ASSESSMENT OF THE INSECTICIDAL POTENCY OF NEEM (*Azadirachta indica*) SEED KERNEL METHANOLIC AND AQUEOUS EXTRACTS ON THE MALARIA VECTOR *Anopheles gambiae*

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ABSTRACT

The potency of aqueous and methanolic extracts of neem (Azadirachta indica A. Juss) seed kernel, in inhibiting and disrupting development of Anopheles mosquito was assessed in the laboratory. Different concentrations of aqueous and methanolic extracts were tested on eggs, larvae and pupae. Both extracts were found to cause mortality on the specimens used and the level of mortality was concentration dependent. Mortality increased as the concentration increased. Methanolic extract of the neem seed kernel caused significantly higher (P = 0.05) mortality than aqueous extract. Few adults emerged among those treated with lowest concentration of 1 %(wt./vol.) aqueous extract, whereas no larvae or pupae survived to next stage in methanolic extract of the same concentrations. At highest concentration of 5 % (wt/vol), both extracts caused 100% mortality of larvae although the methanolic extract gave a quicker kill (12 hours) than the aqueous extract (24 hours). No egg hatched in all concentrations of both extracts.

Keywords: Potency Assessment, Neem seed kernel, *Anopheles gambiae*

INTRODUCTION

Man since his arrival on earth has been faced with the problem posed by insects. Insects constitute pest of field crops, stored products and some are parasites or vectors of many parasites that cause human and animal diseases (Collins and Paskewitz, 1995). Among these insects, mosquito seems to be the most important. They are man's worst enemy because their females suck blood and in the process transmit the deadly malaria parasite - *Plasmodium* sp. Malaria is wide spread in the tropical and subtropical Africa, parts of Asia and Americas. About three million people are at risk of infection in malaria endemic countries (WHO, 2008). It is estimated that 350-500 million cases are reported annually, and between one and three million of such cases end up in death (WHO, 2008). Majority of the victims of malaria attacks in sub-Saharan Africa are pregnant women and young children below the age of five years (Snow *et al.,* 2005). Africa alone has about 90 percent of such malaria related deaths especially among under fives (WHO, 2008).

Nigeria account for a quarter of all malaria cases within the African region (WHO, 2008). In Nigeria the main vectors of malaria parasite include the following *Anopheles* species: *An. arabiensis*, *An. bronchieri*, *An. constani*, *An. flavicosta, An. funestus, An. gambiae, An. hancocki, An. hargreavis, An. melas, An. moucheti, An. nili, An. paludis* and *An. pharoensi*, (WHO, 2006). These vectors breed in wells, over head tanks, or ground level tanks, used tires, cisterns, roof gutters, coconut shells, open used beverage tins and small pools of water on road surfaces (Harrell *et al.,* 2001).

Such sites and their proximity to human habitation encourage human-mosquito contact and consequently opportunity for malaria transmission during blood meal of the female *Anopheles*.

In view of the enormous problem posed by malaria both health-wise and socioeconomically, there have been concerted efforts to break the cycle of transmission of malaria from mosquito to man. Such efforts range from: (i) the use of drugs targeted against the protozoa parasite- *Plasmodium* sp., (ii) development of vaccine that can provide durable potent immunity and therefore protect human host against the parasite, (iii) development of transgenic or genetically modified *Plasmodium* resistance mosquitoes (Tan and Sung, 2008), (iv) use of sterile mosquito technique and (v) use of biological agents such as natural enemies like bacteria (*Bacillus thuringiensis israelensis)* and fishes (like *Tilapia* sp, *Carp* sp and Guppies) to eat up the larval stages of the vector (Ito *et al.,* 2002). However, the last four methods have not been largely successful because of the challenges bordering on technicality, acceptability and application (Tan and Sung, 2008).

The common method that has since been in use is the use of conventional insecticides against adult mosquitoes (Cao *et al.,* 2004). These conventional insecticides have recorded tremendous success but their use have generated concerns because; of environment pollution (Killean, *et al.,* 2002), insect resistance (Okumu *et al.,* 2007), neuro-toxic effect on man and his domesticated animals (Killean, *et al.,* 2002), high cost (Barat *et al.,* 2004) and unavailability to the poor who are at risk of infection (Barat *et al.,* 2004). Any effective alternative malaria vector control measure must: (i) address the epidemiological situation and the risk factor involved in transmission, (ii), be appropriate for controlling the specific vector species, (iii) be simple to understand and apply, (iv) be affordable and based on locally available resources and technical skills, (v) acceptable and compatible with local customs and practices, and (vi) be safe for the user and the environment.

To ensure effective mosquito control there is the need to develop and incorporate new alternative tools for integrated vector management where methods aimed at reducing adult mosquito bites and control of their aquatic larval stages are combined.

