Evaluation of Some Plants for their Repellency against Some Anopheline and Culicines Mosquitoes in Koka-Negewo (Central Ethiopia)

By

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TABLE OF CONTENTS

Tables

pages

II. TABLE OF CONTENTS.....i
III. LIST OF TABLES.....iv
IV. LIST OF FIGURES.....v
V. LIST OF APPENDICES.....vi
VI. ABSTRACT.....vii
I. INTRODUCTION.....1
1.1. MALARIA IN ETHIOPIA.....2
1.2. THE VECTORS OF MALARIA IN ETHIOPIA.....4
1. 3. VECTOR CONTROL METHODS.....5
1.3.1. Chemical Control and Resistance to Chemical nsecticides.....5
1.3.2. Biological control.....8
1.3.3. Environmental Management.....9
1.3.4. Insecticide Treated Mosquito Nets (ITN).....10
1.3. 5. Insect Repellents.....10
1.3.5.1. Synthetic Repellents.....11
1.3.5.2. Natural Repellents.....12
1.3.5.2.1. Essential Oils.....12
1.3.5.2.2. Traditional Repellents.....14
1.4. VECTOR CONTROL IN ETHIOPIA.....15
2. OBJECTIVES.....17
2.1. GENERAL OBJECTIVE.....17
2.2. SPECIFIC OBJECTIVES.....17
3. MATERIALS AND METHODS.....18
3.1. THE STUDY AREA.....18
3.2 SELECTION AND COLLECTION OF TEST PLANTS.....19
3.3 DESCREPTION OF TEST PLANTS.....19
3.4. REPELLENCY TEST PROCEDURE.....21
3.5 DATA ANALYSIS.....23
4. RESULTS.....24

4.1 MOSQUITO COLLECTION 24
4.2 REPELLENCY STATUS OF THE PLANT PARTS.. 24
4.2.1 Direct Burning of Seeds of *S. molle, M. azedarach* and *R. challepensis*......................24
4.2.2. Direct Burning of Leaves of *S.molle, M. azedarach* and *R. challepensis*...............26
4.2.3. Direct Burning of Leaves of *P. abyssinica, O. lamifolium* and *E. globulus*...............28
4.2.4. Direct Burning of Leaves of flowers, and seeds of *L. camar*................................30
4.2.5 Thermal Expulsion of volatiles from Seeds of *S. molle, M. azedarach* and *R. challepensis*...32
4.2.6. Thermal Expulsion of volatiles from Leaves of *S. molle, M. azedarach* and *R. chllepensis*...34
4.2.7. Thermal Expulsion of volatiles from leaves of *P. abyssinica, E. globulus* and *O. amifolium*...36
4.2.8. Thermal Expulsion of volatiles from Leaves, Flowers and Seeds of *L. camara*.........38
4.2.9. Effect of Fresh Leaves of *S. molle, M. azedarach* and *R. challepensis*.....................40
4.2.10. Effect of Fresh Leaves of *P. abyssinica, E. globulus* and *O. lamifolium*...............42
4.2.11. Effect of Fresh Seeds of *S. molle, M. azedarach* and *R. challepensis*...................44
4.2.12. Effect of Fresh Leaves Flowers and Seeds of *L. camara*.................................46
4.3 Comparison of Direct Burning, Thermal Expulsion and Fresh Plant 48
Parts. 48
5. DISCUSSION.. 52
6. CONCLUSIONS AND RECOMMENDATIONS 56
REFERENCES 57
APPENDICES 66

LIST OF TABLES

Tables Page

Table 1 Percent repellency of direct burning of seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicines..25

Table 2 Percent repellency of direct burning of leaves of different test plants against *An. arabiensis*, *An.pharoensis* and culicines..27

Table 3 Percent repellency of direct burning of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicine..29

Table 4. Percent repellency by direct burning of leaves of different test plants against *An. arabiensis*, An. pharoensis and culicines..31

Table 5. Percentage repellency of direct burning of different parts of *L. camara* against *An. arabiensis*, *An. pharoensis* and culicines..33

Table 6. Percent repellency of thermal expulsion of seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicines..35

Table 7. Percent repellency of thermal expulsion of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines..37

Table 8. Percent repellency of thermal expulsion of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines..39

Table 9. Percent repellency of fresh leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines-..41

Table 10. Percent repellency of fresh leaves of *S. molle*, *M. azedarach* and *R. challepensis* against *An. arabiensis*, *An. pharoensis* and culicines..43

Table 11 Percent repellency of fresh seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicine-..45

Table 12. Percent repellency of fresh seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicine..47

IV. LIST OF FIGURES

Figure 1 Repellency by direct burning of plant parts 49
Figure 2 Repellency rate of thermal expulsion of plant parts 50
Figure 3 Repellency rate of fresh parts of plants 51

V. LIST OF APPENDICES

Appendix 1. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant seeds (*S. molle, M. azedarach* and *R. challepensis*) using direct burning 66
Appendix 2. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant seeds (*S. molle*, *M. azedarach* and *R. challepensis*) using thermal expulsion 66
Appendix 3. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*S. molle*, *M. azedarach* and *R. challepensis*) using direct burning........................ 67
Appendix 4. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*S. molle*, *M. azedarach* and *R. challepensis*) using thermal expulsion 67
Appendix 5. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*S. molle*, *M. azedarach* and *R. challepensis*) by placing fresh leaves 68
Appendix 6. Summary table for analysis of variance (ANOVA) for Repellency of mosquitoes by plant seeds (*S. molle, M. azedarach* and *R. challepensis*) by placing fresh seeds..................... 68
Appendix 7. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*P.abyssinica, E. globulus, and O.lamifolium*) using thermal expulsion............... 69
Appendix 8. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*P.aybssinica, E. globulus, and O.lamifolium)* by placing fresh parts. 69
Appendix 9. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves (*P.abyssinica, E. globulus, and O.lamifolium*) using direct burning..................... 70
Appendix 10. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by *L. camara* (seeds, flowers, and leaves) using placing of its fresh. 70
Appendix 11. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by *L. camara* (seeds, flowers, and leaves) using thermal expulsion. 71
Appendix 12. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by *L. camara* (seeds, flowers, and leaves) using direct burning...................................71
Appendix 13. Test plants..72
Appendix 14. Experimental test tukuls...73
Appendix 15. Human baits..74

VI. ABSTRACT

To evaluate and compare the repellency of 7 different local plants against different mosquitoes, investigations were made by using field experiments in Koka Negewo village about 94kms on the road between Addis-Ababa and Awassa. The experiments were conducted inside houses by using four human volunteers in four replicates tewelve different times in the total of 48 days. Burning of the plants was made by placing parts on burning charcoal stoves while the thermal expulsion was done by placing the plant parts on small metal plates on burning stoves. Fresh part was tested by placing them on a plate and raised 35cms in front of the mosquito collectors. Direct burning of seeds of *R. challepensis*, *S. molle* and *M. azedarach* provided 78.9%, 58.3% and 54.3% repellency against *An. arabiensis* respectively. Highest repellency showed with the seed of *R. challapensis* ($P<0.001$). The seed of this plant showed similar effect on *An. pharoensis* and Culicines. In the contrary *An. arabiensis* was more repelled by burning the leaves of *S. molle* ($P<0.05$) than the leaves of *R. challepensis* and *M. azedarach*.

