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LARVICIDAL EFFECTS OF *EUCALYPTUS CAMALDULENSIS* LEAVES EXTRACT ON *CULEX QUINQUEFACIATUS* LARVAE

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ABSTRACT

Mosquitoes have worldwide distribution and adapted to tropical and sub-tropical regions of the world. They are vector for variety of infectious diseases affecting millions of people per year, particularly in Sub-Saharan Africa. In the present study, larvicidal efficacy of *Eucalyptus camaldulensis* was tested against *Culex quinquefaciatus*. The most effective concentration is 100 percent extract with 100% mortality in 12 hours of exposure, while least effective concentration was 30% extract with 0% mortality in 12 hours of exposure until 24 hours of exposure with about 93% mortality. In all the treatment, mortality increased with increased exposure period of time. Treatments were significantly different ($P < 0.05$) when compared in conformity to the controls. The findings therefore suggest that *Eucalyptus camaldulensis* extracts have potential in the control of the mosquito commonly known as tropical house mosquito.

KEYWORDS: *Culex quinquefaciatus*, Mosquitoes, Diseases transmitted by mosquitoes, Malaria, Plant pesticides, *Eucalyptus camaldulensis* etc.

INTRODUCTION

Mosquitoes have worldwide distribution and adopted to tropical and sub-tropical region of the world (Fang, 2010), due to significant rainfall and consistent high temperature and humidity, along with stagnant waters which provide mosquitoes with conducive environment needed for continuous breeding (prothero and mansell, 1999), Mosquitoes are vectors for variety of infectious diseases affecting millions of people every year, particularly in sub-Saharan Africa. Conservative estimate put more than 7 million people at risk annually in

Africa, South America, Central America, Mexico, Russia and most of Asian countries with million cases resulting in death (WHO, 2000). Mosquito transmitted diseases such as malaria, filariasis, dengue fever, yellow fever, encephalitis, West Nile virus (Deore and Khadabadi, 2009; Kaufman and Briege, 2004; Naznin *et al*., 2001; Vasudevan *et al*., 2006), also cause morbidity, economical loss, social disruption and nuisance that contributed significantly in poverty and social debility in tropical countries (Vasudevan *et al*., 2009). Recently, it has been reported that mosquito can also transmit hepatitis B virus (Naznin *et al*., 2001).

The mosquito *Culex quinquefaciatus* (tropical house mosquito) is a known vector of lymphatic filariasis, which is also widely distributed tropical disease with around 120 million people infected worldwide and 40 million people have common chronic manifestation (Benhard *et al*., 2003). The disease is a major health problem in other 73 countries in the tropical world (Abdul-Rahman *et al*., 2008). Mosquito control is one of major problem of the world today. Most of the widely used mosquitoes control methods are attempted by synthetic insecticides-based (Bishms and Zeev, 2005). Larval control in particular is frequently dependent on continued application of organophosphate and insect growth regulator (IGR) (Yang *et al*., 2002). But the effect of synthetic insecticides to non-target insects which are beneficial in pollination, as well as source of food to other organisms and effect of these chemicals bring about environmental pollution and residue in food, potential chronic toxicity on environment and non-target animals.

This has led to a continued search for alternatives for vector control measure that are cheaper, eco-friendly, easily biodegradable, easily available non-toxic to non-target animals and which required little or no sophisticated technology in application but give excellent results.

It has been estimated that more than two thousand plant species have been known to produce chemical components and metabolites of value in pest control programmes (Vasudevan *et al*., 2009; Naznin *et al.*, 2001). Many plants of terrestrial origin have been reported to suppress mosquito larval population and suggested to be advantageous for field use in mosquito control programmes (Saraf and Dixit, 2002).

In the present study we have evaluated the efficacy of *Eucalyptus camaldulensis* leaves extracts against larvae of tropical house mosquito *Culex quinquefaciatus*.

MATERIALS AND METHOD

Study Area

This research was conducted in Usmanu Danfodiyo University, Sokoto, Sokoto state which is located at $13^0$06`15N, $5^0$ U`50E, altitude 250 – 300m above sea level; mean temperature is 14 to 45^0C; average rainfall is about 732mm.

