Larvicidal Activity of Aqeous Extract of *Moringa oleifera* Seeds on *Anopheles gambiae* and its Effects on *Poecilia reticulata.*

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ABSTRACT

Malaria is a major public health problem and its prevalence is dependent on mosquitoes. There is an increased interest in developing plant-based insecticides as sustainable alternatives to chemical insecticides for the control of mosquitoes. The potential of Moringa plant as a larvicide against the malaria vector, *Anopheles gambiaes. S* was evaluated and its effects on mosquito fish were also studied. Toxic components in *Moringa oleifera* seeds were extracted using; five aliquots of concentrations ranging from 1160-8700 µg/ml were prepared by serial dilutions from the extract. Three independent laboratory procedures were run in quadruplicate in which third instar larvae of *Anopheles gambiaes. S* was exposed to different concentrations and a control group was exposed to distilled water. The fishes were exposed to three graded treatment of the aqueous extract in two experiments run in triplicate. Behavioral changes were observed for 24 hours. From the larval bioassay, the extract presented a 72hour- LC50 and LC90 of 1885.4 µg/ml, 3902.4 µg/ml respectively, ($p<0.05$), causing 99.33±1.15%mortality at 8700µg/ml. The toxicity assay showed that the guppy fishes exhibited varying degrees of changes as a result of the exposure to treatment unlike in the control. The results obtained show that Moringa seed extract has larvicidal potentials for *Anopheles gambiaes. S* with observed little or no toxicity effects on the non target, guppy fish. The effectiveness of Moringa seed extract makes it a viable larvicide. Moringa-based larvicides may provide cheap and readily available alternative to synthetic insecticides.

Keywords: Malaria, mosquito control, Guppy fish, Larvicidal activity, *Moringa oleifera* seeds.

INTRODUCTION

Malaria is a severe public health problem that is primarily transmitted by mosquitoes belonging to the genus Anopheles. It is a leading cause of death and disease in mostly developing countries including Nigeria, where young children and pregnant women are the groups most affected and accounts for 25% of U-5 mortality, 30% of child mortality and 11% of maternal mortality. At least 50% of the population has at least one episode of malaria annually, while children that are aged below 5 years (about 24 million) have 2 to 4 attacks of malaria annually. It is also the reason for hospital attendance in 7 out of every 10 patients seen in Nigerian hospitals (FMH, 2005).

Vector control is recognized as the major means of combating mosquito-borne diseases of public

health importance. However, prolonged exposure to synthetic insecticides increases development of resistance mechanisms in the mosquitoes, hence leading to the resurgence of malaria and mosquito-borne diseases in different communities, deleterious effects on non target organisms, while other limiting factors include cost and environmental pollution concerns (Brown 1986; Junwei *et. al.,* 2006)

Plant-based insecticides have been long proposed as alternatives to synthetic chemical insecticides for vector and pest management because they pose little threat to human and environmental health (Choochote *et. al*., 1999, Murray, 2005). Several plants around the world have been identified with insecticidal (larvicidal, pupicidal and adulticidal) properties against mosquitoes either in the seeds, fruits, barks, roots or in their root exudes. However such compounds must be evaluated with respect to their toxicological effects under laboratory conditions, in accordance with international standardized procedures (Zucker, 1985, OECD, 2004).

Moringa oleifera (Lam) belongs to the family Moringacae and is commonly called drum stick tree, horse radish West India Bern tree or "Miracle tree". In Nigeria, it is called Ewé ilé (Yoruba), Okochi egbu (Ibo) and Zogale (Hausa). It is a multipurpose, rapidly growing crop indigenous to North West India (Cidamis*et al*., 2003). It is commonly planted in Africa as a living fence tree (Von Maydell, 1986). This "Miracle Tree" is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates which have been reported to have anti-hypotensive, anticancer and antibacterial activities (Bharah*et al.,* 2003; Fahey, 2005; Adedapo *et al.,* 2009,)

This study was therefore designed to determine the larvicidal effects of aqueous extract of *Moringa oleifera* seeds on the larvae of *Anopheles gambiaes. s*., the primary vector of malaria in Nigeria and to study its impact on mosquito fish, a common non-target organism found in mosquito habitat.