Direct burning *P. abyssinica, O. lamifolium* showed remarkable repellency against *An. arabiensis* ($P<0.05$). However, less protection was exhibited to the other two mosquito species. Less repellency against all the three mosquito species has been obtained by *E. globules* in any test methods. Burning of flowers, leaves and seeds of *L. camara* exhibit similar but significant repellency ($P<0.05$) against the two Anopheline species. However, no remarkable repellency was exhibited against the culicines.

Only seed of *R. challepensis* showed good repellency to *An. arabiensis* than the seeds of *S. molle* and *M. azedarach* in the application of thermal expulsion of their volatiles. In contrast three of the plant seeds were found less protective to *An. pharoensis*. On the other hand thermal expulsion of volatiles from leaves of same plants well protected *An. arabiensis*. Less repellency effect was obtained by thermal expulsion of volatiles from leaves *P. abyssinica*, *E. globulus* and *O. lamifolium* against the three mosquitoes. All parts of *L. camara* significantly repelled *An. arabiensis* only.

Fresh leaves of *R. chalepensis* provided appreciable protection against the two Anopheline species. *An. pharoensis* also well protected by fresh leaves of *M. azedarach* and *S. molle*. *O. lamifolium*, *P. abyssinica* and *E. globulus* failed to protect well *An. arabiensis*. However, the former two repelled *An. pharoensis*. On the other hand all the fresh parts of *L. camara* failed to

protect against the three mosquito species. However, significant repellency potential has been obtained from placing the seed part of the plant, but only to *An. pharoensis* ($P<0.05$).

As the plants are locally available they can be easily used for mosquito protection by the local communities, it is suggested to integrate them with other vector control options. Moreover, further investigations are required to evaluate the oils extracted from these plants.

I. INTRODUCTION

Malaria is prevalent in about 100 countries. Africa takes the largest and the regions of southern Sahara appears to be the most affected (WHO, 2000). Worldwide malaria incidence is estimated to be about 300-500 million cases each year, with about 90% of these occurring in Africa, south of the Sahara. *Plasmodium falciparum* is the major cause of the disease (WHO, 1995; 2000). Worldwide 80% malaria cases and 90-95% of malaria and malaria related deaths are estimated to occur in Africa. Moreover, more than one million deaths each year occur in the continent, of which most of them are children under 5 years age. One out of every 20 children born in the continent die due to this disease and its related illness before the age of five (Samba, 2000).

Malaria is becoming more difficult to manage. Improvement of transportation facility highly increases the risk of getting malaria outbreaks by escalating population mobility from non-endemic areas to malarious areas and the vice-versa. For instance, during the 1970's and 1980's the resurgence of imported malaria in some of the developed countries, like the U.S.A., Europe and the U.K. was reported (Gilles and Warrell, 1993).

Malaria has also an impact on economic loss especially in the developing world. It causes significant economic cost in some poor countries. For instance in 1997 alone, health economists estimated that the African region lost more than $2 billion because of malaria and malaria related diseases (Samba, 2000). Liese (1998) estimated that families with malaria clear only 40% as much land for crops and the yield is 30% below normal. In addition, single bout malaria estimated to cost the equivalent of more than ten working days in the continent.

Furthermore, widespread resistance of malaria parasites to anti-malarial drugs and insecticide resistance of the vectors have resulted in flare-ups of malaria and complication of its control. The number of *Anopheles* species resistant to insecticides is increasing. In 1992 about 55 resistant *Anopheles* species have been recorded and 21 of which were found to be very important vectors of malaria (Gilles and Warrell, 1993). All members of *An. gambiae* s.l. have shown various degrees of resistance to DDT and other organo-chlorine insecticides. The increasing resistance of vectors to the conventionally used insecticides raises concern. In the history of vector control, insecticides have been the mainstay to treat human habitations and fabrics. Although there are other alternative vector control tools, there is less practice to make them in use.

Generally, in many countries today, malaria is still responsible for serious illnesses and numerous deaths. The ever growing concern for the protection of vector species and the downward trend in availability of the broad spectrum pesticides for public health are forcing people to look for other economical means of control of malaria vectors (Gupta and Rutledge, 1994). The cost of pesticides is increasing from time to time and it is becoming unaffordable for most of the less developed nations like Ethiopia. The increasing insecticide resistance of vectors to the currently used compounds is a growing problem. Therefore, the use of insect repellents is an alternate means of providing relief when other conventional vector control methods are not feasible. Though there are many types of synthetic repellents so far developed, their uses are limited because of their cost and are not widely used. The best way now appears to develop botanical derived repellents based on traditional knowledge and practice, on which this work has emphasized.

1.1. MALARIA IN ETHIOPIA

Nearly three quarters of the land in the country is considered to be malarious and about 65% of the populations are at risk of the disease (Tulu, 1993; MOH, 2002). The country is situated in the tropical zone which comprises mass of central high lands, girdled by low lying, hot and arid regions. Thus, the distribution and transmission dynamics of malarias is related to the diversity of the physical geography, variations in climate, Socio-economic patterns of the population (MOH, 2002).

In 1958 a serious out break of malaria in the highlands of Ethiopia occurs. This resulted in the case of 3 million and death of 150,000 people (Fontaine *et al.*, 1961). In areas of higher altitudes the disease is occurring mostly from September through November after the long rainy season. In contrary (Gebermariam, *et al.*, 1984) noted that there is also often out break of malaria following the short rainy season of March and April. The distribution of the disease in Ethiopia is determined by altitude, topographical and climatic factors. Unstable malaria in the highlands of the country is occurring predominantly. Moreover, periodic malaria epidemic is common in Ethiopia (Gebremariam, *et al.*, 1988). Tulu (1993) reviewed the climatic conditions of the country and categorize as dega or cold zone (above, 2500m altitude), free of malaria and no records of indigenous transmission so for obtained. This may be due to the inhabitant climatic factors are inconvenient for both the vector species and the survival of the parasites inside the vectors (Ghebreysus, *et al.*, 2000). The second one is the weynadega (temperate) zone, malaria occurs but most often below 2000m. Due to exposure-related communal immunity, it has low endemicity and very unstable with frequent outbreaks which results in high morbidity and mortality. On the other hand, places such as Setit-Humera, Metema, Metekel, Gambella, Gode and Awash valley are representative of the lowland areas of the country where the disease is endemic. Thus, transmission of malaria from low land to high land areas by immigration of different people is a common phenomenon (Tulu, 1993). In addition, this endemicity of the disease in the lowland areas is one of the reasons for the scarce population in the lowlands and over crowded population in the highland areas for many centuries.