Preparation of Materials

The leaves of *Eucalyptus camaldulensis* where collected from Usmanu Danfodiyo university main campus Sokoto state Nigeria, using hand picking at 7:30am. The leaves were then washed after the collection with tap water and shade dry at room temperature. Local blander or mortar was used to powder it. Leaves powder was kept in a container until used. Standard solution is made by dissolving 200g of powder in one liter of distilled water, kept for 24 hours and then filtered with A_1 filter paper and stored in a 1000ml capacity conical flask, in laboratory at room temperature the flask is then covered with maskin tape to avoid contaminations with impurities and also to avoid escape of some volatile component of the extract.

Maintenance of culture stock

Breeding site of mosquito were selected with the aid of visual identification of gutter, reservoir, septic tank, dug well, stagnant water and river. The breeding site of mosquito identified on the basis of presence of adult mosquito on the surface of water indicate the probability of eggs of the same species and the presence of larvae were also observed to confirm the breeding site of mosquitoes. Several eggs rafts of *Culex quinquefaciatus* were collected from septic tanks and some well around Usmanu Danfodiyo University hostel area in wide mouth glass jars. During collection, a standard dipping technique (simple scoop) was used with a dipper of 8cm in diameter (400ml) and 150cm long handle. The eggs were brought to the laboratory and maintained in algae rich water for hatching and emergence of larvae. All the hatched larvae were sorted with Pasteur's pipette and identified to species using identification keys of Russell (1996) and Harrison (2005) before being transferred into four glass beaker (1000ml). Each beaker contains 500ml of water that collected together with eggs from their natural breeding sites (septic tanks and well).

Treatment

From the stock solutions of the plant extract prepared above (as aqueous solution), separate serial dilutions were made by measuring 3ml, 5ml, 7ml and 10ml to give 30%, 50%, 70% and

100% respectively in each 1000ml beaker capacity. Twenty larvae of *C. quinquefaciatus* (obtained from the larval stock were released into each beaker containing the different concentrations of same extracts). Control was also set up with twenty larvae per beaker maintained in 500ml of water from the mother stock, the effect of each extract concentration was monitored by counting the number of dead larvae at interval of 12, 24, 48 and 72 hours of larvae exposure. Each experiment was replicated three times including control. Larvae motility calculated in percentage, while treatment means were calculated using ANOVA at 5% level of significance.

RESULTS

Result obtained from the first, second and third trials (repeated experiments) where various concentrations of extract of *Eucalyptus camaldulensis* plant leave (aqueous) was tested against larvae of *Culex* mosquito presented in Table 1, 2, 3, while Table 4 comprise the average of all three repeated experiments. It is evident from Table 1 that out of four different concentrations of *Eucalyptus* extracts used, the extract of almost all concentrations used proved to be effective against the larval stages of *Culex* mosquito. The extract of highest concentration proved to be most effective than the lowest concentration. In all concentrations of extracts the mortality was time and dose dependent.

It is observed from Table IV that twelve hours after the application of the 30% extract, more than 70% of the larvae were looking paralyzed, though they were alive (response against touch). After 24 hours, 93.3% of the larvae were dead and 36 hours later, all the larvae were observed dead, resulting in 100% mortality.

In the trials, where 50% *Eucalyptus* extract was used, the mortality among larvae started after twelve hours of application with 35.5% and all larvae were observed dead within fourty eight (48) hours.

Table IV also shows that 50% of the larvae were dead after twelve hours of treating with the extract of 70% concentration. Total mortality was recorded within 24 hours of treatment.

In trials where 100% extract was used, 100 percent of the larvae were observed dead. A rapid increase in mortality follows an increase in concentration of the extract.

The lower the concentration, the longer the time it took before the extract action started. Thirty five point five percent (35.5%) was the least mortality recorded in twelve hours after

the application of extract of 50% concentration, while the highest mortality was 100% in twelve hours after the application of 100% concentration.

The five percent (5%) mortality recorded in the control medium after 48 – 72 hours was assumed to be natural death since the extract was not applied to it.

