Comparative Evaluation of Larvicidal Potentials of Three Plant Extracts on *Aedes aegypti*

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Abstract: The activities of ethanol extracts of *A. indica*, *O. gratissimium* and *C. citratus* on *Ae. aegypti* larvae were investigated at 96 hours exposure. Mortalities were observed to increase with concentration ($P \leq 0.05$).The larvae of *Ae. aegypti* exhibited differential susceptibility to the extracts of the three plants.. In *C. citratrus*, mortality was recorded all through the exposure period of 96hours comparing to *A. indica* and *O. gratissimum* in which activities ceased at 48 hours. Comparative evaluation of the LC_{50} of three plant extracts showed significantly high toxicities with *A indica* showing the greatest toxicity having LC_{50} at 8.32mg/ml, while on the other hand *O. gratissimum* and *C. citratus* had LC_{50} 19.50mg/ml and 34.67mg/ml on *Aedes aegypti* respectively. There were also variations in the composition of the phytochemicals in the 3 plants with *A. indica* having highest amount of all the phytohemicals relative to other plants except flavonoids, while glycosides were completely absent in the 3 plants. [Journal of American Science 2010;6(10):435-440]. (ISSN: 1545-1003).

Keywords: Aedes aegypti, Extract, Larvae, Ethanol, Mosquito, Plant

1. Introduction

Mosquitoes are vectors of many different disease agents around the world. Depending on the species, mosquitoes are vectors of protozoa (e.g *Plasmodium* species) that cause malaria, the nematode worms that cause filariasis (e.g *Wuchereria brugia*) and a large number of arboviruses (e.g yellow fever and dengue viruses) including two of great impact in the tropical and subtropical regions (i.e yellow fever and dengue viruses). Service (1990) recorded 2 million people primarily in tropical countries as being at risk from mosquito - borne diseases. Though, mosquito – borne diseases currently represent a greater health problem in tropical and subtropical climates, no part of the world is immune to this risk (Fradin and Day, 2002).

Control of mosquito – borne diseases is becoming increasingly difficult because of increasing resistance of mosquitoes to pesticides (Ranson, 2001). However, the discovery of the insecticide Dichloro – diphenyl – trichloroethane (DDT) in the 1940s was a major breakthrough in the control of vector borne diseases. The insecticide was highly effective for killing indoor – resting mosquitoes when it was sprayed on the walls of houses. Moreover, it was cheap to produce and active over a period of time. The application of these chemical insecticides, which has been in use for decades, has met with tremendous setbacks in the light of the development of vector resistance and some attendant environmental hazards. Don – Pedro and Adegbite (1995) showed the ease with which resistance develops in their study in the health center and surroundings of the University of Lagos, where despite spraying Dichlovos insecticides on a two weekly basis for four years, a high mosquito population still persisted. In addition, Don – Pedro (1976) reported other deleterious effects of insecticides on non – target organisms. With these problems in focus, it becomes increasingly necessary to search for an alternative in the development of environmentally safe; biodegradable, low cost, indigenous methods for mosquito control which can be used with minimum care by individuals and communities in specific situations. This work aims at determining the comparative efficacy of *A. indica*, *O. gratissimium* and *C. citratus* extracts on *Aedes* mosquito.

2. MATERIALS AND METHOD

Study site

Imo State is located between latitude 5^0 12 and 5^0 56 North of equator and longitude $6^0$38 and $7^0$25 East of the Greenwich meridian. It is located in the tropical rain forest zone of West Africa with climatic conditions favoring the proliferation of arthropods including mosquitoes. Average temperatures are 34^0C during the dry season and 30^0C in the rainy season while the relative humidity ranges between 75% and 85%. Imo State occupies a landmass of 5,100sq kilometers with a population of 2.4million persons and an annual growth of 2.8%, distributed in the 27 local government areas situated in the 3 geopolitical zones (MIC, 2000) The State has the tropical rainforest zone characterized by heavy rainfall and high and equable temperatures. The dry season is between October and March while the rainy

season is from April to September. The wet season supports the growth and development of larvae and pupae stages and abundant recruitment of the young adults and higher prevalence levels of mosquito - borne diseases. The onset of rainfall supports the development of additional mosquito breeding sites, hatching of eggs following oviposition and growth of vegetation cover and shaded environment enhance further development of aquatic stages and recruitment of young adults and their survival.

