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Larvicidal Activity of Two *Ocimum* species (Lamiaceae) Crude Leaf Extracts against the Larvae of *Culex quinquefasciatus* SAY (Diptera: Culicidae)

[1*]**Akinnibosun, H. A.,** [2]**Rotimi, J. and** [3]**Igbinidu, O. C**
[1]Department of Plant Biology and Biotechnology, University of Benin, P.M. B. 1154, Benin City 300283, Edo State, Nigeria.
[2]Department of Animal and Environmental Biology, University of Benin, P.M. B. 1154, Benin City 300283, Edo State, Nigeria.
[3]Department of Environmental Science, University of Benin, P.M. B. 1154, Benin City 300283, Edo State, Nigeria.
*Corresponding author e-mail: henry.akinnibosun@uniben.edu

ABSTRACT
This study was conducted to evaluate the larvicidal activity of *Ocimum basilicum* and *Ocimum gratissimum* aqueous leaf extracts against the larvae of *Culex quinquefasciatus* mosquito. The leaf extracts were hydrodistilled in the laboratory to obtain various concentration dosage of 500 ppm, 1500 ppm, 2500 ppm and 5000 ppm which were used in running the bioassay under laboratory conditions. The results obtained showed that the leaf extracts of the two plants exhibited larvicidal activity against the mosquito larvae. About 100 % larval mortality occurred at leaf concentrations of between 2500 ppm and 5000 ppm within 24 hr of the bioassay tests. *O. gratissimum* leaf extract which gave LC_{50} value of 920.0 ppm was more potent than *O. basilicum* leaf extract which produced LC_{50} of 1000 ppm. The results from this study suggest that aqueous leaf extract of *O. basilicum* and *O. gratissimum* are promising as larvicides against *C. quinquefasciatus* and could be useful as biodegradable larvicidal natural resource for the control of mosquitoes.

Keywords*:* Larvicidal activity; *Culex quinquefasciatus*; Leaf extract; LC_{50}; *Ocimum* species

INTRODUCTION
Culex quinquefasciatus are mosquitoes belonging to the order Diptera and family Culicidae. This mosquito is very much domestic in its breeding places and the larvae are abundant in barrels, tins, tanks, discarded machinery, motor tyres and in domestic utensils (Sarojini and Ramalingam, 2008). The importance of mosquitoes in the warmer parts of the world stems primarily from their role as vectors of malaria. In Africa, some species also play a major part in the transmission of bancroftial filariasis and the yellow fever virus. Mosquito-borne diseases contribute to a large proportion of health problems of developing countries (Pushpanathan *et al*., 2006; Fradin and Day, 2002). Worldwide, mosquitoes have been declared as "public enemy number one" because they are responsible for the transmission of various dreadful diseases (WHO, 1996).

Efforts to eradicate malaria and other diseases transmitted by mosquitoes have focused on the elimination of the mosquito vector, one successful way is by attacking the larvae in their breeding places (Gluber, 1989). Carvalho *et al.* (2003) have suggested that the ideal control method is thus the systematic treatment of their breeding places through larvicides.

Although synthetic insecticides have shown high degree of success as larvicides, there are however serious environmental concerns about the use of chemical larvicides worldwide due to their adverse toxic effects on non-target organisms and the environment (Chandre, 1998); There is the need therefore to redouble efforts to produce effective and environmentally friendly means of control, one such important means is the use of plant products. This study is therefore aimed at assessing the larvicidal potential of cold water extracts of the leaves of two plant species, *Ocimum basilicum* and *O. gratissimum* against the larvae *of Culex quinquefasciatus.*

MATERIALS AND METHODS

Description of Plants

Two plant species (*O. basilicum* Linn and *O. gratissimum* Linn.) belonging to the family Lamiaceae, were used for this study. *Ocimum basilicum,* also known as sweet or hairy basil is a bushy plant growing to about 1ft-3ft in height and 12 ft – 18 ft wide. The leaves are green in colour. It is widely known and cultivated for its highly aromatic leaves which are used as spice. The strong cinnamon-like flavor of the plant makes it popular for flavouring hot drinks and food. It has been used locally in the treatment of gonorrhea, cough, constipation, dysentery, ring worm, hypertension and as anti-helmintics (Gill, 1992).

Ocimum gratissimum is an herb which grows 1 - 3 meters in height. The stem of the plant is dark brown bearing green leaves from top to bottom. The leaves are narrow and oval in shape. The strong aroma of the leaves is used in flavouring soups and spicing meat products (Gill, 1992). Components of this herb are used in producing mosquito insect repellants as well as in treating skin infections and cough. The leaves contain volatile oil which is above 3 % by weight. The constituent of the oil include Hymol (75 %), Evgenol and terpenses.

Collection and Processing of Plants

Fresh and fully developed leaves used for the tests were collected from the botanical garden of the department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The fresh leaves of the two plant species were washed with tap water, weighed and categorized into 5.0 g, 15.0 g, 25.0 g and 50.0 g by means of a Metler balance The leaves were blended using an electric stainless steel blender with 1 litre of cold water to give an equivalent concentration of standard extract solutions of 500 ppm, 1500 ppm, 2500 ppm and 5000 ppm respectively. The sieved filtrate was used for the experiment.

Culture of mosquito larvae

Culex mosquito colony and larvae were cultured and maintained in the laboratory at 27±1 °C and 80 – 85 % relative humidity. The larvae were fed with dog biscuits and yeast powder in the ratio of 3:1 as recommended by Mullai and Jebanesan (2007).