Periodic malaria epidemic is common because of the unstable behavior of the disease. As a result frequent occurrences of moderate to sever epidemics are known in Ethiopia (Fontaine *et al.*, 1961). In some of the lowland areas where there is ample environment for mosquito breeding perennial malaria transmission exists (Tulu, 1993).

In Ethiopia four *Plasmodium* Species are known to cause malaria, such species include *P. falciparum, P. vivax, P. malarae*, and *P. ovale.* Of these *P. vivax* has wider distribution, but *P. falciparum* takes about 60% malaria transmission followed by *P.vivax* (40%). *P. malarae* comprises less than 1%and is mainly reported from Arbaminch area and recently also reported from Nathreth. *P. ovalae* is reported from few patients in Humera, Gambela, and Gamu-Gofa (Gebre-Mariam, 1984).

Most frequent and fatal cases of malaria is caused by *P. falciparum* in Ethiopia; about 10% case fatality rate in hospitalized adults and nearly 33% in children less than 12 years old. Seventy percent of malaria illness and death especially during epidemics is also caused by *P. falciparum* (Armstrong, 1978; cited in Gebre-Mariam, *et al*., 1988).

Recently the high population increase, frequent drought, famine, and agro-industrial developments in the highlands make people more interested to resettle in the lowlands. This resettlement and agro industrial expansion undertaken in lowland areas changed the pattern of malaria transmission in the country especially in Gambella (Nigatu *et al*., 1992).

1.2. THE VECTORS OF MALARIA IN ETHIOPIA

Gebre-Mariam *et al.* (1988) indicated that presently there are 42 *Anopheles* species in Ethiopia. Worldwide 422 species of *Anopheles* mosquitoes exist, of which only 70 are so far recognized as potential vectors of malaria (Service, 1993).Till now there are only two members of *An. gambiae* complex found in Ethiopia, *An. arabiensis* and *An.qaudriannulatus* species B (White *et al*., 1980; Hunt *et al*., 1998). *Anopheles qaudriannulatus* species A is found in South Africa (Hunt *et al*., 1998), thus the total members of species in the *An.gambiae* complex now increased to seven. The most important vector of malaria in Ethiopia is *An.arabiensis*, despite the fact that the Anopheline fauna is highly diversified (White *et al*., 1980; Abose *et al*., 1998) and the distribution of this species is concentrated in the lower rainfall zones, which represent the drier savannah areas, in collection sites where annual rainfall <1000mm (Coetzee *et al*., 2000). Larvae of this species prefer small, temporary, and sunlit water bodies with emergent vegetation and are a very important vector where malaria is unstable (Fontenille and Lochouarn, 1999). In addition, it primary feeds outdoors and rests indoors. *Anopheles quadriannulatus* sp. B is not a malaria vector as it feeds mainly on cattle (White *et al*., 1980; Gilles and Warrell, 1993).

Anopheles pharoensis is the second most frequent and widely distributed (Tulu, 1993).It bites man or animals both indoors and outdoors, but rests predominantly indoors (Bruce-Chewatt, 1985). This species prefer large, permanent and shaded water bodies with emergent vegetation such as irrigation canals, rice fields, and lake shores.

Anopheles funestus is the third common vectors of malaria in Ethiopia and has a good vectoral capacity (Fontelline, *et al.* 1961). It bites man predominantly, but also bites domestic animals feeds both indoors and outdoors. The larvae of this species also occurs in more or less permanent waters, especially with vegetation, such as swamps, marshes, edges of streams, and rivers (Bruce-Chwatt, 1985).

Anopheles nili is the list common species, and it is more localized and confined to southern, western and north western parts of the country (Tulu, 1993). It breeds in the edges of rivers, and in some areas of high population density, along rivers, and they exhibit good vectoral capacity (Fontenille and Lochouarn, 1999).

1. 3. VECTOR CONTROL METHODS

Vector control is an essential component of malaria control programs. However, there is little or no participation of communities to understand the value and relevance (WHO, 1995). This led to poor use of available alternative control tools, inappropriate use of insecticides, inadequate resources and over all poor management. The problem is aggravated by changing environmental conditions in areas in which exploitation of natural resources and development activities are taking place (Sloof, 1987; Tulu, 1993; WHO, 1995; 2000).

Though some of the methods are not practical in many counties for many reasons, there are several vector control options, and these are, chemical control, biological control personal protection methods (use of repellents and insecticide treated mosquito nets) and environmental management.

1.3.1. Chemical Control and Resistance to Chemical Insecticides

Despite the growing problem of resistance of many mosquito vectors and the present concern with the effect of some of the compounds to the environment; chemical insecticides are still the mainstay of most vector control programmes. Pyrethrum was the first insecticide which was implemented in 1935 in South Africa and in parts of India, but DDT and other insecticides replaced it after Second World War (Bruce-Chwatt, 1985). Indoor residual spraying of insecticides to kill adult mosquitoes is one of the components of the different vector control method which aims at spray the sprayable surface of individual houses at the right time, with the right insecticide and sufficient dosage so that longevity of the anopheline vector will be reduced to the level that the mosquitoes would not to live long enough to transmit the parasite (MOH, 2002). Basically the effectiveness of this method depends on resting behavior of the mosquito vector, the toxicity and persistence of the residual insecticide, and the customs of people with regard to housing and night shelters (Bruce-Chwatt, 1985). Moreover, indoor residual spraying is more appropriate for epidemic prone areas, areas of economic importance, in refugee camps and initial protection of non immune settlers in development areas (WHO, 2000). On the other hand since most anopheline vectors breed in different rain pools, stream margins, forest pools etc., or where source reduction is impossible, it is very important to use insecticides to control the larvae of the vectors (Gratz and Jany, 1994).

In Ethiopia, the primary choice of insecticide for indoor application is DDT, especially where local mosquitoes are less resistant to the insecticide, this is because it is relatively cheap, has long residual effect and safe to humans and animals. However, in places where the local vectors are DDT resistant, an alternative insecticide Malathion is used (MOH, 2002).

Resistance to chemical insecticides refers to the ability of a population of insects to tolerate doses of insecticide that would prove lethal to the majority of individuals in a normal population of the same species; and this biological phenomenon develops as a result of selection pressure (Bruce-Chewatt, 1985). Insecticide resistance is becoming a major problem in many areas of the world (Hemingway, *et al.*, 1988; 1990; Roberts and Andre, 1994). In 1992, 73% of DDT by weight has been implemented or used for indoor spraying in the globe (WHO, 1995). According to different studies level of resistance to DDT differs among the different species of mosquitoes and within the same species which are found in different places. This is due to the frequency of exposure of the vectors to the chemical. Appearance of resistance can be a major factor in deciding whether a compound continues to be used for vector control in addition to the price and ecological factors (Gratz and Jany, 1994; Roberts and Andre, 1994). DDT resistance is associated with metabolic or physiological changes in the insect /vectors/ with either increase the rate of degradation of insecticides to non- toxic products or decreases the sensitivity of target site to inhibition (Herath, *et al.*, 1988). Generally resistance to most pesticides is very likely to develop after vector populations have been exposed to them for a long but varied period of time, depending on the genetic make up of the target population and the extent to which genes that cause resistance are present(Roberts and Andre,1994).

