Current Research Journal of Biological Sciences 4(2): 220-224, 2012
ISSN: 2041-0778

Submitted: December 16, 2011 Accepted: January 13, 2012 Published: March 10, 2012

Evaluation of Water and Ethanol Extract of *Eucalyptus globulus* Labillardiere (Myrtaceae) Leaves against Immature Stages of Filarial Vector *Culex quinquefasciatus* Say (Diptera: Culicidae)

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Abstract: Aim of the present study was to evaluate larvicidal and pupicidal effect of aqueous and ethanol extract of *Eucalyptus globulus* leaves against immature stages of *Culex* mosquitoes. The experiment was conducted in the Botany Laboratory, University of Gondar, Gondar from February 2011 to May 2011. The standard WHO protocol was used to check five concentrations such as 100, 250, 500, 750 and 1000 ppm against II, III, IVth instar larvae and pupae of *Culex* mosquitoes, respectively. Irrespective of the concentrations tested and the period of exposure, 100% larval mortality was recorded in 1000 ppm concentration after 72 h exposure period followed by 96% in 750 ppm and 90% in 500 ppm. The percentage of larval mortality was based on concentration of the extract and the exposure period. When both are increased the percentage of larval mortality was also increased. After 72 h, 100% pupal mortality was recorded in 500, 750 and 1000 ppm concentration. Study reveled that aqueous and ethanol extract of *Eucalyptus* leaves is having strong insecticidal activity and it could be useful for the control of larval and pupal stage of *Culex* mosquitoes in small breeding places. Further, effective simple formulation and field evaluation of this plant extract is suggested for successful implementation of large scale use in mosquito control program.

Key words: Aqueous, *culex*, ethanol, extract, *Eucalyptus globulus*, larvicidal, pupicidal, mortality

INTRODUCTION

Culex species are most significant vector for the transmission of *Wuchereria bancrofti* which is responsible for lymphatic filariasis (Subra, 1981; Rajagopalan *et al*., 1987). Lymphatic filariasis, known as elephantiasis, puts at risk for more than a billion people in more than 80 countries. Over 120 million people have already been affected by it; over 40 million of them are seriously affected and disfigured by the diseases. One third of the people infected with disease are live in India, one third are in Africa and most remainder in South Asia, the Pacific and Americas (WHO, 2005). In South-East Asian countries, it is one of the most major public health problems and eight out of ten countries in the regions are known to be endemic for filariasis (WHO, 2004).

Chemical control is an effective strategy used extensively in mosquito control program. Many kinds of toxic chemical compounds to mosquitoes are includes organochlorine, organophosphorus, carbamates, pyrethroids, respectively. However, indiscriminate usage of chemicals in the breeding site and also used in the form of adulticides, fumigants, repellents and residual spray the rate of mosquito breeding are increased (Kumar and Hwang, 2006). The continuous usage of chemicals disrupts natural enemies and also let to outbreaks of some insect species (Katade *et al*., 2006). The problems of high cost and development of resistance in many vector mosquito species to several synthetic insecticides have revived interest in exploiting the pest control potential of plants (Grainge and Ahamed, 1988). The chemicals derived from plants have been projected as weapons in future mosquito control programme as they are shown to function as general toxicant, growth and reproductive inhibitors, repellents and oviposition deterrent (Sukumar *et al*., 1991). The Genus *Eucalyptus* (family Myrtaceae) represented over 700 species distributed throughout the World and the oil has been known for hundreds of years as having antibacterial, antifungal and antiseptic properties (Brooker and Kleinig, 2006). *Eucalyptus* essential oils have a wide spectrum of biological activity against fungi, bacteria, insects, mites and weeds and provide a simple, inexpensive and environmentally friendly alternative to pest control (Mossi *et al*., 2011). Present paper reports the effectiveness of aqueous and ethanol extract of *Eucalyptus globulus* leaves against the immature stages of *Culex quinquefasciatus*.

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MATERIALS AND METHODS

The experiment was conducted in the botany laboratory, Faculty of Natural and Computational Sciences, University of Gondar, Gondar from February 2011 to May 2011.

Collection and maintenance of *culex* larval mosquitoes: *Culex* larvae were collected from the stagnant water with rich organic pollution in and around Tewodros campus and also near by places around University of Gondar. Mosquito larval collection was done from the breeding site by using large kitchen strainer and transferred to large plastic container and transported to the laboratory for further study. In the laboratory the larvae was kept in tap water and provided powdered dog biscuit along with yeast powder (3:1 ratio) as a feed. After acclimatization of the mosquito larvae in the laboratory conditions, subsequent experiments were conducted.