Preparation of Plant Extracts

Control trials were set up using – *Azadirechta indica (neem), O. gratissimium* and *C. citratus*. Fresh leaves of these plants were obtained in and around Owerri, Imo State, South eastern, Nigeria. The leaves were transported to the Chemistry laboratory of the Project Development Agency, Enugu, Enugu state, Nigeria for the extraction and phytochemical analysis. The leaves were washed in tap water, shade-dried for 5 days and ground into fine powder. Ethanol extract was made by extraction of 150g of the powder in 2500mls of ethanol using soxhlet apparatus. The extracts were concentrated in a rotary vacuum evaporator to yield dark residue which were further reduced to pastes by heating. Stock solution was prepared by dissolving 5g of the extract in 150mls of water and 3 drops of acetone were used to emulsify the oils in water and then making it up to 250ml by mixing with distilled water in standard flask. All the test solutions were made by pipetting 2.5mg/ml, 12.5mg/ml, 25mg/ml, 37.5mg/ml, and 50.0mg/ml of the stock solution and introduced into 250mls of water in separate labeled 500ml bowls.

Rearing of mosquito larvae

The mosquito larvae were recruited from the egg colony held at the Arbovirus Vector Research Center, Enugu, Enugu State, where they were reared in the laboratory. The eggs were washed with 0.01% formaldehyde solution for 30 – 40 minutes as recommended by Al - Masghadani et al (1980). This is necessary as a precaution against possible microsporidian infections which might interfere with the normal development of the immature stages of mosquitoes (Anosike and Onwuliri, 1992) and soaked in water to facilitate hatching. After hatching, first instar larvae were distributed in bowls 30cm in diameter and 12.5m in depth. Care was taken to prevent overcrowding until development to early 4^{tth} instar larvae required for the study.

The larvae were kept in the plastic buckets half filled with tap water and fed with quaker oat once a day initially and twice during the later stages of development. Water in rearing container was refreshed every day by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface.

Bioassay

Standard methods for testing the susceptibility of mosquito larvae to insecticides as stipulated by WHO (1996) were followed. The bioassay were performed at a room temperature of 27 ± 1^{0}C, Relative humidity 70-85%, Photoperiod 14:0 (light: dark) and pH 7.0 of distilled water. Twenty five (25) larvae selected by means of rubber pipette were placed in 4 small separate specimen bottles containing 25ml distilled water, and then exposed to each of the concentrations of the extracts in a final volume of 245ml distilled water taken in 500ml plastic bowls. This is done by tripping the contents of smaller specimen bottles into the 500ml plastic bowls. The larvae in all the bowls were fed every twenty four hours on equal amount of quacker oat powder which was spread evenly across the water surface.

Four replicates for each of the test concentration and control (without plant extracts) were tested for anti – larval effects. The larval mortalities were recorded at intervals of 24, 48, 72 and 96 hours exposure using NAO / NAVC / 2000 form (used for biological pesticide tests) obtained at the National Arbovirus Research Center, Enugu. All the mortalities were counted and recorded as in percentages. Probit analysis (Finney, 1971) was used to determine the median lethal concentration $LC_{50.}$ (Woolf, 1968, Wardlaw, 2000).

1 Glycosides (Evans, 2002)

Two drops of the plant extract were placed in a small beaker. 15ml of distilled water and 3mls of 10% sulphuric acid were added and the mixture boiled for 15 minutes. The boiled mixture was then made alkaline by adding 10ml solution of 5% Potssium hydroxide. 10% of Fehling's solution was added and boiled for 3 minutes. The occurrence of a brick precipitate was indicative of the presence of glycosides.

2 Tannins

The presence of tannins was determined using the method described by Evans (2002). The filtrate obtained from boiling 2g of the samples with 20ml of 45% ethanol for 5 minutes was used for these tests.

a. Ferric chloride test.

One ml of filtrate was diluted with 2ml of distilled water and 2 drops of ferric chloride solution

added and observed for transient greenish to black colour.

b. Lead acetate test

1ml of filtrate was added to 3 drops of 5% lead acetate solution and observed for gelatinous precipitate.

3. Alkaloids (Evans, 2002)

a. Dragendorff's test prepared

Two drops of the extract were dissolved in 1% dilute sulphuric acid and boiled. The mixture was filtered hot and a drop of freshly prepared Dragendorff's reagent was added. The formation of pink or red precipitate was taken as a positive test.

b. Mayer's test

Two drops of the extract were dissolved in 10% dilute H_2SO_4 and boiled .The mixture was filtered hot and a few drops Mayer's reagent were added. A white or yellow precipitate was taken as a positive test.

4 Saponin

The Frothing test was used. 0.1g of the powdered sample was boiled with some distilled water for 5 minutes and decanted while hot. 1ml of the filtrate was diluted with 4ml of distilled water and the mixture shaken vigorously and observed for stable froth on standing (Evans, 2002).

