LABORATORY EVALUATION OF ETHANOLIC AND METHANOLIC EXTRACTS OF *OCIMUM GRATISSIMUM* AGAINST LARVA OF *ANOPHELES GAMBIAE* AND NON-TARGET ORGANISMS

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ABSTRACT: Methanolic and ethanolic extracts of *Ocimum gratissimum* leaves were evaluated for larvicidal activity against larva of *Anopheles gambiae* and non target organisms in the laboratory. Photochemical analysis of leaf showed presence of tannins, alkaloids, flavonoid, cyanogenic glycosides, cardiac glycosides and saponin. The organisms were exposed to different test concentrations ranging from 20mg/ml to 100mg/ml for 96hours. Larvae of *A. gambiae* were susceptible to the extracts. Ethanolic extracts of the leaf was more active with LC_{50} and LC_{90} of 60.9mg/ml and 464.4 mg/ml respectively while the LC_{50} and LC_{90} of methanolic extracts of leaf were 73.6mg/ml and 1021mg/ml respectively. The extracts exhibited insect growth regulating activity and were found to be safe to non target organisms. Results showed that larvicidal effect of the methanolic extract can be compared to that of neem leaf extracts.

KEY WORDS: *Ocimum gratissimum* leaves, methanolic and ethanolic extract, larvicidal activity, *Anopheles gambiae,* non target organisms.

Mosquitoes are important vectors of diseases and nuisance pests (Azhari et al., 2009). Mosquito-borne diseases remain a major problem in the world particularly in tropical and subtropical regions (Bernard, 1999). Over two million people are at risk of diseases caused by mosquitoes (Odalo et al., 2005). Repeated use of synthetic insecticides for mosquito control has resulted into development of resistance in many vector species, undesirable effects on non-target organisms, environmental and human health concerns (Das et al*.,* 2007). These problems coupled with high cost of the insecticides have revived interest in exploiting the pest control potentials of plants (Grainge & Ahmed, 1988). Despite their application as general toxicants against immature stages of mosquito, their phytochemicals may have potential uses as growth and reproduction inhibitors, repellants and oviposition deterrents (Savignaname & Kalyanasundaram, 2004).

Many species of *Ocimum* belonging to the Family Lamiaceace and Order Lamiales (USDA, 2008) a perennial scented shrub with lime fizzy (Wagner et al*.,* 1999) have been reported to have larvicidal activity, *O. basilicum (*Azhari et al*.,* 2009) *O. sanctum* (Vinayagam et al., 2008) and *O. americana* (Cavalcanti et al., 2004). Oil extracts of *O. gratissimum* leaves have been reported to have repellant effect on adult mosquitoes (Oparaocha, 2008). Traditionally, its fresh leaves are placed on a lighted lamp and the fumes have been reported to repel adult mosquitoes from target organisms. *Ocimum gratissimum* have been reportedly

used in the treatment of fever and diarrhea (Oliver, 1980), convulsion, stomach pains and catarrh (Oparaocha, 2008) among many other uses. However, there is still dearth of information on its effects on immature stages of mosquitoes. This study examines the effects of *O. gratissimum* on the larvae of *Anopheles gambiae* and other non-target organisms.

MATERIAL AND METHODS

Preparation of stock solution of plant leaf extract

Fully developed leaves of *O. gratissimum* were collected in the month of June, 2011. The leaves were thoroughly washed, shade dried and ground with the warring blender. Ground leaves were sieved to get fine powder. Methanolic and ethanolic extracts were obtained by soaking 100gm of finely ground plant material in 500ml of 70% methanol and ethanol respectively for 24hr with periodic shaking. The content of each set up was filtered using Whatmann's No 1 filter paper and the residue evaporated to dryness using the soxhtet apparatus. After this, the extract was allowed to cool and then each... was put into a container and labeled accordingly. 20mg, 40mg, 60mg, 80mg and 100mg of each of these were mixed with 100ml of ethanol or methanol as the case may be to get test concentrations to be used.