MATERIALS AND METHODS

Plant material and Extract preparation

Recently collected mature dry seeds were dehulled manually and crushed into powder using an electric blender. Distilled water was added to powdered seed in the proportion of approximately 220mg per 10 ml of distilled water (Gerdes, 1997; Ferreira *et.al*., 2009). A magnetic stirrer was used to stir the whole mixture for 60 minutes at room temperature (25°C) and then filtered through Whatman No. 1 paper. Soluble solids concentration of the AEMOS was taken into consideration.

Experimental Animals

Mosquito larvae breeding

The larvae of *Anopheles gambiae S.* was used in this study and was obtained from the Institute of Medical Research, Yaba, Lagos. These larvae were cultured and maintained at 25-30^{o}C, with relative humidity 60-70% under a photoperiod of (12h: 12h) following standard operating procedures for mosquito maintenance (WHO, 1975) and modified by Adebayo *et al* (1999). The larvae were free of exposure to pathogens, insecticides or repellents and were maintained in tray by providing dog biscuit and yeast powder.

Guppy fish

Poecilian reticulata, commonly called guppy fish were used for the toxicity test and were obtained from the environs 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 , with access to commercial fish feed.

Data Collection

Evaluation on larvae of *Anopheles gambiae S.*

Different concentrations of the AEMOS (between 1160, 1450, 2900, 5800and 8700 ug/mL) were prepared using distilled water. The mosquito larvae were treated with extract according to the methodology described by WHO (1981). Twenty larvae of *Anopheles gambiae* were introduced in different test concentration of the extract along with control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in the laboratory, five replications were maintained for each concentration and mortality observed and recorded 24 hours for 3 days at room temperature. Larvae were considered dead if they were immobile and unable to reach the water surface.

Toxicity Evaluation on *Poecilia reticulata*,

Twenty guppies per treatment were exposed to different concentrations of the AEMOS (10, 20 and 30 mg/mL) with control containing non chlorinated water without any test solution. Treatment was introduced randomly, two independent tests in triplicates were setup for the experiment. The behavioral conditions and mortality in each setup was observed so as to define the response of the test organism to aqueous extract of *Moringa* seed. The behavioral parameters were monitored and recorded hourly for the first six hours and there after three hourly for the rest of 24hours period. The investigational protocol was carried out in controlled environmental conditions so as to define the response of the test organism to the *Moringa* seed extract and it was in accordance with international standard on the care and use of experimental animals (EEC Directive, 1986; American Public Health Association, 1987; Ferreira, 2009).

Statistical Analyses

Data were analyzed using descriptive statistics, the control mortality was corrected using Abbott's formula (WHO, 2003) and Log-probit analysis was carried out to determine the median (LC50) and 90% lethal concentrations (LC90) values, their 95% confidence intervals were obtained by the method of Finney (1971). SPSS Software version 15 was used for the analysis.

RESULTS AND DISCUSSION

Larvicidal activity

Assessment of the efficacy of different phytochemicals obtained from various plants have been carried out by researchers on different species of mosquito in the field of vector control (Promisiri *et al.,* 2006, Okumu *et al.*, 2007 and Ferreira *et al.,* 2009).

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 gambiae* and the mosquito larvae exposed to the different concentrations showed significant behavioral effects such as inability to swim to the top within 45 minutes of exposure. All the concentrations were lethal to the larvae but with different degree of effectiveness as was observed from (Figure1). Okumu *et.al* (2007) reported that neem oil formulation was toxic to third instar larvae of *A. Gambiae* while the effectiveness of the extracts of *Piper nigrum* Linn. against *Culexpipien, Aedesaegypti* and *Aedestogoi* have been established(Park *et. al*., 2002). Leaf extract of *Lantana camara* and *Catharanthusroseus* were found to be highly toxic to *Aedesaegypti* even at low doses (Remiaand Logaswamy, 2010). *Moringa oleifera* extract in combination with *Trichoderma* soil sprinkle (a bio-control agent) was highly effective against *Sclerotiumrolfsii*, the causative agent of the damping off and stem rot disease in cowpea with more than 94% and 70% efficacy in disease control in the green house and field respectively (Adandonon*et.al*. 2006). Njom and Eze (2011) reported that *M*oringa seed extracts were larvicidal to *Anopheles gambiae*larvae exposed to different concentrations.