5 Flavonoids

The Shinoda test was used. 0.5g of the powdered sample was extracted in ethanol by boiling in a water bath for 5 minutes, this was filtered and cooled. To the filtrate was added 4 pieces of Magnesium fillings followed by few drops of concentrated HCL. A pink or red colour indicated the presence of flavonoids (Harborne, 1984)

6 Steriods and Terpenes

5g of the powdered sample was extracted by maceration with 50ml of ethyl alcohol (95%), filtered and the filtrate evaporated to dryness and used for the Liberman acid test. A portion of the organic extract was treated with drops of acetic anhydrate, and then concentrated H_2SO_4 acid was carefully added by the side of the test tube. The presence of a brown color at the boundary of the mixture was taken as positive result (Evans, 2002).

STATISTICAL ANALYSIS

A 3 × 6 factorial experiment in completely randomized block design analysis of variance (ANOVA) was performed. Fisher's least significant difference (FLSD) was used to separate the means of the main factors (plant extract, concentration and their interactions).

3. Result

Table 1: Mean mortality of *Aedes aegypti* larvae after treatmeat with different levels of botanical (*A. indica*, *C. citratus* and *O. gratissimium*) extracts on 24 hourly intervals.

		Mean mortality			
Botanical Material	Levels of application (mg/ml)	24 hours	48hours	72 hours	96 hours
Azadrichta Indica	2.5	5.0^{e}(20)	8.0^{c}(32)	0.0^{a}(0)	0.0^{a}(0)
	12.5	6.0^{d}(24)	10.0^{b}(40)	0.0^{a}(0)	0.0^{a}(0)
	25.0	8.0^{c}(32)	13.0^{a}(52)	0.0^{a}(0)	0.0^{a}(0)
	37.5	10.0^{b}(40)	13.0^{a}(52)	0.0^{a}(0)	0.0^{a}(0)
	50.0	14.0^{a}(52)	10.0^{b}(40)	0.0^{a}(0)	0.0^{a}(0)
	Control	0.0^{f}(0)	0.0^{d}(0)	0.0^{a}(0)	0.0^{a}(0)
Cymbopogon Citrates	2.5	0.0 ^{d}(0)	2.0^{d}(8)	4.0^{c}(16)	0.0^{c}(0)
	12.5	1.0 ^{c}(4)	1.0^{d}(4)	5.0^{b}(20)	2.0^{b}(8)
	25.0	1.0 ^{c}(4)	2.0^{c}(8)	6.0 ^{a}(24)	0.0 ^{c}(0)
	37.5	3.0 ^{b}(12)	3.0^{b}(12)	6.0 ^{a}(24)	2.0^{b}(8)
	50.0	5.0^{a}(20)	4.0^{a}(16)	5.0 ^{b}(20)	3.0^{a}(12)
	Control	0.0^{d}(0)	1.0^{d}(4)	1.0^{d}(4)	0.0^{c}(0)
Ocimium gratissimium	2.5	3.0^{e}(12)	3.0^{c}(12)	0.0^{a}(0)	0.0^{a}(0)
	12.5	6.0^{d}(24)	3.0^{c}(12)	0.0^{a}(0)	0.0^{a}(0)
	25.0	9.0^{c}(36)	5.0^{a}(20)	0.0^{a}(0)	0.0^{a}(0)
	37.5	10.0^{b}(40)	4.0^{b}(16)	0.0^{a}(0)	0.0^{a} (0)
	50.0	13.0^{a}(52)	1.0^{c}(4)	0.0^{a}(0)	0.0^{a}(0)
	Control	1.0^{f}(4)	2.0^{d}(8)	0.0^{a}(0)	0.0^{a}(0)

Means in the same column under the same botanical material and having the same letter (superscript) are not significantly different at $P \leq 0.05$ (LSD). Numbers in parenthesis represent percentage mortalities

Results of the activities of *A. indica*, *O. gratissimum* and *C. citratus* on the *Aedes aegypti* larvae at 96 hours exposure are shown in Table 1. ANOVA analysis showed significant difference in the mean mortality of larvae among the various treatment concentrations of the three plant extracts ($P \leq 0.05$). The activities of the extracts of *A. indica* and *O. gratissimum* were pronounced only at 24 and 48 hours respectively. At 72 and 96 hours the extract of the two plants seemed to have lost their potency on the larvae of *Aedes* as no further death was recorded. Similarly, treatment level 50mg/ml exhibited highest mortality rates for the two extracts. This was significantly different from the mean mortalities recorded for the other concentrations. In *C. citratus*, the activity of the extract seemed to increase with

exposure time as higher percentage of larvae died at 48 and 72 hours than 24 hours. The residual effect of the extract is significant with time (P< 0.05). The table also showed that the concentration 50mg/ml recorded the highest mean mortality of *Ae. aegypti* at the various time intervals. The various concentrations of *C. citratus* were significantly different from the mean mortality of the larvae recorded in the control.