According to Prasittisuk and Curtis (1982) DDT has been a safe and effective insecticide for malaria control with long residual life and is one of a few methods which are sufficiently cheap to be usable under present conditions on a large scale in rural areas of developing countries. Resistance to DDT was relatively slow but is now widespread and in some mosquitoes there is evidence that this resistance can cause cross- resistance to permithrin (Prasittisuk and Curtis, 1982). When DDT spraying was suspended, the pressure of natural selection against DDT spraying resistant genes in some *Anophelines* seem to be weak (Curtis *et al.*, 1978: cited in Parasittisuk and Cirtis, 1982). Zaim (1987) reported the status of DDT resistance in A*nopheline* vectors of malaria in Iran: *Anopheles stephensi*; *Anopheles culicifacies*, *Anopheles maculipennis* and *Anopheles sacharovi* were to be resistant to DDT.

In Ethiopia despite the need of evaluating the resistance status of *An. arabiensis* country wide in view of the prolonged usage of DDT, the records are only for a few localities (reviewed by Abose *et al.*, 1998). The WHO bioassay standard test kits have been in use to detect resistance. The records indicated low (10%) to higher (60-70%) levels of resistance (Ameneshewa, 1995; Abose *et al.*, 1998; Balkew *et al.*, 2003). Gambella and Arbaminch were the two places with higher resistance. Where there is higher resistance such as Alamata and Sheraro woredas in Tigray, DDT is being replaced by Malathion (Abose *et al.*, 1998; Ghebreyesus, *et al.*, 2000). In other places, DDT remains the major insecticide for indoor application, as it is relatively cheap and persistent (MOH, 2002).

1.3.2. Biological Control

Biological control is one of the mosquito vector control methods by which living organisms are used as natural enemies in the form of predators, competitors and pathogens, which interact with vectors and their environment. Though the effective use of this method needs good knowledge of bionomics of the vector species and of local ecological conditions; in recent years, it is indicated as alternative to chemical pesticides (Service, 1983; Bruce-Chwatt, 1985)). The great potential of biological methods lies in their use together with environmental manipulations, with agricultural practices and even with some pesticides. Nowadays, attention is given to two bacteria and their products; such bacteria are *Bacillus thuringiensis* serotype H-14 and *Bacillus sphaericus*. The former forms spores and produces toxin which is a potent gut poison when ingested by mosquitoes and other aquatic insects but harmless to plants, animals, and man. The use of *B. thuringiensis* is operational program is limited due to its short residual effect. On the other hand *Bacillus sphaericus* which also produces a toxin in its spore envelope can multiply in polluted habitats (Bruce-Chwatt, 1985; Gilles and Warrell, 1993; Lacey and Orr, 1994). This toxin is also harmless to man and animals and is more potent and more specific to many mosquitoes.

Some species of fungi (*Coelomomyces iliensis*) and nematodes are capable of causing massive mortalities in larvae of *Anopheles* mosquitoes. However, there is problem of cultivation and practical use but for the future this natural pathogen can be a potential interest. Several species of mosquitoes in the genus *Toxorhynchites* are also considered as possible biological control agents (Castner and Bailey, 1987). The larvae of which feed on other mosquito larvae. They may be effective on distruction of container-breeding species but not so effective in field condition (Bruce-Chwatt, 1985; Castner and Bailey, 1987).

The use of larvivorous fish has been the most successful biological mosquito control methods and has been implemented in parts of the world. This fish are natural enemies of mosquito larvae and have been more utilized as mosquito control in different countries of the world. Naturally, the use of larvivarous fish is limited to some special situations where the water and other conditions are suitable. Cisterns, shallow ponds, small streams, ornamental pools are ideal places for mosquitoes control by fish. Field trials like in Karnataka state in India indicated promising results but they were not utilized at operational scale (Lacey and Orr, 1994). In another field trial in wild rice field in California, *Gambusia affinis* was shown to control *Aedes aegypti* (Kramer *et al.*, 1987). Work done in Asseb, Eritrea (when it was part of Ethiopia) by Flecher *et al.* (1992) showed an indigenous fish, *Aphanius dispar* to be very effective in controlling mosquito larvae in including the local mosquito vectors, *An. culicifacies adanensis* in their breeding habitats such as cisterns, wells and barrels.

1.3.3. Environmental Management

Environmental management for vector control has been defined as the planning, organization, carrying out and monitoring of activities for the modification and/or manipulation of environmental factors on their interaction with man, with the view of preventing or minimizing vector propagation and reducing man-vector contact (Bruce-Chwatt, 1985). It is one component of the integrated vector control which in long run is cost effective, environmentally safe and community oriented control strategy. Three approaches are comprised in this method: I) creating a permanent change in a vector's habitat (environmental modifications), II) using temporary or repeatitive measures to manipulate the natural factors that limit vector reproduction, survival or abundance (environmental manipulation); and III) reducing man vector contact with infective vectors by zooprophylaxis, modification of human habitations (Ault, 1994). The use of salt water marsh drainage, ditch clearing and other source reduction measures have successfully reduced the *Anopheline* vectors in Haiti, Israel, and the United states (Schliessmann, *et al.*, 1973; Kitron and Spielman, 1989: cited in Ault, 1994). In areas of high vector resistance problem this method can be used as alternative method of control; moreover environmental management is effective in urban areas and where development activities are taking place. It is better to undertake these activities during the transmission season as a regular sanitation program. However, the quantity, quality and effectiveness of these program activities are difficult to measure. for instance that the size of breeding sites drained, filled, cleared and the number of community participants are regularly reported in Tigray from the concerned officials of the communities (Ghebreyesus, *et al.*, 2000).

1.3.4. Insecticide Treated Mosquito Nets (ITN)

This method is less effective for exophilic and exophagic mosquitoes and when people are not protected during the active biting time of the vectors, because the treatment of bed nets with insecticides mainly aimed at killing mosquitoes coming in contact with the net or biting through the net. Therefore, in order to apply this method for mosquito control, understanding of the feeding and resting habits of the vectors, the cultural practices and sleeping habits of the target people is crucial as they are determinants of the efficacy of this method (WHO, 1995). It was found successful to control malaria morbidity and mortality in many places of Africa and western pacific regions (WHO, 1995). Moreover, in Africa south of the Sahara use of ITN is gradually increasing with a shift from project based to operational implementation.