Collection and extraction of plant materials: *Eucalyptus* leaves were collected from the vicinity of Tewodros campus, University of Gondar in the month of February 2011. The collected plant leaves were washed with tap water to clean the dust and other materials and allowed to dry under shade in the botany laboratory. The dried plant leaves were powdered by using local coffee powder making iron mortal and pestal. Finally, fine powder was collected from the powdered leaves by sieving through the kitchen strainer and used for extraction.

Method of extraction: Twenty gram of powdered plant material was kept in 200 mL of conical flask and added 100 mL of solvent such as water and ethanol individually. The mouth of the conical flask was covered with aluminum foil and kept in a shaker for 12 h for continuous agitation for thorough mixing of powder in to solvent. Then the extract was filtered by using muslin cloth followed by Whatman no 1 filter paper and the solvents from the extract were removed by using rotary vacuum evaporator. Finally the residues were collected, weighed and used for the experiment.

Preparation of concentrations: Stock solution of 1000 ppm concentration was prepared by adding 1gm of plant powder with 5 mL of acetone and make up to 1000 mL by adding tap water. From the stock solution 0.1% of soap powder was added for emulsification purpose. From the stock solution four different concentrations such as 100, 250, 500 and 750 ppm, respectively was prepared by serial dilution method. All these concentrations were tested against immature stages of *Culex*.

Evaluation of larvicidal activity: Larvicidal activity of water and ethanol extract of *E. globulus* was evaluated by using WHO method (1996) with modifications. Ten second, third and fourth instar larvae were released in to 100 mL glass test tube. In each test tube concentration of water and ethanol extract was maintained at 100, 250, 500, 750 and 1000 ppm, respectively concentration with the final water volume of 50 mL individually. In control experiment except plant materials remaining all was added as mentioned in the preparation of concentration. The larval mortality rates were recorded continuously for 24, 48 and 72 h. The dead larvae in five replicates were counted individually and converted in to percentage of mortality. Dead larvae were identified when they failed to move when the water was disturbed. The experiment was replicated five times and the percentage mortality was calculated. The corrected percentage of mortality was calculated by using Abbott's formula (Abbott, 1925).

$$\text{Corrected \% mortality} = [\%\ \text{mortality in test} - \%\ \text{mortality in control}]/[100 - \%\ \text{mortality in control}] \times 100.$$

Evaluation of pupicidal activity: Pupicidal activity of the plant extract was tested with same concentration and methods followed in the larvicidal activity. For pupicidal activity 10 numbers of freshly emerged pupae were released in each concentration individually and the percentage of mortality was recorded continuously for 24, 48 and 72 h. The experiments were replicated five times and the percentage of mortality was calculated. The corrected percentage of pupal mortality was calculated based on Abbott's formula as mentioned earlier.

Statistical analysis: The data collected from the five replicates were subjected to statistical analysis to derive mean and standard deviation. Statistical significance of different concentration and exposure period was confirmed by two way analysis of variance (ANOVA) by using Microsoft Excel program.

RESULTS

Ethanol and water extracts of *E. globulus* plant leaves were yields 4.6 and 2.6 g of crude extract respectively. These extracts were tested at 100, 250, 500, 750 and 1000 ppm, against immature *Culex* mosquitoes. Mean percentage of larval mortality of IInd instar larvae was minimum in all the concentration after 24 h exposure period (Table 1). After 48 h exposure period, maximum mortality of 96% was observed in 750 ppm concentration. After 72 h exposure period, 100% mortality was recorded in 750 ppm and 94% was recorded in 1000 ppm

Table 1: Mean percentage mortality of IInd instar larvae of *C. quinquefasciatus* exposed to water and ethanol extract of *E. globulus* leaves at different concentration and different exposure period

	Water extract			Ethanol extract		
Tested concentration in ppm	24 h	48 h	72 h	24 h	48 h	72 h
100	6±5.4	6±5.4	12±8.3	6±5.4	24±5.4	30±0
250	18±13.0	24±8.9	24±8.9	12±9.5	64±5.4	78±16.4
500	32±16.4	74±11.4	82±8.3	32±4.4	80±10	90±10.0
750	38±13.0	96±5.4	100±0	30±12.2	70±18.7	96±5.4
1000	30±14.4	84±8.9	94±5.4	60±27.3	96±5.4	100±0

Values are percentage mean and standard deviation of five replications; Two way ANOVA showed statistical significance ($p<0.05$) within the concentration and the exposure period

