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Effectiveness of red water tree (*Erythrophleum suaveolens*) plant barks and roots extracts in controlling mosquitoes (*Anopheles gambiae*) larvae in Songea district, Tanzania

Mnguu Y.O.[1], Nkomera V.A.[2] and Malila M.N.[1]
[1]: Institute of Rural Development Planning (IRDP), P.O. Box 138, Dodoma, Tanzania,
[2]: Songea Municipal Council, P.O.Box 14. Songea, Tanzania
Corresponding author email: yomnguu@gmail.com , Mobile phone: +255755966250

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1 ABSTRACT

Malaria is one of the most important parasitic diseases and the largest killer disease in Tanzania. The conventional control measures for the disease vector involved the use of inorganic insecticide spray like *Dichloro Diphenyl Trichloroethane* (DDT) which poses a lot of environmental as well as health problems. This paper intended to assess effectiveness of organic insecticide extracted from *Erythrophleum suaveolens* plant barks and plant roots in the control of larvae of *Anopheles gambiae* by determining the lethal dose and lethal time at LD 50 and LD 95 of plant bark and roots extract solution and determining the availability of plant in the study area. The experimental design used was randomized block with 10 treatments of extract concentration from plant bark and roots. The treatments also comprised of control arm and standard arm. Larvae of *Anopheles gambiae* at 3rd and 4th instars were subjected to various concentrations of the extracts solution. Mortality was counted and recorded after one hour and 24 hours exposure to the chemical. The results from the study revealed that *Erythrophleum suaveolens* plant barks extract have greater lethal effects to larvae than extracts from plant roots. Mortality of 100% was recorded after one hour of exposure at dosage of 70 mls to100mls per litre for plant barks extract solution and mortality of 100% after twenty-four hours of exposure at dosage of 10mls to 100 mls per litre for plant barks extract solution. The availability of the *Erythrophleum suaveolens* plant showed a distribution index of 1.76 indicating regular distribution. Basing on the findings of this study, it was concluded that plant barks extract solution is more effective in controlling mosquito. The study recommends further analysis to evaluate an appropriate formulation and assess whether there are adverse effects for the use of extracts from *Erythrophleum suaveolens* plant bark.

2 INTRODUCTION

Malaria is one of the most important parasitic diseases in the world, which is thought to be responsible to over 500 million of illness and up to 2.7 million deaths annually (Holt *et al.*, 2002). It ranks among the three major health and developmental challenges facing most of the poorest countries in the tropical and sub-tropical regions of the world (Pilula, 2010). In Tanzania, Malaria remains the largest killer disease and an enormous public health problem-ranking number one in both outpatient and inpatient statistics and accounts

for over 30% of the national diseases burden (Maegga *et al.*, 2005; URT, 2008; Masanja, 2011). This disease is widely transmitted by mosquito specie known as *Anopheles gambiae;* the major vector of malaria parasites (*Plasmodium falciparum)* in Africa and one of the most efficient malaria vectors in the world. Earlier efforts in the control of this disease focused on use of synthetic pesticides for vector control, which formed an important component of the World Health Organization (WHO) Global Strategy for Malaria Control. Synthetic pesticide such as *Dichloro Diphenyl Trichloroethane* (DDT) is widely used to control a wide range of insects both in residential houses and in agricultural crops. Scientists estimated that indoor spray with DDT have freed almost a billion people of endemic malaria since 1945 by preventing the transmission of diseases such as malaria, bubonic plague, sleeping sickness and typhus (Robert & Tren, 2011). The use of DDT has however, raised more concern on its ecological effects such as bioaccumulation and its non-selective nature. In addition, most of these pesticides have adverse environmental effects to flora and fauna. For instance, despite the fact that DDT has a low acute toxicity, its chemical stability results to accumulation in the environment through food chains and in tissues of exposed organisms, including people living in treated houses, giving more concern in relation to its possible long-term toxicity (WHO, 2007). Although many Governments are still debating on the use of DDT, the WHO declared its use as been more effective in malaria control particularly due to its long term effects in killing the vector of malaria (WHO, 2006). However, the sustainability of DDT use has also started to raise some questions as its effectiveness in killing malaria vector has recently shown to decrease due to resistance of some species of mosquito. The study by Jean (2010) on *Anopheles gambiae* resistance to *Deltamethrin* showed that between the year 2007 and 2010, the proportion of the insects with a genetic resistance to *Deltamethrin* rose from 8% to 48%, respectively. The use of synthetic insecticides in mosquito control is also constrained by the fact that many rural communities cannot afford the costs for buying these chemicals and that majority lack appropriate knowledge on its use posing greater health risks on its use. Bio-insecticides have shown some potential to replace synthetic insecticides in malaria vector control if adequate evaluation is conducted to ensure user-friendly formulations. Previous study by the World Health Organization in Vietnam established that non- DDT malaria controls were significantly and more effective than DDT use (WHO, 2000). The study on the effectiveness of bio-insecticides by Kumar and Maneemegalai (2008) on larvicidal of methanol and ethanol extract from leaves and flowers of *Lantana camara* plants from the family of Verbanaceae was effective on third and fourth instar for mosquito species of *Aedes aegypti* and *Culex quinquefasciatus* after being investigated for 24 hours. Their results showed that with 1.0 mg/ml and 3.0mg/ml concentrations of extracts of *Lantana camara*, 100% mortality was observed in *Aedes aegypti* and *Culex quinquefasciatus* when exposed for 24 hours, respectively. This study was however, confined in mosquito species of *Aedes aegypti* and *Culex quinquefasciatus* and information on other mosquito species like *Anopheles gambiae* is lacking. Experience in Songea district in Tanzania has shown that, *Erythrophleum suaveolens* plant bark and roots have insecticide effects for killing bollworms in farms and in the preservation of grains from beetles and weevils. The present study was therefore aimed at to assess the effectiveness of *Erythrophleum suaveolens* plant barks and roots extracts in the control larvae of mosquitoes *Anopheles gambiae*. Specifically, the study aimed at: determining the lethal dose at LD 50 and LD 95 of extracts solution of *Erythrophleum suaveolens* plant bark and root to control larvae of *Anopheles gambiae* mosquitoes; examine effect of time the larvae of *Anopheles gambiae* mosquito are exposed to the lethal dose of extracts from plant bark and root of *Erythrophleum suaveolens* and; to examine