In Ethiopia ITN is a new control method, which was introduced as a component of other malaria control methods (MOH, 2002). It is also used singly for travelers and temporary residents against malaria. However, disseminating the program has got challenges such as difficulty of re-impregnation of the nets, poor awareness, and low purchasing ability of the local communities. In addition increased tax of the nets and the pesticides make the program to be slow to implement. Therefore, to avoid this problem the government is encouraging private investors in the production of the nets and the pyrethroid chemicals and minimizing tax both for the nets and the chemicals (MOH, 2002). Despite the program is new it has provided promising protection against malaria in different regional states in the country (MOH, 2002). For instance, in Tigary this program exhibited good protection for the demobilized and returnees from Eritrea (Ghebreyesus, *et al.*, 1999). However, re-impregnation of the nets failed after two years due to the war with Eritrea.

1.3. 5. Insect Repellents

Repellents prevent human mosquito contact by making the vectors to fail to locate their hosts. They are applied directly to the skin or clothing or other fabrics. They are recommended for people standing or sleeping out side (out doors) at night for work or leisure and those working in plantations and may be at work during day time (Fradin, 1998). There are different kinds of repellents which are used or applied in various forms and these different types of repellents can be categorized in to synthetic and natural repellents.

1.3.5.1. Synthetic Repellents

Synthetic repellents are those repellents, which are synthesized and manufactured. They can play an important role in the interruption of transmission of insect borne diseases at individual as well as community level by preventing mosquitoes and other blood-sucking insects from biting (Schmidt and Schmidt[2,] 1969; Wirtz *et al.*, 1986; Kalyanasundaram *et al.*, 1994; Rozendal, 1997; Mittal and Subbarao, 2003).

Though their efficacy is not equal, there are many types of synthetic repellents so far manufactured, such as N, m-diethyl–m-toluomide (DEET), dimethyl phthalate (DMP), N- diethyl phenyl acetamide (DEPA). Of these repellents, DEET is the most effective and widely distributed (Frances, 1987). DEET was discovered in 1946 by the U.S Department of Agriculture and was patented by the U.S army and it was subsequently registered for the general public use in 1957 (EPA, 1996; Ware, 1999). The product is relatively safe, but it can be toxic if it is misused. DEET can be available in 5% to 100% concentration in multiple formulations; however, it is recommended only in 5% to 30% because more concentrations of the product cause problem by percolating through the skin in to the blood stream especially in children (Fradin, 1998). Durrheim and Govere (2002) reported topical application of 15% DEET to feet and ankles reduced the over all biting rate of *An. arabiensis* by 69%. Yap *et al.* (1998) showed that DEET gave complete protection during the first four hours against *Culex* mosquitoes.

Mixture of DEET with other repellents also gave good results against mosquito biting. When DEET based repellents are applied in combination with permithrin-treated clothing, nearly 100% protection can be achieved from the bites of mosquitoes (Frances, 1987). Lindsay *et al.* (1998) reported that mixture of DEET with thanaka (*Limonia acidissma*) gave 65 and 85% protection against *An. minimums* and *An. maculatus*, respectively. This could show the presence of variation in susceptibility of different species of mosquitoes to a particular or group of repellents. Kumar *et* *al* (1992) tested three repellents (DEET, DEPA and DMP) against the hard tick *Rhipicephalus sanguineus* and the soft tick *Argas persicus* by topical application on rabbits and it was found that a 25% concentration of DEET gave >90% repellency. In the same experiment, dimethyl phthalate (DMP) gave the list repellency. DMP has been evaluated as repellent against different species of sand flies, but did not give adequate protection as that of DEET (Schmidt and Schmidt[2], 1969; Kalyanasundaram *et al.*, 1994). Kalyanasundaram, *et al.* (1994) reported the comparative repellency effect of DEPA (N, N-diethyl phenyl acetamide) to *Phlebotomes papatasi*. In another experiment made by Writz *et al.* (1986) against *P. papatasi*. DMP gave similar protection with that of DEET. Kulkarni (1977) has tested different synthetic repellents including DMP and DEET against larvae of mites and the result was promising especially when DMP is used in combination with DEET. Meher *et al.* (1986) showed that DMP were more effective against soft tick than any other commercial or experimental repellents. Curtis *et al.* (1987) reviewed the importance of DMP against different species of mosquitoes.

1.3.5.2. Natural Repellents

Natural repellents are those repellents obtained from plants and plant products. They are used as smokes, impregnation of clothing or nets, topical applications, and as fresh or live plants (Kokwaro, 1976; Curtis *et al.*, 1991; Fradin, 1998; Palsson and Jaenson, 1999; Seyoum *et al.*, 2002 a, b).

1.3.5.2.1. Essential Oils

The essential oils or volatile oils are found in many different species of plants. These oils are distinguished from fatty oils by the fact that they evaporate or volatilize in contact with air and may posses a pleasant and strong aromatic odor. They can be removed from plant tissues without any change in composition; and they have complex chemical nature (Hill, 1952). Some types of essential oils such as basil oils are found in the international market (Nigist and Berhanu, 1989).

Though various plants have been reported to possess repellent activity against mosquitoes (Sukumar, *et al*, 1991), the most studied plants include *Azadirachta indica*, *Eucalyptus* species.

Lantana camara, *Cymbopogon* spp., *Mentha pipetita* and *Tagets minuta* (Mittal and Subbarao, 2003). On the other hand, plant extracts of *Lippia javanica, Cimpopogon excavatus* (rose geranium) and *Plargonium reniforme* (lemon grass) have been tested on humans against *An. arabiensis* mosquitoes from South Africa (Govere *et al.*, 2000). The results where reported as follows: *L. javanica* provided 76.7% protection after four hours but after five hours the repellency was reduced to 59.3% . The other plants, rose geranium and lemon grass gave 63.3% and 66.7% protection, respectively.

The essential oils of different species of *Ocimum* were tested for their repellency against different species of insects (Padilha, 2003). Oil of *O. selloi* showed good repellency effect against mosquitoes (Padilha, 2003). The oil of *O. sancutum* has been used against mosquitoes of different species (Mittal and Subbarao, 2003). Tawatsin and Waratten (2001) reported that oil of *O. americanum* (hairy bail) provided about four hours protection against *Aedes aegypti* six hours protection against *An. dirus* and eight hours protection against *Culex quinquefaciatus*.

Essential oils extracted from the seeds of *O. suave*; *O. kilimandscharicum* and *O. kenyense* have also provided good protection against stored product insect pests (Jembere, 1995; Bekele *et al.*, 1996; 1997).