Even though the mortality rate remained the same in both the two larvae stage (early and late), the time of action varies. It was observed that among nearly hatched larvae, reaction was more quick than the late (matured larvae).

Table 1: Mortality among *Culex quinquefaciatus* larvae treated with different concentrations of *E. camaldulensis* leaves extract

S/N	Conc. of *E. camaldulensis* leave extract	No. of larvae per beaker	Mortality in % (Number of larvae died) Period of exposure (hrs)				
			12	24	48	72	Mean ($\bar{x}$)
1.	30%	20.00	0.00	90.00	100.00	100.00	72.50
2.	50%	20.00	30.00	95.00	100.00	100.00	81.30
3.	70%	20.00	50.00	100.00	100.00	100.00	87.30
4.	100%	20.00	100.00	100.00	100.00	100.00	87.50
5.	0% (control)	20.00	0.00	0.00	5.00	5.00	100.00
Mean ($\bar{x}$)		20.00	36.00	77.00	81.00	81.00	-

Table 2: Mortality among *Culex quinquefaciatus* larvae treated with different concentrations of *E. camaldulensis* leaves extract

S/N	Conc. of *E. camaldulensis* leave extract	No. of larvae per beaker	Mortality in % (Number of larvae died) Period of exposure (hrs)				
			12	24	48	72	Mean ($\bar{x}$)
1.	30%	20.00	0.00	95.00	100.00	100.00	73.50
2.	50%	20.00	35.00	95.00	100.00	100.00	82.50
3.	70%	20.00	50.00	100.00	100.00	100.00	87.50
4.	100%	20.00	100.00	100.00	100.00	100.00	100.00
5.	0% (control)	20.00	0.00	0.00	5.00	5.00	2.00
Mean ($\bar{x}$)		20.00	37.00	77.00	81.00	81.00	-

Table 3: Mortality among *Culex quinquefaciatus* larvae treated with different concentrations of *E. camaldulensis* leaves extract

S/N	Conc. of *E. camaldulensis* leave extract	No. of larvae per beaker	Mortality in % (Number of larvae died) Period of exposure (hrs)				
			12	24	48	72	Mean ($\bar{x}$)
1.	30%	20.00	0.00	95.00	100.00	100.00	73.50
2.	50%	20.00	35.00	95.00	100.00	100.00	82.50
3.	70%	20.00	50.00	100.00	100.00	100.00	87.50
4.	100%	20.00	100.00	100.00	100.00	100.00	100.00
5.	0% (control)	20.00	0.00	0.00	5.00	5.00	2.00
Mean ($\bar{x}$)		20.00	37.00	78.00	81.00	81.00	-

Table 4: Average of all the three repeated experiments

S/N	Conc. of *E. camaldulensis* leave extract	No. of larvae per beaker	Mortality in % (Number of larvae died) Period of exposure (hrs)				
			12	24	48	72	Mean ($\bar{x}$)
1.	30%	20.00	0.00	93.30	100.00	100.00	73.34
2.	50%	20.00	35.50	95.00	100.00	100.00	82.13
3.	70%	20.00	50.00	100.00	100.00	100.00	87.50
4.	100%	20.00	100.00	100.00	100.00	100.00	100.00
5.	0% (control)	20.00	0.00	0.00	5.00	5.00	0.75
Mean ($\bar{x}$)		20.00	36.70	77.70	81.00	81.00	-