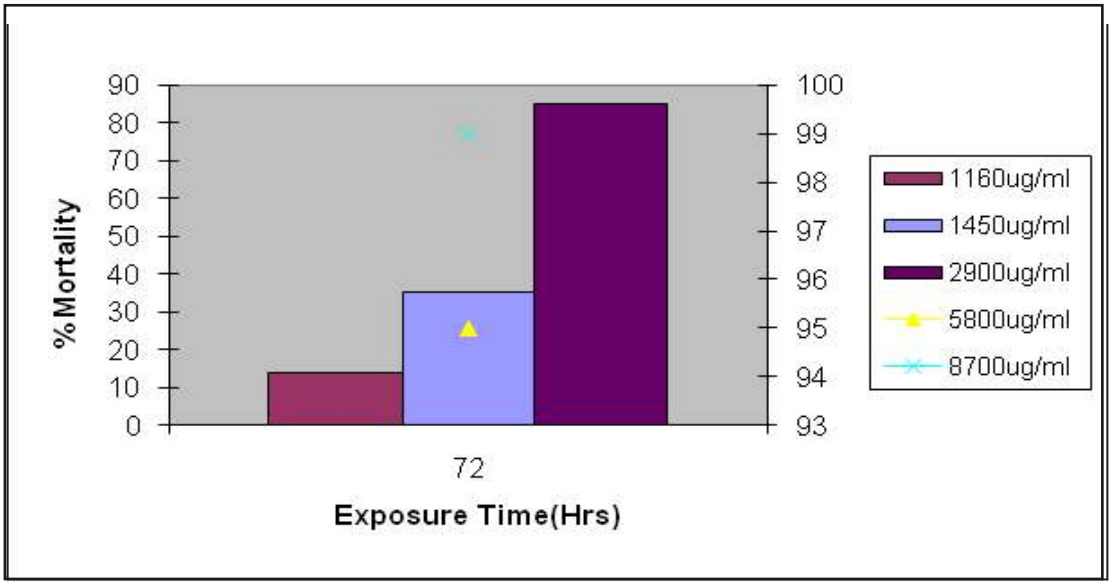

Fig 1: Larvicidal effect of aqueous extract of *Moringa oleifera* on larvae of *A. gambiae* at 72 hours of exposure.

There was an increased progression toward larvae death over the period of exposure of the larvae to the treatments in a dose dependent manner (Figure 2). Studies on plant derived insecticides have shown that some plant extracts have dose dependent mode of action against target organisms. The larvicidal activity of neem oil formulation against *Anopheles gambiae* was observed to increase as the dose increased (Okumu *et a*l. 2007). Also, similar trend was observed in the investigation of the efficacy of two botanicals against *Aedesaegypti* (Remia and Logaswamy, 2010).

