while others are pests of stored or field crops (Collins and Paskewitz, 1995). Among these insects, mosquitoes seem to be very important because they transmit a number of diseases such as malaria, filariasis, dengue, Japanese encephalitis etc (Jaswanth *et al.,* 2002). Malaria is wide spread in the tropics affecting about 350 – 500 million people worldwide resulting in the death of more than a million annually (WHO, 2008).

One of the malaria control measures is the use of synthetic insecticides which repeated use has resulted to many well known and serious problems such as development of resistance by the insects (Brown, 1986), environmental pollution (Killeen *et al.*, 2002) and toxic effect on man and his domesticated animals (Barat *et al.,* 2004). Environmental protection agencies have banned or placed severe restrictions on the use of many synthetic pesticides which were formerly used in mosquito control programmes and there are now fewer adulticides available than there have been for the last 20 years (Carvalho *et al.,* 2008), even there are indications that effective synthetic insecticides may not be available in the near future (Poonam *et al.,* 2002). These problems have stimulated the search for biologically based alternatives. Accordingly biological insecticides based on natural compounds from plants are expected to be a possible alternative. They have broad spectrum activity, are relatively specific in mode of action and easy to source and process (Belmain *et al.,* 2001). Natural product of plant origin have been shown to act as insect larvicide, ovicides, adulticides, growth regulators, repellent and ovipositor attractant (Babu and Murugan, 1998; Venketachalam and Jebasan, 2001; Anyanwu *et al*., 2001; Cetin *et al.,* 2004; Lucantoni *et al.,* 2006).

Moringa oleifera Lam. (Moringaceae) commonly called "horseradish" is one of such plants with great potentials. Its leaf extracts show antioxidant and hypocholesterolaemic activity (Chumark *et al.,* 2008). The seeds possess antimicrobial properties (Ali *et al.,* 2004), ovicidal and larvicidal effects on *Ae. aegypti* L. (Paulo *et al*., 2009). Information on effects of *Moringa oleifera* on the malaria vector *Anopheles gambiae* have been limited in this part of the world despite the havoc the vector is causing. This research amongst others intends to assess the toxicity and growth regulatory effects of *Moringa oleifera* on the larvae of *Anopheles gambiae* (Diptera: Culicidae).

MATERIALS AND METHODS

Mature dry seeds of *Moringa oleifera* were collected from Professor Okafor, J. C. of Botanical Research Center, Enugu, Nigeria. The seeds were dehulled manually. Spoilt seeds were discarded while quality ones were ground into fine powder. Powdered seeds were measured out in 1, 2.5 and 5g, each mixed with 100 ml of distilled water in three different 250 ml conical flasks. Several sets were prepared. The mixtures were left overnight and later each was stirred for 2 hours with a magnetic stirrer at room temperature. Each stirred mixture was filtered with double layered cheesecloth. The various flasks constitute concentrations of 1, 2.5 and 5 %, respectively.

Mosquito collection and maintenance: A stock of adult *Anopheles gambiae* was obtained from National Arborvirus Research Center, Enugu. The mosquitoes were kept and maintained in a 60 x 30 x 30 cm^3 net-screened wooden cage. Mosquitoes were fed with 20% glucose solution in three 30 cm enamel bowels placed at strategic positions within the cage. After 4 days of rearing all mosquitoes were starved for 12 hours and later provided mammalian blood meal (with de-haired rats placed in resting cage) for them to feed on. Oviposition traps made of black coloured 6 x 4 x 4 cm^3 plastic cups containing wet filter papers were randomly placed inside the cages and left for 72 hours. Eggs laid on the filter papers were harvested carefully with aid of stereo-microscope and placed in an enamel bowel with white back ground and allowed to hatch into larvae. The mosquito stages were maintained at temperature of 28 ± 2 ^{0}C, 75 – 80 % relative humidity, with 12:12 light and dark photoperiod. Larvae were fed fish fingerling feed (10g per 25 larvae) daily with regular change of water to avoid pollution from uneaten feed.

Assay for larval mortality: Larval mortality was tested with third instar larvae using 1, 2.5 and 5 % concentrations of *Moringa oleifera* seed water extracts in three 500 ml conical flask, each containing 200ml of distilled water. Each concentration received 25 larvae and each treatment replicated three times. Control larvae for mortality test were kept in 200 ml of distilled water. Larvae were fed with fish fingerling feed at the ratio of 25 larvae per 2g of feed. Mortality was recorded every hour for 12hrs. Mortality was calculated using Abbot's formula (1925). Abbot's formula = percentage of test mortality - percentage of control mortality ÷ 100 – Percentage of control mortality x 100.

Larval life span assay: Larval life span was tested using first instar larvae. Twenty larvae were placed in three 500ml glass beaker with 200 ml of distilled water. Each set of beaker received 0.5%, 1.0%, and 2. 5% concentrations of water extracts of *Moringa oleifera*. Larval life span was recorded in days. Difference in larval mortality and larval life span were analyzed using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The toxicity of Water Extract of *Moringa oleifera* seed (WEMOS) was evaluated on larvae of *Anopheles gambiae* by observation. The result showed that the extract exhibited toxicity activity on larvae and also delayed pupal development from surviving larvae. The toxicity activities were concentration dependent (Table 1). At highest concentration of 5.0 % (g/100ml) mortality was 80%, 2.5 % (g/100ml) concentration recorded 41.3% mortality while least concentration of 1.0% had 9.33% mortality (Table 1).

Table 1: *Anopheles gambiae larva*e mortality in different concentrations of *Moringa oleifera* seed water extract

Extract concentration (g/100ml H_2O)	Hourly mortality												Total larval mortality	% mortality	Mean mortality
	1	2	3	4	5	6	7	8	9	10	11	12			
5.0	7	11	6	5	8	3	4	5	3	4	4	0	60	80	5 ± 0.78
2.5	4	1	2	3	1	2	5	2	4	4	1	2	31	41.33	2.58 ± 0.40
1.0	0	0	1	0	1	2	1	1	0	0	1	0	7	9.33	0.78± 0.19
Control	0	0	2	0	0	1	0	0	0	0	1	0	4	5.33	0.67± 0.23

Table 2: *Anopheles gambiae* larval life span after treatment with *Moringa oleifera* seed water extract

Extract concentration (g/100ml H_2O)	Larval life span (days)
5.0	0.0 ± 0.0*
2.5	0.0 ± 0.0*
1.0	14.3 ± 0.3
Control	9.2 ± 0.4

**Larvae died before pupal stage.*

Pupal life span was prolonged in the least concentration of 1.0% compared to the control (Table 2). In the other concentrations (2.5% and 5.0%) no larvae survived up to pupal stage (Table 2). These agree with findings of (Paulo *et al.,* 2009) who demonstrated toxicity of water extracts of *Moringa oleifera* seeds on *Ae. aegypti.* Plants extracts have been shown to have insecticidal properties (Senthill and Kalaivani, 2005; Lucantoni *et al.,* 2006; Kamsuk *et al.,* 2007). They are easy to source and are environmentally friendly. *Moringa oleifera* extract has properties which can be exploited and used for malaria vector control.

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