Table 2: Mean percentage mortality of IIIrd instar larvae of *C. quinquefasciatus* exposed to water and ethanol extract of *E. globulus* leaves at different concentration and different exposure period

	Water extract			Ethanol extract		
Tested concentration in ppm	24 h	48 h	72 h	24 h	48 h	72 h
100	10±10.0	18±8.3	34±8.9	10±10.0	18±8.3	40±10.0
250	10±10.0	44±20.7	68±16.4	10±10.0	44±20.7	48±13.0
500	36±5.47	42±10.9	54±20.7	36±5.4	42±10.9	74±5.48
750	44±18.1	58±4.47	66±11.4	44±18.1	58±4.4	78±8.3
1000	52±4.4	64±15.1	88±13.0	52±4.48	64±15.1	86±5.4

Values are percentage mean and standard deviation of five replications; Two way ANOVA showed statistical significance ($p<0.05$) within the concentration and the exposure period

Table 3: Mean percentage mortality of IVth instar larvae of *C.quinquefasciatus* exposed to water and ethanol extract of *E. globulus* leaves at different concentration and different exposure period

	Water extract			Ethanol extract		
Tested concentration in ppm	24 h	48 h	72 h	24 h	48 h	72 h
100	4±5.48	18±13.0	24±18.1	8±8.37	26±11.4	40±15.8
250	4±5.48	22±13.0	34±18.1	10±12.2	24±16.7	36±18.1
500	14±5.48	26±5.44	4±5.4	26±15.1	38±16.4	48±21.6
750	22±13.0	34±18.1	50±17.3	38±13.0	70±10.0	90±10.0
1000	48±30.3	66±29.6	82±14.8	92±13.3	100±0	100±0

Values are percentage mean and standard deviation of five replications; Two way ANOVA showed statistical significance ($p<0.05$) within the concentration tested and the period exposure

Table 4: Mean percentage mortality of pupal stage of *C.quinquefasciatus* exposed to water and ethanol extract of *E. globulus* leaves at different concentration and different exposure period

	Water extract			Ethanol extract		
Tested concentration in ppm	24 h	48 h	72 h	24 h	48 h	72 h
100	22±8.3	64±5.48	94±5.4	44±16.7	76±5.4	94±5.4
250	38±17.8	82±4.48	98±4.4	42±25.8	80±14.1	98±4.4
500	30±12.2	80±0	98±4.4	58±8.3	82±4.48	100±0
750	30±17.3	72±17.8	100±0	82±29.4	100±0	100±0
1000	28±10.9	92±13.0	100±0	84±23.0	98±4.4	100±0

Values are percentage mean and standard deviation of five replications; Two way ANOVA showed statistical significance ($p<0.05$) with the concentration and the period exposure

concentration. In ethanol extract, 60% larval mortality was recorded in 1000 ppm concentration after 24 h exposure period. After 48 h exposure period, maximum mortality of 96% was recorded in 1000 ppm concentration followed by 80% in 500 ppm and 70% in 750 ppm. After 72 h exposure period, 100% larval mortality was recorded in 1000 ppm concentration followed by 96% in 750 ppm and 90% in 500 ppm. The percentage of larval mortality was based on the concentration of plant extract and the period of exposure. The two way analysis of variance showed statistical significance within the period of exposure, within the concentration tested ($p<0.05$).

Mean percentage mortality of IIIrd instar larvae was recorded at the miximum of 52% in 1000 ppm concentration of water extract after 24 h exposure period. When the concentration of plant extract was increased and the percentage of larval mortality was also increased. After 48 h exposure period maximum of 64% mortality was noted at 1000 ppm concentration. The maximum percentage larval mortality of 88% was recorded in same

1000 ppm concentration after 72 h exposure period. When the larva exposed to ethanol extract at 1000 ppm concentration the percentage of larval mortality was on par with water extract after 24 and 48 h exposure period. However, after 72 h exposure period 86% mortality was recorded in 1000 ppm concentration followed by 78% in 750 ppm and 74% in 500 ppm concentration (Table 2). The percentage of larval mortality was clearly dose dependent and also exposure period. Two way ANOVA results showed statistical significance ($p<0.05$) within the exposure period and within the concentrations tested.