On the other hand, lemon eucalyptus oil has also been known to have strong repellency effect against mosquitoes (Curtis *et al.*, 1991; Trigg, 1996). The most important essential oil found in lemon eucalyptus is quwenling that possesses the ingredient para mentane diol. Its efficacy increases proportionally with its concentration and dosage (Curtis *et al.*, 1991). In Tanzania, a repellent made from quwenling showed comparable efficacy and duration of protection with that of DEET against *An. gambiae* and *An. funestus* (Trig, 1996). Similar effect was shown against *Aedes aegypti* and *Aedes albopictus* (Curtis *et al.*, 1991).

Oil of citronella is extracted from the grass, *Cymbopogon nardus* and it contains the active ingredient, citronellol. Citronellol is commonly found in natural or herbal insect repellents (Fradin, 1998; Fradin and Day, 2002). In India it is a popular repellent (Curtis *et al.*, 1991) and it is one of the few natural repellents commercially available in Europe and North America. The oil provides acceptable protection when frequently applied to external human body with recommended amount but gave shorter protection time than DEET (Fradin and Day, 2002). For example, it was experimentally shown that 0.01µmol of DEET per liter of air was sufficient to prevent 90% of mosquitoes from landing on their targets; a 100 fold higher concentration of citronella was required to achieve similar effect (Fradin and Day, 2002).

Other studies carried out in India and elsewhere have shown the repellent action of *A. indica* (neem) oil. Topical application of 2% neem mixed in coconut oil produced varying degree of protection against different vector species especially the *Anopheline* species (Mittal and Subbarao, 2003).. Sharma and Dhiman (1993) evaluated the repellent action of neem oil against *P. argentipes* under laboratory and field conditions and concentration of 2% neem mixed with coconut oil provided 100% protection. Dhiman and Sharma (1994) evaluated the different concentrations of neem oil against *P. papatasi* in rooms using human volunteers and a 97.6% protection was obtained for ten hours using 2% neem oil.

1.3.5.2.2. Traditional Repellents

Traditional repellents are those plant based repellents used by local communities to reduce or prevent biting insects. Such repellent plants are used as smokes of leaves, barks, roots, and placing of fresh parts in rooms and potted plants. Use of traditional repellents is a common practice in different communities (Curtis, *et al.*, 1991). Traditionally, people in some parts of the world use plant parts and plant derived substances to repel or kill mosquitoes and other domestic pest insects. For instance, people in the Far East were reported to cover their legs with sledges and barks to reduce insect biting nuisance (Curtis, *et al.*, 1991). Recently, interest has grown in scientific evaluation of traditional repellent plants.

In Guinea-Bissau, Palsson and Jaenson (1999) have experimentally proved that *Hyptis suavolense* and *Daniella oliveri* are mosquito repellents. The same study showed that *Ocimum canum, Senna accidentalis* provided 29.4-85.4% repellency against mosquito bites. Smouldering and fresh leaves of *H. suavolense* gave 85.4% and 73.2% repellency effect respectively. On the other hand smoke of *Eucalyptus* leaves gave 72.6% and fresh leaves *O. canum* provided 63.6%

and burning leaves of *Azadirachta indica* exhibited 76.0% repellency. Herbs of the basil family (Labiatae) are commonly used traditionally by the African and Asian people. In East and West Africa they are also used as mosquito repellents (Kokwaro, 1976). Smoke produced by burning of dried leaves of *A. indica* has been used for the protection against mosquitoes since ancient times (Mittal and Subbaroa, 2003). Smoking of leaves of *H. suavelens, Daniella oliveri, Elaeis quineensis, Parkia biglobosa, A. indica* and *Eucalyptus* spp. are among the few plants traditionally applied. Seyoum, *et al.* (2002a) conducted ethno botanical survey in western Kenya and reported that the most commonly used plants as repellents are *Ocimum americanum, L. camara, Tagetes minuta, A. indica,* and *O. basilicum.* Another ethnobotanical survey had been also conducted in Zimbabwe by Lukwa *et al.* (1999). Accordingly they reported that 50% of the people interviewed use *Lippia javanica* in all age groups and the leaves, barks, flowers and seeds of the plant are commonly used as smokes and fresh. In experimental trials, placing of fresh leaves of *E. citriodora* was found to be effective against *An. gambiae (*Seyoum, *et al.* 2002a). *Ocimum basilicum* is traditionally used by Luo communities to drive away mosquitoes by placing branches in houses (Kokwaro, 1976).

Seyoum, *et al.* (2002b) also evaluated potted live plants for their mosquito repellency inside a green house and reported satisfactory results from *O. americium; L. camara* and *Lippia ukambensis* which gave an average of 39.7%, 32.4% and 33.3% protection against *Anopheles* mosquitoes, respectively. Seyoum. *et al.* (2003) also conducted field experiment, on *O. americanum, L. camara* and *Lippia ukambensis* in live and *Corymbia citrodora*. Seeds and leaves of *O. kilimandscharicum* and *O. suave* were tested by thermal expulsion on traditional stoves; and all plant species showed significant repellency against *Anopheles gambiae s.l.*

1.4. VECTOR CONTROL IN ETHIOPIA

Vector control has been one of the major components of malaria control. In Ethiopia this program mainly focused on the use of indoor residual spray of DDT to control the principal vector because of the understanding of its partial endophilic behavior (Gish, 1992). The program was successfully undergoing in its initial phase, however, successful control couldn't be achieved due to the complexity of the disease control process, the expensiveness of the control program, variations of disease patterns and transmission dynamics from place to place, frequent climate changes and environmental modifications (Tulu, 1993). Appearance of drug resistance of the parasites (Mengesha *et al.* 1998; Ghebreyesus and Alamrew, 1998) and insecticide resistance vectors (MOH, 2002) is also becoming a threat to malaria control efforts. Moreover, environmental concerns and natural resource development programs have necessitated the utilization of appropriate technological and management techniques in an integrated approach to bring about an effective degree of vector suppression. Hence, the search for control tools that are cost effective, environmental friendly and effective in reducing man vector contact is very much desired. Plant based mosquito repellents are crucial to be investigated. Therefore, this study was focused on traditional means of mosquito repellency by local plants.

The other option was source reduction which refers to in broader term any method of physical alteration of a pest or a vector production site to render it unsatisfactory for the completion of the vector life cycle (Ault, 1994). It is also the second important approach in vector control in the country. The method is an important feature of environmental management mainly aims at vector suppression through the destruction of the breeding sites and resting places of the target vectors (Gubler and Clark, 1994). It is one of the components of the integrated vector control, which is in the long run cost effective, more or less environmentally safe and community oriented control strategy (Sloof, 1987). Its target is reduction of aquatic stages of vector mosquitoes. The method can be used as supplementary or even as alternative measures of control in areas where there is a serious vector resistance problem (Sloof, 1987). In Ethiopia this method is applied in several regional states especially in urban and semi-urban areas, in refugee, development projects and irrigation schemes. Such method is found effective in Tigray, Amhara and Oromiya regional states (MOH, 2000). Therefore, as it is cost-effective, environmentally safe and easy to be handled by communities this method remains one of the best alternative approaches to control malaria.