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the availability of the *Erythrophleum suaveolens* plant in Songea District

3 RESEARCH METHODOLOGY

This study was conducted in the laboratory of Songea Municipal Council (Tanzania) located near 11°17'42"S 34°48'7"E in the western part and its eastern point is located near 10°17'40"S 40°10'43"E. The research adopted a randomized complete block design. The larvae of mosquito *Anopheles gambiae* were exposed to 10 different extract concentrations from tree bark and roots that formed the experimental treatments (Table 1). In each experimental unit, 10 larvae were exposed different extract concentrations for both 1 hour and 24 hours.

Table 1: Treatment Codes and Descriptions

Treatment Code	Description
T1	10 mils of bark or roots extract
T2	20 mils of bark or roots extract
T3	30 mils of bark or roots extract
T4	40 mils of bark or roots extract
T5	50 mils of bark or roots extract
T6	60 mils of bark or roots extract
T7	70 mils of bark or roots extract
T8	80 mils of bark or roots extract
T9	90 mils of bark or roots extract
T10	100 mils of bark or roots extract
Standard	5 mils of bacillus *Thiphelicus isliensii*
Control	No extract solution

The mosquito larvae in their 3rd and 4th instars were reared in the laboratory whereby 1280 larvae were sampled. The transparent pipette was used to remove larva one by one from rearing tray into testing container. The experiment comprised of test arm, standard arm and a control arm. The adult female *Anopheles* mosquitoes were collected from the field by using mouth aspirators (indoor catching). The collection was made during the night from 21hrs to 7 hrs in the morning. Collected mosquitoes were transferred in paper cups with 5% of glucose solution and were taken to the laboratory. The mosquitoes kept in the special cage for laying the eggs in the special containers under room temperature of 28º to 30ºC after being fed with the blood of the rabbit; the larva were fed by glucose. During the 3rd and 4th instars of larva (three days after hatching), they were tested by *Erythrophleum suaveolens* plant barks and plant roots solutions. The larvae of *Anopheles gambiae* mosquitoes were subjected to various concentrations of plant bark, roots extract solution and mortality were counted and recorded.

3.1 Extracts from *Erythrophleum suaveolens* plant barks and roots: The barks and roots collected from trees in the forest located at Mhukuru Village in Songea District. The barks were grinded into powder. A 100gm of *Erythrophleum suaveolens* powder was soaked into one litre of water for 24 hrs, one day before the experiment. The filtered stock solutions were kept into a beaker. The plant roots were crushed by using mortar and pestle then filtered using clean and sterile pieces of clothes to obtain the extract solution (Lemmens *et al.*, 1995).

3.2 Lethal concentration of *Erythrophleum suaveolens* plant barks roots : From the prepared solution, different concentrations were used in two experiments (one experiment using extracts from plant bark and the other using extracts from plant roots).