Mean percentage mortality of IVth instar larvae was at the maximum of 48% was noted in 1000 ppm concentration of water extract after 24 h exposure period. However, after 48 and 72 h exposure period, larval mortality was 66 and 82%, respectively. When the larvae exposed to ethanol extract maximum of 92% larval mortality was recorded in 1000 ppm concentration after 24 h exposure period. After 48 h exposure period, 100% mortality was recorded in the same concentration. After 72 h exposure period, 100% mortality was recorded in 1000 ppm concentration followed by 90% mortality in 750 ppm concentration (Table 3). The percentage larval mortality was clearly based on the period of exposure and the concentration of the plant extracts tested. Two way analysis of variance clearly showed statistical significance within the period of exposure and within the concentration tested ($p<0.05$).

Mean percentage mortality of pupal stage was at the maximum of 38% in water extract after 24 h exposure period. After 48 h exposure period, 92% mortality was recorded in 1000 ppm followed by 82% in 250 ppm; 80% in 500 ppm; 72% in 750 ppm, respectively. However, after 72 h exposure period 750 and 1000 ppm concentration showed 100% pupal mortality. At lower concentration of 100 ppm showed 94% mortality followed by 98% in both 250 and 500 ppm concentration. When the pupa exposed to ethanol extract after 24 h exposure period the maximum mortality of 84 and 82% was recorded in 1000 and 750 ppm concentration respectively. After 48 h exposure period, 100% mortality was recorded in 750 ppm concentration followed by 98% in 1000 ppm; 82% in 500 ppm; 80% in 250 ppm and 76% in 100 ppm concentration, respectively. After 72 h exposure period in 500, 750 and 1000 ppm, respectively concentration showed 100% pupal mortality (Table 4). Two way analysis of variance showed that the pupal mortality in water and ethanol extract within the period of exposure and within the concentrations tested were statistically significant ($p<0.05$).

DISCUSSION

Mosquito borne diseases are one of the most public health problems in the developing countries. It can be controlled by preventing mosquito bite using repellent causing larval mortality and killing mosquitoes. There are several methods practiced world wide to control mosquitoes one of the approach is to control the mosquitoes at larval stage in order to minimize the adult populations. In larval control using plant products is one of the safer alternative methods. Many plant based products are widely used for their insecticidal/ repellant properties for control of mosquitoes/protection from mosquito bites (Jacobson and Crosby, 1971; Sukumar *et al*., 1991).

Present study the effect of water and ethanol extract of *E. globulus* leaves were studied in a dose dependent manner with different exposure periods. Irrespective of the concentration tested and the period of exposure both water and ethanol extracts showed strong larvicidal and pupicidal activity against immature stages of *Culex* mosquitoes. Our findings are in agreement with Senthil Nathan (2007) who has reported that higher larvicidal activity of *Eucalyptus tereticornis* oil (leaf extract) with increased doses on *Anopheles stephensi*. In addition, further observed that the first and second instar larvae were most susceptible to all treatments. Our findings are also showed similar results in early instar stages of *Culex* larvae.

Present study after 72 h exposure period 100% pupal mortality was recorded in 750 and 1000 ppm concentration. It is clearly showed that percentage of mortality is related to the contraction of the plant extract and the duration of the exposure period. It is in agreement with the report of Batish *et al.* (2008); they have reported that pesticidal activity of *Eucalyptus* oil has been related to the presence of components such 1,8-cineole, citronellal, citronellol, citronellyl acetate, ecamalol, limonene, linalool etc. Among the various compounds 1, 8-cineole was the most important and in fact, a characteristic compound of the genus *Eucaluptus*, being largely responsible for a variety of pesticide property (Duke, 2004). Our findings are also in agreement with these findings because *Eucalyptus* leaves which are mostly having the oil that is responsible for the rate of mortality.

From this study it is very clear that the *Eucalyptus* leaves extract is having strong insecticidal activity and it could be useful for the control of larval and pupal stage of the *Culex* mosquitoes. Many literatures are also reported that the compound 1,8-cineole present in the leaves is responsible for effectiveness and the compound is also easily biodegradable. However, biosafety of the plant extracts, improved formulation technique to increase the insecticidal potential, stability and cost effects need to be analyzed before going to implement in large scale. Even though, aqueous extract of the present study was showed promising result and it can be useful for small manmade breeding places to reduce the breeding rate of *Culex* mosquitoes thereby reducing the rate of filariasis causal organism transmission.

CONCLUSION

Eucalyptus globulus are growing extensively in Ethiopian highlands. The commercial exploitation surely be a safer alternative and contribute much towards resource poor communities in this region and the country as whole. Further research activities are focused on to develop simple formulation techniques and field evaluation to confirm the present findings for large scale application.

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