Plant derived products are usually readily available, safe and more ecologically acceptable (Senthill and Kalaivani, 2005). Some of them have been shown to have larvicidal , growth regulatory, repellence and egg laying inhibition effects on larvae and adults of some insects respectively (Lucantoni *et al.,* 2006; Kamsuk *et al.,* 2007). Such plants and their derived products have been shown to contain various active ingredients with different modes of action which is believed to lessen the chance of resistance developing in target insect populations.

Among plants that have evoked interest as a source of bio-insecticide are members of the family Meliaceae. They have been shown to possess anti-feeding and growth regulatory properties on certain insects (Saxena *et al.,* 1984; Jacobson, 1987; Schmutterer, 1990; Gianotti, 2008). A member of the family, *Azadirachta indica* A. Juss commonly known as neem has been showed to be a promising source of plant derived insecticide. Content of its leaves, seeds, and bark showed marked insecticidal properties on a wide range of insect species (Schmutterer, 2002; Isman, 2006). The trees are widely spread in many parts of Nigeria almost in every village. The seed kernels have insecticidal properties because they contain approximately ninety nine active compounds the most potent of which is azadirachtin, present in a concentration of about 5mg/g of kernel (Schmutterer, 1995). Though there have been works on the use of neem plants and other plants extracts for the control of certain insects, information on impact of neem plant extracts on *Anopheles gambiae* mosquito and have been limited. This research therefore as its objective intends to assess the potency of aqueous and methanolic extracts of neem seed kernel on egg, larvae and pupae of *Anopheles gambiae* S.L (Diptera: Culicidae) - the malaria vector.

MATERIALS AND METHODS

Neem Seed Collection, Processing and Extraction: Fallen ripped fruits of *Azadirachta indica* A. Juss, were picked. The bulk was sorted; rotten and poor quality fruits were removed while the good ones were washed with distilled water to remove soil and other dirt. The fruits were depulped manually and seeds shelled. Seed kernels were picked, oven dried to constant weight at 50^0C for 72 hours. Dried kernels were pounded with wooden pestle and mortar into a finer powder which was later divided into two equal parts (A and B).

Part A of the neem powder was weighed into 1, 2.5 and 5 grams and placed in three different 250ml flasks. Each was added 100ml of distilled water to give concentrations of 1%, 2.5%, and 5% solutions (wt/vol.); shaken vigorously and left overnight. The suspension was later strained through fine cheesecloth and kept as stock A.

Part B was soaked in 250 ml of petroleum ether over night and later agitated with a magnetic stirrer for two hours to remove neem oil. The solution was filtered with a Whatman filter paper Number 1 and the residue extracted with absolute methanol in a soxhlet extractor. Extracted samples were collected in a pre- weighed 250 ml beaker. Extracting solvent (methanol) was evaporated using a Rota-vaporizer. The semi-solid extracts were dried to solid powder in an oven at 50^0C for 2 hours. One, 2.5 and 5 grams were weighed out and added 100ml of methanol each in three different 250ml flasks to make 1, 2.5 and 5% solution (wt/vol.) which constitute the stock solution B.

Mosquito: A stock of adult *Anopheles gambiae* S.L was obtained from National Arborvirus Research Center Enugu. The mosquitoes were kept and maintained in a 60x30x30cm wooden cage net-screened on all sides. Mosquitoes were fed with 20% glucose solution in three 30cm enamel bowels placed at strategic positions within the cage. After 4-days of rearing all mosquitoes were starved for 12 hours and later provided with de-haired rats placed in resting cage to feed on. Later oviposition traps made of black coloured 6 x 4 x 4 cm^3 plastic cups containing wet filter paper 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. Some were isolated in a separate dish and used to assay for ovicidal/egg hatching inhibitory effects, while others were placed in an enamel bowel with white back ground and allowed to hatch into larvae. The mosquito stages were maintained at an average temperature of 28 $\pm$ 2^0C, 75 – 80% relative humidity, with 12:12 light and dark photo period. Larvae were fed on fish fingerling feed; 10g per 25 larvae.

For hatching inhibition and ovicidal effects, two sets of three glass Petri dishes (6cm diameter) were filled with 100ml of distilled water. The first set were labeled AE_1, AE_2 and AE_3 while the second set ME_1, ME_2 and ME_3 for 1, 2.5 and 5% concentrations of aqueous and methanolic seed kernel extracts respectively. Each dish received 30 eggs and every concentration was replicated three times. A control was kept for each set with water and methanol as treatment substance respectively. The set up was observed for 8 days for any egg hatch. Inability of egg to hatch was deemed as egg mortality.