Effects of plant leaf extract on mosquito larva

Ten active larva were put into 500ml transparent containers which have 249ml of dechlorinated water, fed with glucose and allowed to acclimatize. 1ml of each test concentration was added to each container and labeled accordingly. Two replicates for each test concentration ranging from 20mg/ml, 40mg/ml, 60mg/ml, 80mg/ml, 100mg/ml and a control(made up of 249ml of dechlorinated water and 1ml of ethanol(or methanol) were setup and observed under room temperature of 30°C ± 2°C.

Mortality of larvae were observed at 6hr intervals for 96hr. the larvae were also observed for effect of extract on their growth, other abnormalities like sluggishness and reduced swimming activity in each container of the different test concentration for 96hrs.

Effect on non-target organisms

The experimental set-up for the larvae was also used against non-target organisms. The organisms used were water skater, water strider (*Alierris remigis*) and tadpole of toad (*Bufo regularis*). One organism per container was used to avoid predation and each set-up observed for mortality and other abnormalities like reduced swimming activity, sluggishness etc for 24hrs.

Phytochemical screening of plant leaf material

Phytochemical screening of leaf material for saponins, tannins, flavonoids, alkaloids, cynogenic and cardiac glycosides was carried out according to Amadi et al. (2004).

RESULTS

Methanolic and ethanolic extracts of leaves of *O. gratissimum* showed some larvicidal activity on *A. gambiae* tested at concentrations ranging from 40mg/ml to 100mg/ml. No mortality was recorded for each at test concentrations less than 40mg/ml. The results are as shown in tables 1 and 2.

Ethanolic extract of leaf material was more effective than methanolic extract of leaf material of *O. gratissimum* as shown in table 3.

It was observed that the leave extracts tested on the mosquito larva affected the growth rate and swimming activities negatively. Sluggishness and other morphological abnormalities were observed. The time onset of observed behavioral changes reduced with increasing extract concentration (table 4).

The effect of the plant extract increased with increasing test concentration. No abnormal behavior was observed in the control. Pupal emergence occurred after 4days in the control while it occurred after 5days in the 20mg/ml test concentration experiment and there were no other observed effects. Pupal emergence in the 40mg/ml assay emerged 6days for ethanol extract and 7days in methanol but the emerged pupa were not as active as those emerging in the control, they could not molt to the imago stage but died later on. Those that survived in 60mg/ml, 80mg/ml and 100mg/ml (methanol) after 96hr could not molt to pupa but also die later on.

Phytochemical screening result revealed the presence of tannins, saponins, alkaloid, flavonoid, cyanogenic and cardiac glycosides in varying concentrations / ranges (table 5).

Saponins occurred in trace amounts while flavonoids concentrations were high.

There were no observed adverse effects on non-target organisms. No abnormality or mortality was observed on the non-target organisms after 24hr exposure to different test concentrations of both methanolic and ethanolic extracts of *O. gratissimum* leaf.

DISCUSSION

The study has shown that methanolic and ethanolic extracts of *O. gratissimum* leaf have growth and larvicidal effects at high concentrations have growth regulatory and larvicidal effect on *Anopheles gambiae*. The result has shown that *O. gratissimum* leaf extracts have larvicidal effects on *A. gambiae* with 100% mortality at 100mg/ml of leaf extract. Larvicidal effects of other species of *Ocimum* such as *O. basilicum* on *Anopheles gambiae* (Azhari et al., 2009), *O. sanctum* on *A. stephesi* (Vinayagam et al., 2008) and *O. americana* on *Aedes aegypti* (Cavalcanti et al., 2004) have been reported. Mortality could not be linked to the solvents ethanol and methanol as no death was recorded in the control containers. The Larvicidal effect could be linked to phytochemicals present in the leaf. The presence of alkaloids in plants may be associated to their biological activities (Basu & Basa, 1972). Crude extracts of saporium from fruit pods of *Sivartzia madagascarinsis* produced high mortality in *Anopheles gambiae* larvae (Minija & Sarda, 1986).