2. OBJECTIVES

2.1. GENERAL OBJECTIVE

This study aims at evaluating the potential repellency of some selected local plants that may be suitable for use by rural communities against mosquitoes.

2.2. SPECIFIC OBJECTIVES

a) To evaluate some local plants for their repellency against three mosquito species namely *An. arabiensis*, *An. pharoensis* and culicines by applying different methods.

b) To compare the test plants for their repellency potential against the different mosquitoes.

c) To compare the effectiveness of different application methods (direct burning, thermal expulsion and placing of fresh parts).

3. MATERIALS AND METHODS

3.1. THE STUDY AREA

The field experiments were conducted in Koka-Negewo village. The village is located at N 8°41" E 39°35" about 94 kms south from Addis Ababa to Awassa on the right side of the Awash River Bridge. The area is one of the lowland malarious areas where irrigation farming is taking place and produce mostly tomatoes.

Over flooding water from the Awash River during the rainy season accumulates in the fields creating swamps that appear favorable for the breeding of mosquitoes. The inhabitants belong to the Oromo ethnic groups who live principally on farming. They live in huts constructed of mud walls and thatched roofs. They keep domestic animals, the majority being bovid.

The village was selected because of its proximity to Addis Ababa, availability of the majority of the test plants and the presence of mosquitoes involved in malaria transmission as confirmed in a preliminary investigation. The experiments were undertaken from mid June to the end of the first week of August 2004.

3.2 SELECTION AND COLLECTION OF TEST PLANTS.

Based on empirical, local and literature information seven plant species were selected to evaluate their mosquito repellency property. These were: *Schinus molle, Melia azedarach, Lantana camara, Ruta challepensis, Pychnostachys abyssinica, Ocimum lamifolium,* and *Eucalyptus globules*. The first three plants were collected from the experimental place where as *E. globules*. *O. lamifolium*, and *P. abyssinica* were obtained from Addis Ababa. *R. challepensis* was purchased from the market in Mojo.

3.3 DESCRIPTION OF TEST PLANTS

Melia azedarach (Meliaceae) commonly known as 'chinaberry' or 'false neem' is a large tree native to pertia, India but is now naturalized in a number of countries (Borges *et al.*, 2003). It commonly grows in many areas of Ethiopia and is used to treat malaria in some parts of the country (Dr. Teshome Gebre-Michael Aklilu Lemma Institute of Pathobiology pers. Comm.). It also contains compounds that repel mosquitoes and different extractions based on petroleum ether, chloroform, acetone and ethanol exhibited about 90% of repellency against *Culex pipiens* (Mansour *et al*; 1998). However, the repellent effect of its smoke or its fresh leaves/branches against *An. arabiensis* or other malaria vectors in Ethiopia is not yet known. Smoking leaves of a related and well known repellent plant *A. indica* showed moderate repellent action against *An. gambiae* in Kenya (Seyoum *et al.*, 2002a) but was highly effective against unspecified mosquitoes (Palsson and Jaenson, 1999).

Ruta challepensis (Rutaceae) locally called Tena-Adam (Amharic) is a shrub growing up to one meter high. It occurs widely in Ethiopia and the leaves and fruits are important medically to flavor milk, cottage cheese, coffee and tea. The leaves and fruits are also used in the preparation of the Ethiopian condiment 'berberi' (Gilbert, 1989b).

Schinus molle (Anacardiceae) is an evergreen plant commonly called true-man tree, planted in dry warm climates throughout the world. It is drought resistant and is also widely planted in

Ethiopia (Gilbert, 1989a). The plant is used as firewood, charcoal, spice (fruit), its leaves as insect repellent (Bekele *et al.*, 1993).

Ocimum lamifolium (Labietae) is a shrub plant and in Amharic it is commonly called 'Yefiyel Zikakibe'. It occurs widely in Ethiopia and its leaves are medically used at home to give remedy to different ailments like the common cold (Fitchl and Adi, 1994). Many related species of *Ocimum* have been shown to have repellent effect against agricultural pests and medically important vectors. For example, *O. suave* has been found to be an effective repellent against the malaria vector, *An. gambiae* s.s in Kenya (Seyoum *et al.*, 2002a). The oil of O. suave is known to repel and kill all active stages of the tick *Rhipicephalus appendiculatus* (Mwangi *et al.*, 1995). Essential oil extracts from the seeds of the same plant and other related species (*O. kilimandscharicum O. Kenyense*) also provided greatest protection against stored product insect pests (Jembere *et al.*, 1995; Bekele *et al.*, 1996; 1997). In east Africa, *Ocimum basilicum* is traditionally used by Luo communities to drive away mosquitoes, by laying branches in houses (Kokwaro, 1967). *O. canum* is also reported to be used extensively by the people of Guinea-Bissau (Palssson and Jaenson, 1999). Moreover, 63% repellency to mosquitoes was exhibited from the fresh leaves of *O. canum* tested in Guinea-Bissau (Palsson and Jaenson, 1999). In Brazil, *O. selloi* is found an effective repellent against *An. brazililiensis* (Padilha de Paula *et al.*, 2003). However, no published information or otherwise is available on *O. lamifolium* as an insecticide or insect repellent. It would thus be appropriate to examine the potential of this plant for its repellency against *An. arabiensis* in Ethiopia.

Eucalyptus globulus it is also called "Nech bahirzaf) in Amharic is a long tree native to Australia but widely distributed in many countries including Ethiopia. About 100 species of *Eucalyptus* trees are recorded in east Africa (Friis, 1995). *Eucalyptus globulus* and *E. citrodora* (lemon eucalyptus) (Myrtaceae) are widespread species and are cultivated in Ethiopia for the purpose of timber production and oil extraction, respectively (Friis, 1995). In Tanzania, a repellent made from extraction of oil from lemon eucalyptus showed comparable efficacy and duration of protection to deet against *An. gambiae* and *An. funestus* (Trigg, 1996). Placing fresh leaves and smoking the leaves of *E. citrodora* were found to be effective repellent against *An. gambiae* in Kenya (Seyoum *et al.*, 2002a). Although fresh branches of *E. globulus* are traditionally and widely used to repel intruding armies of ants and for 'treatment' of the common cold and coughs

(Dr. Teshome Gebre-Michael Aklilu Lemma Institute of Pathobiology pers. Comm.), its role as repellents to mosquitoes has not been evaluated yet.

Pychnostachys abyssinica (lamiiaceae) is a shrub plant grows in humid margins of forest, bush or coffee plantations or more often plant in hedges and it is endemic to Ethiopia (Ryding, 1997). It is locally called 'Ton-Ton' in 'Sidamigna' around Wondogenet. The plant is widely grown in southern and southwestern Ethiopia (Z. Asfaw and T. Gebre-Michael pers. commun.). The fresh leaves of the plant have a pungent and offensive odor/smell and are used as repellents against ants in southern and southwestern pats of the country (Z. Asfaw and T. Gebre-Michael pers. commun.).