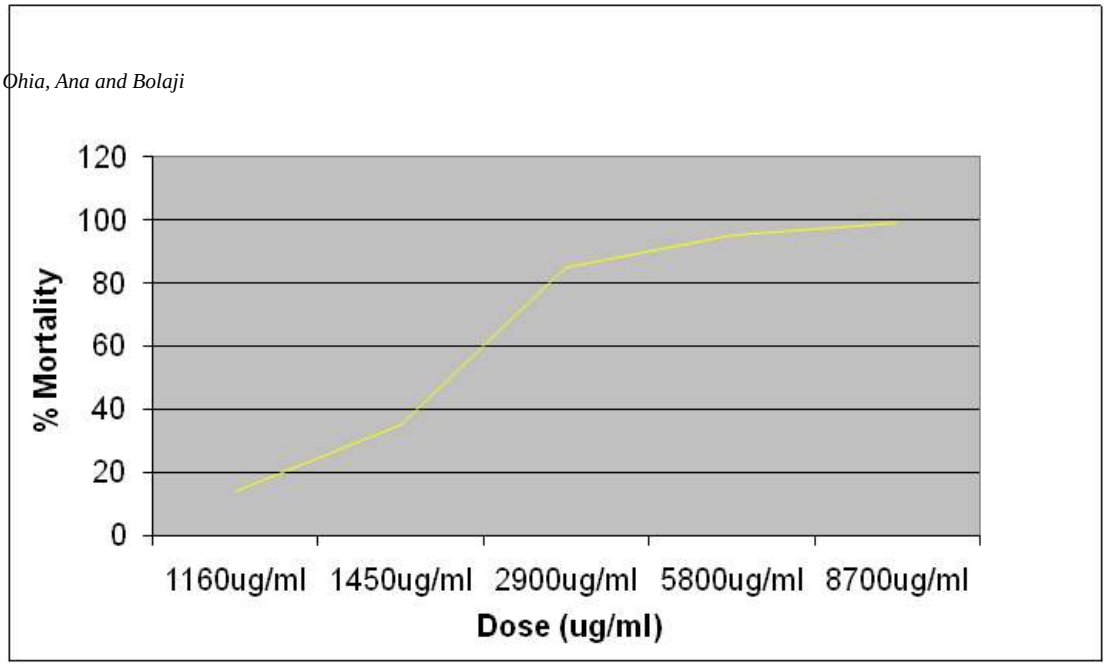

Fig.2: Dose response curve of larvae at 72 hours of exposure to AEMOS

Table 1 shows the toxic effect of aqueous extract of *Moringa oleifera* against larvae of *Anopheles gambiae S.* under 72 hour exposure. The lethal concentration of the extract (AEMOS) was 1885.4ìg/ mL for the larvae. Studies with Water extract of *Moringa oleifera* seeds showed LC50 value of 1260 ug/ml against 3rd instar larvae of *Aedesaegypti* (Ferreira *et. al.,* 2009).

*Table 1 - Toxicity of aqueous extract of Moringa oleifera against larvae of Anopheles gambiae*s. S. *under 72 hour exposure*

		LC	95% Fiducial	
Plant part	stage of exposure	(g/mL)g/ mL)	Upper	Lower
Seed	III instar	1885.4	2481.9	1405.8

[1] LC value were determined by Probit Analysis (Finney, 1971)

All concentrations in µg/ml with 95% confidence intervals.

Also methanolic extract of *Moringa oleifera* seeds was found to be larvicidal against 3rd instar larvae of *An. Stephensis* with LC50 and LC90 values of 72.45ppm and 139.82ppm respectively (Prabhu *et al.*, 2011). In contrast, the present study showed LC50 value of AEMOS at

1885.4ug/ml on *Anopheles gambiaes.S.* Larvicidal activity may vary depending on the mosquito species and geographical location where plant was sourced.

Toxicity assay

The behavioral responses observed in the toxicity assay show that the guppies showed variations in their tolerance to aqueous extracts of *Moringa oleifera (*Table 2) On the ddition of the toxicant, various toxic reactions such as erratic movements, air gulping with prolonged exposure resulted in dullness and discoloration in higher concentrations.

Table 2: 24-Hour Behavioral of *Poecilia reticulate* (Definitive Test)

Exposure Time/ Behavior	1 Hr				6 Hrs			12 Hrs			18 Hrs			24 Hrs		
Concentration(mg/mL)	0	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
Erratic Behavior	+	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-
Air gulping	-+	+	+		-	-	-	-	-	-	-	-	-	-	-	-
Dullness	-	-	-	-	-	-	-	-	+	+	-	-	+	+	+	+
Loss of Reflex	-+	+	+		-	+	+	-	-	+	+	+	-	-	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Key: + =Present
- =Absent

This report agrees with the work of many authors (Balza *et al.*1989, Muniyan and Veeraraghavan 1999, Ayuba and Ofojekwu 2002 and Chung-Min Liao *et al.* 2003 and Ayotunde *et al.*, 2011.) that worked on the toxicity of different chemicals to freshwater fishes. Promisiri *et al.* (2006) reported that *Mammea siamensis, Anethumgraveolens* and *Annonamuricata* demonstrated no or very low toxicity to guppies at concentrations active to mosquito larvae. *Moringa oleifera* seeds have earlier been reported to be non-toxic and recommended for use as coagulant in developing countries (Olsen, 1987, Olayemi and Alabi, 1994 and Schwarz, 2000).