However, *A indica*, *O. gratissimum* and *C. citratus* demonstrated LC_{50} at 8.32 mg/ml, 19.50 mg/ml and 34.67 mg/ml on *Aedes aegypti* (Fig 1, 2&3).

Table 2: Phytochemical composition of the three botanicals (*A. indica*, *C. citratus* and *O .gratissimium*)

Phytochemical Compounds	*A. indica* (%)	*C. citratus* (%)	*O.gratissimium* (%)
Alkaliods	5.24	0.804	1.28
Saponin	0.715	0.038	0.22
Flavonoids	0.98	1.08	0.04
Steriods	1.14	0.039	0.64
Tannins	0.73	0.02	0.18
Glycosides	-	-	-

The result of phytochemical composition of the three botanicals (*A. indica, C. citrats* and *O. gratissimum*) presented in table 2 showed that *A. indica* had greater amounts of all the phytohemicals relative to others except Flavonoids which was 1.08 in *C. citratus* but 0.98 in *A. indica* and 0.04% in *O. gratissImum*. Glycosides were completely absent in the 3 plants. On the other hand, these compounds were found to be higher in *O. gratissimum* than *C. citratus* with the exception of flavonoids. There were variations in percentage composition of these compounds in the 3 plants.

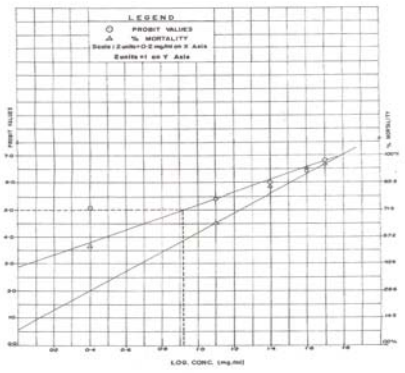

Figure 1.The Activity of *A. indica* on *aegypti* showing $LC_{50.}$

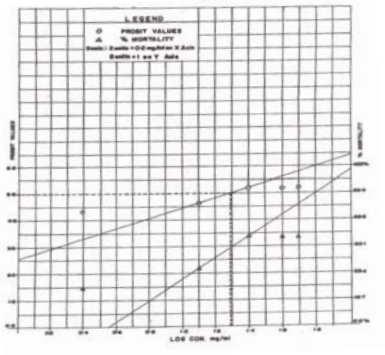

Figure 2. The Activity of O. gratissimium on *A. aegypti* showing LC_{50}

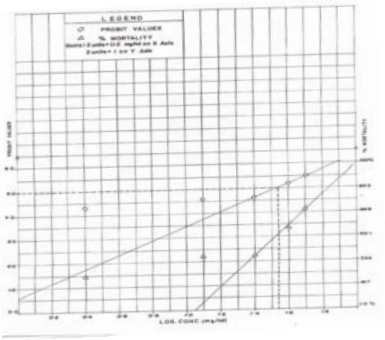

Figure 3. The Activity of on *C. citratus A aegypti* showing LC_{50}

4. Discussion

The extensive use of synthetic organic chemical insecticides results in environmental hazards and resistance in major vector species and this has necessitated the need to develop a more potent and environmentally safe pesticide. Phytochemicals serving as suitable alternative to synthetic insecticide are relatively safe, inexpensive and readily available in many areas of the world. A number of these plants are traditionally used as medicinal plants for the treatments of malarial fevers, liver problems and other symptoms caused by malarial infections. Several studies have focused on natural products for controlling mosquitoes as larvicides and insecticides with varied results (Sivagnaname and Kalyanasundra, 2004; Rajkumma and Jabanesa, 2005; Cavalcanti et al, 2004; Singh et al, 2006 and Patil et al, 2006). This study was carried out to examine the effects of three plants; *Azadirechta indica*, *Ocimum gratissimum* and *Cymbopogon citratus* on *Aedes aegypti*.