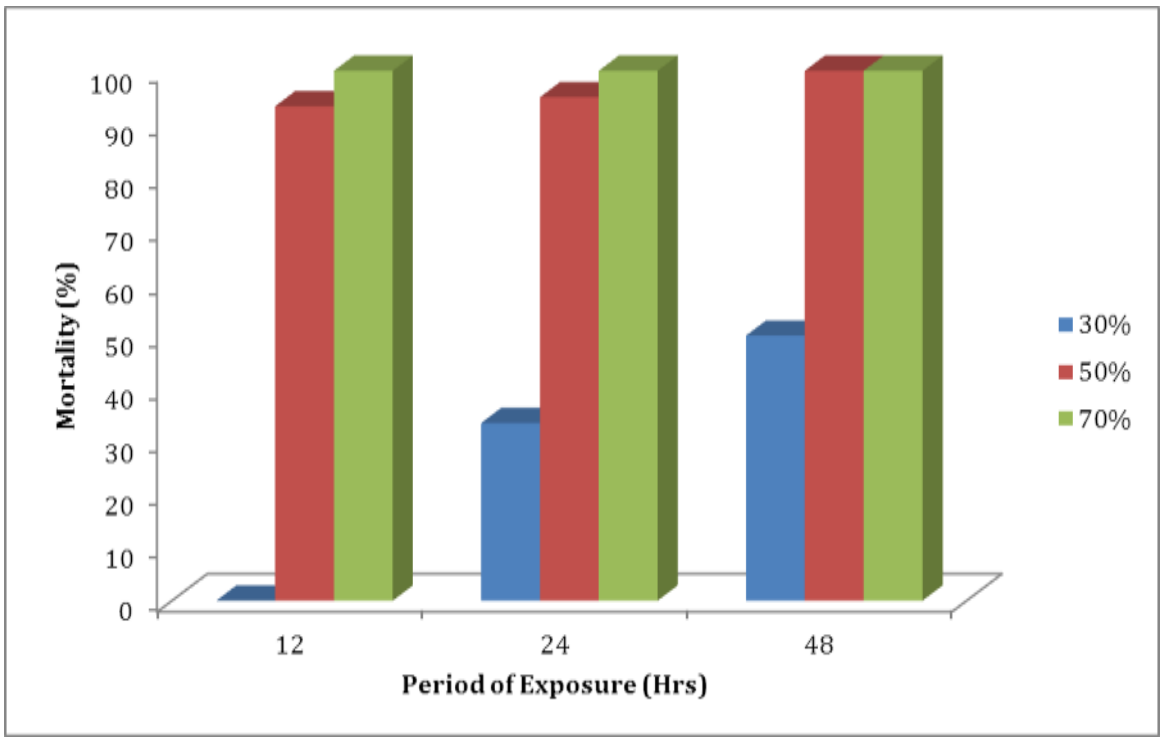

Figure 1:Showing the mortality at 30%, 50% and 70% concentration at given exposure period of time (hrs)

DISCUSSION

It is clear from the results that *Eucalyptus camaldulensis* possess larvicidal effect against *C. quinquefasciatus*. The 100% extract concentration was however, found to be the most effective with lethal values of 100% mortality for those larvae exposed to 12 hours treatment. Phytochemical screening of the crude extract showed that *E. camaldulensis* contain large amount of 1,8-cineole, β-pinene, γ-terpinenene as the major component in leaves (Sumriophon *et al*., 2006), the toxic effects of the extracts on mosquito larvae in the present study may be due to the compounds 1,8-cineole, β-pinene and γ-terpinenene contents, as essential oils with high cineole contents demonstrate good antimicrobial (Gundiza *et al*., 1993) and nematocidal activity (Sangiran, 1990).

The aqueous extract of *Ricinus nasutum* showed LC_{50} values of 5.124 & 9.681mg/L against *C. quinquefaciatus* & *Aedes aegypti* respectively (Chansang *et al*., 2005). The value of lethal concentration were much higher than those obtained in present study, proving the efficacy of extract and also implying that *E. camaldulensis* has a different combinations of bioactive ingredients, or different metabolic pathway or compounds that can rapidly penetrate the soft skin of larvae.

Several studies have established that the activity of phytochemical compounds on target species vary with plant part from which they were extracted, solvent of extraction, geographical origin of the plant and photosensitivity of some of the compounds in the extract are also important factors (Sukuma *et al*., 1991).

Present study also shows that the mortality rate was found to be directly proportional to the concentration of extracts used. Similar observation about *Eucalyptus* oil was also made by (CSIRO, 2005), that *Eucalyptus* oil acts, whatever be the method of application, as fumigant and *Eucalyptus* vapour passes into the trachea of the insects, paralyzing nervous system.

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