CONCLUSION

The diversity of plant species is huge and phytochemicals obtained from them are important sources of safe and biodegradable chemicals which can be screened for mosquito and insecticidal activities and tested for safety in mammals (Mittal *et al*., 2003). Evaluation of the aqueous extract of *Moringa oleifera* seed against third instar larvae of *Anopheles gambiae* in the laboratory showed that it was larvicidal to the mosquito species and with very minimal toxic effects observed in the non-target animal, *Poecilia reticulata*. 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.

REFERENCES

Adandonon, A., Aveling, T. A. S., Labuschagne, N. and Tamo, M. (2006). Biocontrol agents in combination with *Moringa oleifera* extract for intergrated control of *Sclerotium*-caused cowpea damping-off and stem rot. *European Journal of plant pathology 115(4):409-418.*

Adedapo, A. A., Mogbojuri, O. M. and Emikpe, B. O. (2009). Safety evaluations of the aqueous extract of the leaves of *Moringaoleifera* in rats. *Journal of Medicinal Plants research* 3(8):586-591.

American Public Health Association (1987). Standard method for examination of waste water and water (17th ed. WashingtonD.C.) 8910 pp.

Ayotunde, E. O., Fagbenro, O. A and Adebayo, O. T (2011). Toxicity of aqueous extract of *Moringa oleifera* seed powder to Nile tilapia *Oreochromis niloticus* (LINNE I779), fingerlings. *International Research Journal of Agricultural Science and Soil Science* 1(4): 142-150.

Ayuba, V. O. and Ofojekwu, P. C. (2002). Acute toxicity of the Jimson's weed (Daturainnoxia) to the African catfish (*Clariasgariepinus*) Fingerlings *AJOL. J. Aqua. Sci.*17 (2)

Balza, F., Abramowski, Z., Neil, G.H., Towers, Ghn. and Wiriyachitra, P. (1989). Identification of proanthocyanidin polymers as the piscicidal constituent of *Mammeasiamensis, Poylgonumstagninum* and *Diospyrosdiepenhorstii. Phytochemistry,* 28:1827-1830.

Bharah R., Tabassum J. and Azad M. R. H. (2003). Chemomodulatary effect of *Moringa oleifera*Lam. On hepatic carcinogen metaboling enzymes, antioxidant parameters and skin papillomagensis in mice. *Asian Pacific J. Cancer Prev.* 4:131-139.

Brown, A. W. A. (1986). Insecticide resistance in mosquitoes: a pragmatic review. *Journal of America Mosquito Control Association* 2:123-139.

Choochote, W., Kanjanapothi, D. A., Taesotikul, T., Jitpakdi, A., Chaithong, U., Pitasawat, B. (1999) Larvicidal adulticidal and repellent effects of *Kaempferiagalanga. Southeast Asian Journal of Tropical Medicine and Public Health, 30:470-476.*

Chung-Min, L., BoChing, C., Sher, S. M., Chen- Wuing, L. and Bor-Cheng, H (2003). Acute toxicity and bioacummulation of arsenic in tilapia (*Oreochromis mossambicus*) from a blackfoot disease area of Taiwan. *Environ. Toxicol.* 18 (4): 252-259.

Cidamis, A. B., Panga, J. T., Sarwatt, S. V., Chore, B. E. and Shayo, N. B. (2003). Nutrient and antinutrient contents in raw and cooked young leaves and immature pods of *Moringa oleifera,* Lam. *In*; *Ecology of Food and Nutrition,* 42:399-411.

Fahey, J. W. (2005). *Moringa oleifera:* A review of the medical evidence for its therapeutic and prophylactic properties. Part 1. *Trees for Life J*. 1:5-15.

Ferreira, P. M. P., Carvalho, A. F. F. U., Farras, D. F., Cariolano, N. G., Melo, V. M. M., Queiroz, M. G. R., Martins, A. M. C and Machado-Neto, J. G. (2009). Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedesaegypti* and its toxicity upon laboratory animals. Anais da Academia Brasileira de Ciencias (*Annals of the Brazilian Academy of Sciences*), 81 (2): 207-216.

Finney, D. J. (1971). Probit Analysis (Third Edition). 3rd edition. *Cambridge UniversityPress*, UK.