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In both experiments, concentrations used were: 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mls per litre. Each experiment had three replicates. White polyethylene containers used with the volume of one litre of water in each with different concentrations. To reduce overcrowding and congestion, about 10 specimens of larva were placed in each concentration and exposed to the solution and mortality was observed after 60 minutes (1 hr) up to 24hrs. One polyethylene container with the same volume of water and larva without the plant solution was used as control group and one polythene container with the same amount of water, larva and 5 grams of *Bacillus thuringiensis Israelensis*, was used as a standard group. After every exposure time, dead larvae were removed and kept in another tray with the volume of water of 500ml within 24hrs for verification/confirmation. Thereafter, they were recorded as dead or alive. All tests of different concentrations made simultaneously and repeatedly three (3) times with the average temperature of 28^{0C} and pH.6.8 **(**WHO, 2005).

3.3 Availability of Erythrophleum suaveolens plants: The nearest neighbour formula was used to determine the availability of *Erythrophleum suaveolens* plant at Mhukuru village in Songea District.

The formula was:

$$Rn = 2d\sqrt{\{n\}}{\{A\}}$$

Where:
Rn = the description of the distribution
d = the mean distance between the nearest neighbours (m)
n = the number of *Erythrophleum suaveolens* plant in the study area.
A= the area under study (m^2) (Waugh, (2002).

The Nearest neighbour index measures the degree of spatial dispersion in the distribution based on the minimum of the inter-feature distances i.e. it is based on the distance between adjacent point features. The distance between point features in a clustered pattern becomes smaller than in a scattered (uniform) distribution with random falling between the two. The data for the number of mosquito larvae died in each treatment were subjected to the analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 16-computer programme and means were separated using Duncan Multiple Range Test.

4 RESULTS AND DISCUSSION

4.1 Lethal Dose at LD 50 of Extract Solution of Erythrophleum suaveolens : The results in Table 2 showed that concentrations of extract less than 20 mills per litre from plant bark have no lethal effect on the mosquito larvae. However, the results demonstrated that LD50 for extract from plant bark was 60 mills/litre and LD 95 was 70 mills/litre and no such value could be established for plant roots in one-hour exposure to chemicals. These results also showed higher larvicidal effect of the same treatments when the larvae were exposed for 24 hours for all treatments used where the extract from plant bark was used (Table 3). Comparison on the effectiveness of plant parts revealed that plant bark is more effective than plant roots. The results on the extract from plant bark are in parallel with those by Chowdhury *et al.* **(**2008) who observed that protein extracted from mature leaves of *Solanum villosum* plant have larvicide against larvae of *Culex quinquefasciatus*, *Anopheles stephensi* and *Stegomyia aegypti* and recorded at LD 50/LD95 active dose of 60 mills to 100 mills. The implication of these results signifies the possibility of using this bark extract to control *Anopheles gambiae* mosquito larva because both LD 50 and LD 95 were attained.

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Table 2: Effect of plant part at 1-hour exposure to the extracts

Treatment	No. of larvae Died		Difference
	Plant Bark	Plant roots	
10 mls	0a	0a	0ns
20 mls	0a	0a	0ns
30 mls	2b	0a	2*
40 mls	3c	0a	3*
50 mls	4d	0a	4*
60 mls	5e	0a	5*
70 mls	10f	0a	10*
80 mls	10f	0a	10*
90 mls	10f	0a	10*
100 mls	10f	0a	10*
Standard	10f	10b	0*
Control group	0a	0a	0ns

Note: ns = not significant; * significant at 5%; Letters in the same column bearing the same letter are not significantly different

Table 3: Effect of plant part on mosquito larvae at 24 hrs exposure

Treatment	No. of larvae Died		Difference
	Plant Bark	Plant roots	
10 mls	10b	0a	10*
20 mls	10b	0a	10*
30 mls	10b	0a	10*
40 mls	10b	0a	10*
50 mls	10b	0a	10*
60 mls	10b	0a	10*
70 mls	10b	0a	10*
80 mls	10b	0a	10*
90 mls	10b	1b	9*
100 mls	10b	1b	9*
Standard	10b	10c	0ns
Control group	0a	0a	0ns

Note: * significant at 5%; Letters in the same column bearing the same letter are not significantly different

4.2 Effect of time: The results from this study indicated that extract from plant bark performed very well at concentrations ranging from 50- 100 mills/litre in one-hour exposure while at 24 hours all treatments showed equal performance (Table 4). This however, imply that 70 mills/litre can be used to eradicate most of the larvae (95) in just one hour as compared to the use of 10 mills/litter and expose the larvae for 24 hours. The former will have the cost implication and possible bigger environmental concern as more chemicals are exposed to the environment while the later may results to build up of resistance due to extended time of exposure to the chemical. This all together may necessitate another analysis on the appropriate doses that may result to effective eradication of mosquito larvae with little effect to the environment. However, the challenge that needs to be addressed is how to

harvest plant bark without affecting the rejuvenation of the red water plant and therefore affect the population of the tree species. Using extracts from plant roots, results showed that time has very little effect on the death of the mosquito larvae at concentrations less than 100 mills/litre (Table 5). Greater doses may have cost implications and adverse effects to the environment. Therefore, LD 50 and LD 95 could not be established. It can therefore be argued that extracts from plant roots are not appropriate to control mosquito larvae using doses less than 100 mills/litre.