The phytochemicals may also be responsible for the growth abnormalities and delay in the pupal emergence observed in the course of the study. Sivagnaname & Kalyanasundariam (2004) and Mohtar et al., (1999) reported prolonged pre-imago period, for *Aedes aegypti* larva exposed to methanol extract of *Nerium indicum*. The test concentrations recorded larvicidal activities in concentrations from 40 mg/ml and above while concentrations below 40mg/ml were sub lethal. The LC_{50} and LC_{90} for ethanolic extracts were 60.9mg/ml and 464. 1 mg/ml respectively while those for methanol extracts were 73.6mg/ml for LC_{50} and 1021mg/ml for LC_{90}. Some plant extracts have been shown to exhibit larvicidal activity or insect growth regulatory activity against mosquito larvae at concentration above 10mg/ml (Deshmukh & Renapurka, 1987; Thangam&

Kathiresan, 1988). Sivagnaname & Kalyanasundaran (2004) reported LC_{50} values less than 0.2mg/ml for methanolic extracts of Atlantia *monophylla* against larvae and pupae of *Anopheles aegypti* and *Culex quinquefasciatus*. Nevertheless the LC_{50} of 60.9mg/ml of ethanolic extracts of *O. gratissimum* can be compared to LC_{50} of 55-65mg/ml for some Neem extracts as reported by Ascher & Meisner (1989).

CONCLUSION

Ethanolic extracts may be superior to methanolic extracts considering their LC_{50} and LC_{90} values. It could be that ethanol as a solvent extracted more of the phytochemical components than the methanol. It could also be that the more the concentration of leaf materials the more the larvicidal and growth inhibitory activities of the leaf extract. The result revealed that the leaf extract was safe for non-target organisms as no adverse effects or mortality was recorded. This shows that the extract if used in the field where these organisms co-inhabit with the mosquito larvae will only kill the larvae and not harm other organisms.

Resistance of mosquito vectors to synthetic insecticides , high cost of these insecticides and their adverse effects on non-target organisms and environment has been a major concern in vector control. Botanical insecticides are reported to be effective against insect pests, eco-friendly with reduced effect on non-target organisms, cheap, readily available, biodegradable and safe, they may serve as alternative to synthetic ones (Sivagnaname & Kalyanasundaram, 2004; Dehghan et al., 2012; Mufutau, 2012).

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Table 1. Mortality rate of Ethanolic leaf extract of *O. gratissimum* on larvae of *A. gambiae.*

Concentration mg/ml	Total no	No dead	% mortality	Log $_{10}$ concentration	Probit
0.0	10	0	0	0	0
20.0	10	0	0	1.301	0
40.0	10	2	20	1.602	4.16
60.0	10	5	50	1.778	5.0
80.0	10	7	70	1.903	5.52
100.0	10	10	100	2.0	8.09

Table 2. Mortality rate of Ethanolic leaf extract of *O. gratissimum* on larvae of *A. gambiae.*

Concentration mg/ml	Total no	No dead	% mortality	Log $_{10}$ concentration	Probit
0.0	10	0	0	0	0
20.0	10	0	0	1.301	0
40.0	10	2	20	1.602	4.16
60.0	10	3	30	1.778	4.48
80.0	10	6	60	1.903	5.52
100.0	10	8	80	2.0	5.84

NB: Total number and number dead were average of the two replicates for each test concentration.

Table 3. LC_{50} and LC_{90} values of methanolic and ethanolic leaf extracts.

Extract	Y	R^2	LC_{50}	LC_{90}
Methanol	Y=3.502x – 1.538	0.893	73.6mg/ml	1021mg/ml
Ethanol	Y=4.534x – 3.092	0.998	60.9mg/ml	464.4mg/ml

Table 4. Observation time of the abnormalities in *A. gambiae* larvae in different test extract concentrations.

Test concentration (mg/ml)	Time of observation (Hours)	
	Methanol	Ethanol
0.0	nil	Nil
20.0	nil	nil
40.0	84	72
60.0	66	48
80.0	36	24
100.0	18	12

Table 5. Presence of Photochemical in the leaf extract of *O. gratissimum*.

Phytochemical	Range
Flavonoid	++++
Alkaloid	+++
Cyanogenic glycoside	+++
Tannin	++
Cardiac glycoside	++
Saponin	+

- Absence, ++++ High concentration, +++ & ++ Moderate concentration, + Trace concentration.