Forestry/ Fuel wood Research and Development Project (1992). Growing Multipurpose Trees on Small Farms. Bangkok, Thailand: *Winrock International*. 195 + ix pp. (including 41 species fact cards).

Gerdes, C. (1997). Comolimpar e trataraguasuja com sementes de *Moringa oleifera,* Centro de Fortaleza: *Centro de Pesquisa e Assessoria*, 18p.

Junwei, Z., Yanma, Z. X., Ting, L., Kuen, Q., Yuhua, H., Suqin, X., Tucker, B., Schultz, G., Coats, J., Rowley, W. and Aijun, Z. (2006). Adult repellency and larvicidal activity of five plant essential oils. *J. Amer. Mosqu. Contr. Assoc.*, 3: 515-522.

Mittal, P. K.,Subbarao, S. K. (2003). Prospects of using herbal products in mosquito control. *ICMR Bulletin,* 33(1):1-10

Muniyan, M. and Veeraragghavan, K. (1999). Acute toxicity of ethofenprox to the fresh water fish *Oreochromis mossambicus* (PETERS). *J. Environ. Biol*. 20 (2): 153-155.

Murray, B. I. (2006). Botanical Insecticides, Deterrents and Repellents in modern and an increasingly regulated world. *Annual review of entomology,* 51:45-66.

Njom, V. S and Eze, C. S (2011). Toxicity and life xpectancy effects of *Moringa oleifera* seed extracts on the larvae of *Anopheles gambiae. Animal Research International* 8(2): 1388 1391

Okumu, O. F., Knols B. G. J and Fillinger, U. (2007). Larvicidal effects of a neem (*Azadirachtaindica*) oil formulation on the malaria vector *Anopheles gambiae*. *Malaria Journal*, 6:63

Olayemi, A. B. and Alabi, R. O. (1994). Studies on traditional water purification using *Moringa oleifera* seeds. *African Studies Monographs*. 15(3). 135-141.

Olsen, A. (1987). Low technology, water purification by bentonite clay and *Moringa oleifera* seeds flocculation as performed in Sudanese village: Effects of *Schistosoma mansonicercariae. Water Res*., 21:81-92.

Organization for Economic Co-operation and Development. (2004). Guidelines for Testing of Chemicals. Guidelines 202: *Daphnia sp.* Acute Immobilisation Test. *Paris France*, P. 260-275.

Park, I. K., Lee, S. G., Shin, S. C., Park, J. D. and Ahn, Y. J. (2002). Larvicidal activity of isobutylamides identified in *Piper nigrum*fruit against three mosquito species, *J. Agric. Food Chem* 50,1866-1870.

Pradhu, K., Murugan, K., Nareshkumar, A., Ramasubramanian, N. and Bragadeeswaran, S. (2011). Larvicidal and repellent potential of *Moringa oleifera Anopheles stephensis*Liston (Insecta: Diptera: Culicidae). *Asian Pacific Journal of Tropical biomedicine* (2011): 124-129.

Promisiri, S., Naksathit, A., Kruatrachue, M. and Thavara, U. (2006). Evaluations of larvicidal activity of medicinal plant extracts to *Aedesaegypti* (Diptera: Culicidae) and other effects on a non target fish. *Insect Science*, 13: 179-188.

Remia, K. M and Logaswamy, S. (2010). Larvicidal efficacy of leaf extract of two botanicals against the mosquito vector *Aedesaegypti* (Diptera: Culicidae) *Indian Journal of Natural Products and Resources*.1 (2): 208-212.

Roll Back Malaria Initiative/Federal Ministry of Health of Nigeria (2005). Malaria Control for Primary Health Care workers in Nigeria; *a Proceeding at Osun State Health Workers workshop*, March.

Schwarz, D. (2000). Water clarification using *Moringa oleifera.* eschborn: *JDWH Information service* (Online). Available from http://www.gtz.de/gate/gateid.afp

Von Maydell, H. J. (1986). Trees and Shrubs of the Sahel, Their characteristics and uses. Deutsche Gesellschaft fur TechnischeZusammenarbeit (GTZ). *Federal Republic of Germany*. 334-337

World Health Organization (1975). Manual on practical entomology in malaria part 2: methods and techniques. Geneva: *World Health Organization*.