The results from the study showed the three plants exhibited good larvicidal activities on the mosquito with varying susceptibility. The varying susceptibility observed here is in line with reports from previous findings that various mosquito species

showed differential susceptibility to different plant extracts (Pathak et al, 2000). *A. indica* showed very high potency at 24 hours and 48 hours with 92% mortality rate. Mortalities increased with concentration ($P \leq 0.05$). This confirms the report of Shadia et al (2007) that there is a positive correlation between concentration and the percentage of the larval mortality. Singh (1984) found that a concentration of 32.1ppm of deoiled neem seed kernel extract yielded 85% mortality in *Cx. quinquefasciatus* after 12 days of exposure. The results in this work revealed that the cumulative effects which neem showed at 96hours exposure was 68% mortality at 24hours and 96% at 48 hours on *Aedes* respectively, while *O. gratissimum* killed 56% *Aedes* at 48 hours, respectively at 50mg/ml treatment levels. These results were obtained by exposing these larvae at maximum exposure limit of this experiment; it is likely that diluting these concentrations further and extending the period beyond 96 hours would procure much better results than that reported by Singh (1984).

However, *O. gratissimium* exhibited a higher larvicidal potential than *C. citrates* as recorded in the result. This result disagrees with Cavalcanti et al, (2004) who reported that the essential oils of *O. americanum* and *O. gratissimum* were as potent as *L. sidoides* and *C. citratus* in the larvicidal activity against *Ae aegypti* by causing 100% mortality at a concentration of 100ppm. .However, the residual effect displayed by *C. citratus* in relation to the exposure period of 96 hours interval cannot be overlooked, for this could be an advantage it has over others. Significant growth inhibition and mortality of *Ae, aegypti* by *C. citratus* had been reported by Sukumar et al (1991).

The results also confirmed the toxicity of these plants, which have been described as being toxic or are more traditionally used for their toxicity. The toxic activity of the 3 plants could be attributed to a wide range of chemicals including alkoloids, glycosides, tannins, quinines, terpenoids, non – metabolized amino acids which are part of the chemical resistance mechanisms of various plant groups against the degradation of herbivorous insects (Hedin, 1983). However, neem had higher concentration of alkoliods, saponins, tannins and steroids than the other plants; *C. citratus* and *O. gratissimum*. This could account for the observed high activity of neem against the mosquito. The possible reason for the death of the larvae subjected to the extracts could be attributed to the presence of feeding inhibiting substances in the extracts. Schluter and Schulz (1983) reported that azadirechtin (a tetranorterpenoid) which was responsible for causing degradation on larval epidermis preventing the larvae from molting. On the other hand, Al – Sharook et al (1991) reported that the death of treated insects may be due to the inability of the molting bodies to swallow sufficient volume of air to split the old cuticle and expand the new one during ecdysis or to a metamorphosis inhibiting effect of the plant extract which is possibly based on the disturbance of the hormonal regulation Sukumar et al (1991) investigating the analysis of essential oils of *C. citatrus* reported major components to include geranial 60.3% and neral 39.9% (which possibly could have caused the observed growth inhibition on the 3 mosquito species. Cavalcanti et al (2004) reported the essential oil components of *O. gratissimum* to be eugenol 43.7% and cineole 32.7% while Pessoa et al (2002) observed that both eugenol and *O. gratissimum* oil presented anti - helminthic activities against *Haemonchus contortus*, the main nematode of ovines and caprines in Northeastern Brazil.

The results of the LC_{50} of the extracts of the 3 plants showed that *A. indica*, *O. gratissimum*, and *C. citratus* had LC_{50}s at 8.32mg/ml; 19.50mg/ml and 34.67mg/ml on *Aedes* respectively. Since LC_{50} is a measure of a dose effect that quickly kills an organism, it does account for chronic effect. A lower LC_{50} means that a substance is more toxic and would require less of the substance to kill the organism ingesting it.

This result has therefore demonstrated a greater potential of *A. indica* for larvicidal activity on *Aedes* than *O. gratissimum* and *C. citratus* while also *O gratissimium* exhibited greater action than *C. citratus*. However the result in this study significantly contrasts the result obtained with the measurement of LC_{50} of 9 plants widely found in Brazil, the result showed that *O. gratissimum* and *O. americanum* have LC_{50} of 67ppm and 60ppm respectively, compared to 63ppm for *L. sidoides* and 69ppm of *C. citratus* (Cavalcanti et al, 2004). The present result clearly demonstrated close to 100% larvicidal potential of *O. gratissimum* on *Aedes* than *C. citratus* when their LC_{50}s are compared. However, all the 3 plant extracts have shown a high level of larvicidal activities in this work and in fact could serve as effective replacement for the chemical insecticides.

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