Table 4: Effect of bark extract on mosquito larvae

Treatment	Number of Larvae Died		Difference
	1 Hr	24 Hrs	
10 mls	0a	10b	-10**
20 mls	0a	10b	-10**
30 mls	2b	10b	-8**
40 mls	3c	10b	-7**
50 mls	4d	10b	-6*
60 mls	5e	10b	-5*
70 mls	10f	10b	0ns
80 mls	10f	10b	0ns
90 mls	10f	10b	0ns
100 mls	10f	10b	0ns
Standard	10f	10b	0ns
Control group	0a	0a	0ns

Note: Letters in the same column bearing the same letter are not significantly different,
* = significant at 5% and ** = significant at 1%

Table 5: Effect of plant root extract on mosquito larva

Treatment	Number of Larvae Died		Difference
	1 Hr	24 Hrs	
10 mls	0a	0a	0ns
20 mls	0a	0a	0ns
30 mls	0a	0a	0ns
40 mls	0a	0a	0ns
50 mls	0a	0a	0ns
60 mls	0a	0a	0ns
70 mls	0a	0a	0ns
80 mls	0a	0a	0ns
90 mls	0a	1b	1ns
100 mls	0a	1b	1ns
Standard	10b	10c	0ns
Control	0a	0a	0ns

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4.3 The availability of the Erythrophleum suavelens plant: The mean distance between nearest plants in the study area was 2.793 m (Table 6) and forty trees per four hundred square meters. These results conforms with those of Waugh (2002) who also pointed that fewer than thirty trees becomes difficult to say with any confidence that the distribution has regular distribution tendency. The index was computed as follows:

$$Rn = 2 \times 2.793\sqrt{(40/400)} = 1.766$$

Therefore, the computations showed that the distribution of the *Erythrophleum suaveolens* plant was value of 1.766. Using Fig.1,it shows that the tendency was towards regular distribution. This further signifies adequate availability of the tree plant in the study area.

Table 6: Distance to nearest neighbour tree in the study area (m)

Frequency of nearest neighbour distance in the area of 400 m^2							
4	3.7	3	3.3	3.48	3.02	2.12	2.2
2.27	1.77	5	1.81	2.1	2.98	1.93	2.57
4	3.7	3.3	3.48	3.02	2.12	2.2	3.35
1.81	2.1	2.98	1.93	2.57	3.1	2.9	3
3.35	3.1	1.26	1.77	1.26	2.9	2.27	5

d = 2.793 m

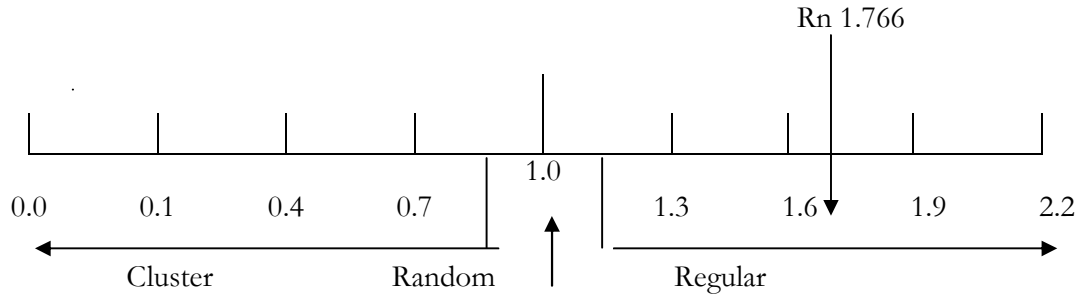

Figure 1: Rn value for *Erythrophleum suaveolens* distribution in the study area

5 CONCLUSION

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he paper concludes that *Erytrophleum suaveolens* plant barks are more effective to larvae of *Anopheles gambiae* mosquitoes under laboratory conditions. The lethal doses (LD50) for extracts from plant bark was 60 mills/litre and LD95 was 70 mills/litter Furthermore, 10 mills/litre of the extract from plant bark when exposed for 24 hours have the same effect as using 70 mills/litre and expose the chemical for 1 hour. It is therefore, recommended to undertake further study to reveal an appropriate formulation and address any environmental effect in the use of extracts from *Erytrophleum suaveolens* plant barks.

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