Bioassay Tests

Bioassay tests were carried out following WHO (1981) methodology. Twenty larvae of *C. quinquefasciatus* were introduced into plastic containers containing the various extract concentrations; the experiment was in three replicates. The control set up contained only dechlorinated tap water with no leaf extract. Treated larvae were held for 24 h at the same conditions used for maintaining the mosquito colony in the laboratory. The numbers of dead larvae were counted every hour for 24 hours and percentage mortality was computed from the average of the three replicates.

Statistical Analysis

The average larval mortality values were subjected to probit analysis for calculating Lethal Concentration (LC_{50}) (Finney, 1971).

RESULTS

Aqueous leaf extracts of *O. basilicum* and *O. gratissimum* were tested for their lavicidal activity against the 4th instar larvae of *Culex quinquefasciatus*, the recorded LC_{50} values are presented in Table 1.

Table 1: LC_{50} values of larvicidal activity of *Ocimum* leaf extract against *Culex quinquefasciatus* in a 24 h bioassay.

Mosquito species	Plant species	Period of bioassay	LC_{50} ppm ± S.E
C. quinquefasciatus	*Ocimum basilicum*	24 h	1000.50 ± 2. 19 (923.86 – 1662.85)
	Ocimum gratissimum	24 h	920.0 ± 2.25 (756.90 – 1723.10)

Based on the 24 hour bioassay results obtained, the leaf extracts of the two plant species exerted effective larvicidal activity against *C. quinquefasciatus. Ocimum gratissimum* leaf extract however exerted more effect than *O. basilicum*. The lethal concentration (LC_{50}) value for the treatment with *O. gratissimum* was 920 ppm (the values ranged from 756.90 to 1723 .10 ppm) (Table 1). The leaf extract of *O. bassilicum* had LC_{50} value of 1000 ppm (the value range from 723. 86 -1662.85 ppm). The toxicity of the leaf extracts was dependent on its concentration, the high concentrations of 2500 ppm - 5000 ppm of the leaf extract solutions of the two plant species produced 100 % mortality, while the lower concentrations of 1500 and 50 ppm caused 86.65 and 23.35 % mortality respectively.

DISCUSSION

Cold aqueous extracts of *Ocimum basilicum* and *O. gratissimum* leaves tested against the 4th instar larvae of *Culex quinquefasciatus* mosquito in this study showed that the different aqueous extracts exhibited larvicidal effects on the *Culex* larvae. The LC_{50} value produced by *O. basilicum* leaf extract was 1000 ppm while that of *O. gratissimum* was 920 ppm. Based on LC_{50}, *O. gratissimum* leaf extract was slightly more potent than the leaf extract of *O. basilicum* when applied against the larvae.

Many authors have also shown the bio activity of some plant extracts against *C. quinque fascitus*. Rahuman and Venkatesan (2008) have shown the larvicidal efficacy of five cucurbitacious plants extract against *Culex* larvae and found that the leaf extracts produced LC_{50} values of between 337.90 ppm and 33 99.03 ppm. Mullai and Jebanesan (2007) had earlier reported that solvent leaf extracts of *Citrullus colocynthis* and *Curcubita maxima* showed LC_{50} values between 75.91 and 171.64 ppm against *C. quinquefasciatus*. Kannathasan *et al*, (2007) also reported that the methanol leaf extracts of four *Vitex* spp used for larvicidal assay produced LC_{50} value ranging from 41.41 ppm to 212.57 ppm against the early fourth instar larvae of *C. quinquefasciatus*. Observations from this study however showed that the LC_{50} values from the bioassay were relatively high when compared to reports of some other authors, the higher values obtained could be attributed to the nature of the solvent used in extracting the leaves since chemical solvent extract of leaves have been shown to be more effective against mosquito larvae than water extract (Sumroiphon, *et al.*, 2006).

In addition to the effect of solvent extract on the potency of plant parts, the high LC_{50} values produced in this study could also be attributed to the fact that *C. quinquefasciatus* prefer polluted water as breeding habitat and could survive in organic polluted water there is therefore the need for higher dose leaf extract to cause larval mortality. This view is in consonant with the view expressed by Sumroiphon *et al*, (2006). In their study the LC_{50} values for mosquitoes like *Aedes aegpti* larvae which breeds on cleaner water was comparatively lower. In a similar vein, Rahuman and Venkatesan (2008) showed that the LC_{50} value of some plant extracts against the larvae of *A. aegpti* were comparatively lower than the values obtained when tested against *C. quinquefasciatus.* However, the larviadal properties contained in *Ocimum* leaf extract was to some extent capable of exhibiting larvicidal effect.

Also, the potency of the leaf extracts in this study was dose dependent the higher concentrations achieved more potency compared to the lower concentrations, invariably as concentration increased the potencies also increased. This activity is comparable to results obtained by Rajkumar and Jebanesan (2005). In their findings, activity of screened plant s in the laboratory using difference species of mosquitoes was dose dependent.

In conclusion, the aqueous lead extract of *O. basilicum* and *O. gratissimum* showed larvidal potential against the early fourth instar larvae of *C. quinquefasciatus*. The promising larvicidal activity of the leaf extract reemphasized the need to explore the possibility of using leaf extract products as complimentary measures to control mosquito larvae. The findings of this study suggest the use of *Ocimum* leaf extract as a local resource in controlling mosquito larvae in small breeding places, this will reduce the use of chemical insecticides and its attendant consequences on the environment.

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