Bioassay: Larval mortality was assessed with third instar larvae using 1, 2.5 and 5% concentrations of both extracts in 250 ml of water. Each concentration received 25 larvae and each treatment was replicated three times. Control was kept for each replicate using water and methanol for aqueous and methanolic sets respectively. Larvae were fed with fish fingerling feed at the ratio of 25 larvae per 2g of feed. Mortality was recorded every 12 hours for 3days (72 hours). Difference in larval mortality was analyzed using ANOVA for both extracts.

Pupal duration was tested using 4 hour old pupae. Twenty Pupae were placed in two sets of three 250ml glass beaker with 200ml of distilled water. Each set of beaker was labeled as above and treated with 1, 2.5 and 5% concentrations of aqueous or methanolic extracts of neem seed kernel. Pupal duration was recorded in days. Difference between treatments was subjected to analysis of variance.

Table 1: Total egg hatch in different concentrations of both extracts

Concentration	Extracts	Number of hatched egg
1.0%	Aqueous	0.0
	Methanolic	0.0
2.5%	Aqueous	0.0
	Methanolic	0.0
5.0%	Aqueous	0.0
	Methanolic	0.0
Control	Distilled water	179

Table 2: Mortality rate of different concentrations of extracts of neem seed kernel on *Anopheles gambiae* larvae

Concentration	Neem Extract	Time (Hours) 12	24	36	48	60	72	Total	Mean	%
1.0%	**methanol**	3	5	9	8	9	12	46	7.67±1.31	61.33
	Aqueous									
2.5%	**methanol**	5	8	10	13	12	15	66	10.5±1.48	88
	Aqueous									
5.0%	**methanol**	75	0	0	0	0	0	75	00±0.0	100
	Aqueous	53	22	0	0	0	0	75	34.50±0.0	100
Control		0	0	0	0	0	0	0	00±0.0	0

Table 3: Total mean pupal duration (days) of *Anopheles gambiae* after treatment with neem seed kernel extracts

Concentration	Extracts	Mean Pupal duration (days)
1.0%	methanol	3.2±0.5*
	aqueous	2.7±0.3
2.5%	methanol	5.6±0.4*
	aqueous	4.8±0.3*
5.0%	methanol	5.7±0.6*
	aqueous	5.6±0.5*
Control	Distilled water	2.6±0.5

RESULTS AND DISCUSSION

The potency of aqueous and methanolic extracts of neem seed kernel on *Anopheles* mosquito egg, larvae and pupae were evaluated by observation and are presented in Tables 1, 2 and 3 respectively. In Table 1, no egg hatched in all the concentrations of both extracts. In Table 2, methanolic extract of neem seed kernel caused 100% larval mortality within 12 hours at highest concentration of 5 % (wt/vol.). Reducing concentration to 2.5% and 1% recorded 88% and 61.33% mortality respectively. For aqueous extract, mortality were (26%), (42.67%) and (100%) in 1%, 2.5% and 5% concentrations (wt/vol.) respectively. Table 3 showed the mean duration (days) of pupal stage of *Anopheles gambiae* after treatment with both extracts. At 1.0% concentration of aqueous extract, few pupae developed into adult stage. While in other concentrations no adult emerged as all pupae died. This study demonstrates the potency of both aqueous and methanolic extracts of neem seed kernel in the control of *Anopheles gambiae*. Exposure of mosquito to both neem extracts caused mortality of third instar larvae of *Anopheles gambiae* and also prolonged pupal duration (in days) of survived pupae compared to control. Mortality effect of both extracts was concentration dependent. Methanolic extract appeared more lethal as it recorded 100%

mortality within 12 hours at the highest concentration of 5%. The same feat was achieved by aqueous extract (at same concentration) but at a longer period of 24 hours. The mortality recorded by aqueous extract might be attributed to oil content of the aqueous extract which was not removed during processing. Oil usually leads to deficiency of dissolved oxygen in water (Aliero, 2003) which probably may have lead to death of eggs, larvae and pupae by suffocation. Mortality of egg, larvae and failure of pupae to develop to adult stage could also be attributed to the action of some neem's bioactive constituents such as azadirachtins which are known to interfere with neuro-endocrine regulation of juvenile and molting hormone titers during insect development (Rembold, 1989; Mordue and Blackwell 1993; Senthill and Kalaivani, 2005).

In conclusion, both neem seed kernel extracts caused mortality of egg, third instar larvae and also prolonged pupal duration of *Anopheles gambiae.* It is therefore a potential source of biological insecticide for the control of *Anopheles gambiae* and other insect pests. The plant can be sourced easily at little or no cost and is environmentally friendly. Aqueous extract of neem seed kernel is therefore recommended for local adoption as it is cheap and easier to prepare.

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