Lantana camara (Verbenaceae) a large shrub growing to the height of 3 m. The plant has dark green leaves; flowers yellow orange and later on become deep red. Originated in South America and it is cultivated as ornamental hedge plant in altitudes between 1300m-2700m. The plant is toxic to livestock (Fichtl and Adi, 1994) but in many places of Kenya and Guinea-Bissau local communities use it traditionally to protect mosquitoes (Palsson and Jaenson, 1999; Seyoum *et al.*, 2002a).

3.4. REPELLENCY TEST PROCEDURE

The experiments were conducted indoors (inside tukuls) in four replicates by placing volunteers human mosquito baits/collectors in four tukuls at twelve different times in a total of 48 nights. All baits/collectors, I and other assistants were on antimalarial prophylaxis, has been given a combination of Sulphadoxine Pyrimethamine (Fansidar) and Chloroquine on weekly basis during the experiment as well as two weeks before and after the experiment as recommended and kindly provided by Ato Fekede Balcha (Aklilu Lemma Institute of Pathobiology). Chloroquine is still the first line of drug for the treatment of uncomplicated falciparum and vivax malaria and Fansidar in the second line for any resistant falciparum malaria (Ato Fekede Balcha pers. Comm.). Two houses were rented from the locals and the other two were constructed in a similar way to the rented tukuls. The experimental procedures for each method are described below. The experiments were conducted by direct burning (smouldering), thermal expulsion and placing of fresh plant parts in accordance to the methods described in (Palsson and Jaenson 1999; Seyoum *et al.*, 2002a). Burning of plants was made by placing parts on burning charcoal stoves while the thermal expulsion was done by placing the plant parts on small metal plates on burning stoves in order to expel volatiles that appear to repel mosquitoes. Fresh plants were tested by placing parts on a plate and raised about 35cms in front of mosquito collectors.

The parts of plants tested were i) Seed and leaves of *R. challepensis, S. molle,* and *M. azedarach*, ii) Leaves of *P. abyssinica, O. lamifolium* and E. *globulus* , iii) Leaves, seeds and flowers of *L. camara*. Four mosquito baits/ collectors dressed in shorts were involved in the experiment: three of the baits/ collectors were made to collect mosquitoes by sitting about 0. 3 meters behind the treatment stoves with one of the plant parts or fresh plants (experimental groups) while one of the collector who served as control sat in a hut without plant treatment. Collection sites as well as treatments were allocated randomly among the four collectors, and each bait/collector was rotated every night in order to allow a bait/collector to take a treatment once and to be placed in a different hut. The changes of treatments and sites were aimed at avoiding any potential differences that could exist among the collectors attractiveness, and for any spatial differences in mosquito abundance among the houses. The influence of external variables such as wind and smoke is considered similar since the experiments were carried out inside tukuls. 20 gm of each of the plant parts was burned, thermally expelled or placed as fresh at intervals of 30 minutes. Landing mosquitoes were collected from 20:00 to 22:00h during their peak biting activities. Mosquitoes landing on legs (below knees) were caught by the human bait using test tubes and a hand torch. Mosquitoes were sorted and identified to the species using keys of Gilles and Coetzee (1987).

The list below indicates the type and set of tests and plant parts distributed on the 48 day period, each test taking four days.

1. Direct burning of seeds of *S. molle, M. azedarach* and *R. challepensis.*
2. Direct burning of leaves of *S. molle, M. azedarach* and *R. challepensis.*
3. Direct burning of leaves of *P. abyssinica, E. globulus* and *O. lamifolium*
4. Direct burning of leaves, flowers and seeds of *L. camara.*
5. Thermal expulsion of seeds of S. *molle, M. azedarach* and *R. challepensis.*
6. Thermal expulsion of leaves of *S. molle, M. azedarach* and *R. challepensis.*
7. Thermal expulsion of leaves of *P. abyssinica, E. globules* and *O. lamifolium.*
8. Thermal expulsion of leaves, flowers and seeds of *L. camara.*
9. Fresh leaves of *S. molle, M. azedarach* and R. *challepensis.*
10. Fresh leaves of *P. abyssinica, E. globules* and *O. lamifolium.*
11. Fresh seeds of *S. molle, M. azedarach* and R. *challepensis.*
12. Fresh leaves, flowers and seeds of *L. camara.*

3.5 DATA ANALYSIS

Percentage repellency (%R) attained from each test was calculated based on the formula described by Sharma and Ansari (1994).

$$\%R=C-T/C*100$$

Where C= number of mosquitoes collected in the control

T= number of mosquitoes collected in the treatment

The number of mosquitoes collected was transformed to log (x+1) to normalize the data and then a one way analysis of variance (ANOVA) was done using SPSS version 10 (Spss Inc., 1999), to observe differences in plant treatments done at the same time. This was done to avoid differences in the population density of mosquitoes in time. When significant differences were observed, the means were separated using Tukey's test.

4. RESULTS

4.1 MOSQUITO COLLECTION

An. arabiensis, *An. pharoensis* and a number of unidentified culicines were collected. A total of 5394 anopheline and culicinne mosquitoes were collected from all the experimental tests. Of these 72.5% were *An. arabiensis* 16% *An. pharoensis* and 11.5% were unidentified culicine mosquitoes. Both *An. arabiensis* and *An. pharoensis* were reported to be vectors of malaria in the neighboring Zeway, although the former species has prime importance (Abose *et al.*, 1998).

4.2 REPELLENCY STATUS OF THE PLANT PARTS

4.2.1 Direct Burning of Seeds of *Schinus molle*, *Melia azedarach* and *Ruta challepensis*

On direct burning of seeds of *S. molle, M. azedarach* and *R. challepensis* provided 58.3%, 54.3% and 78.9% repellency against *An. arabiensis*, respectively showing higher repellency of *R. challepensis* than the other two plant seeds (P< 0.0001). Similarly, the effect of this plant on both *An. pharoensis* and culicines was profound 74.5% for the former and 89% for the later (Table: 1). The protection provided by both *S. molle* and *M. azedarach* against *An. arabiensis* and *An. pharoensis* was also quite significant (%R= 51.1 and 59.6; P<0.05).they were nevertheless, weak against culicines (Table 1).

Table 1. Percent repellency of direct burning of seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicines.

Test plant spp.	Mosquito species	Mean±SEM	No of mosquitoes Caught*	%Repellency	P-Value**
S. molle	*An. arabiensis*	1.34 ± 0.02	83	58.3	0.0001
	An. pharoensis	0.81 ± 0.08	23	51.1	0.03
	Culicines	0.56 ± 0.05	11	38.9	0.46
M. azedarach	*An. arabiensis*	1.36 ± 0.06	91	54.3	0.0001
	An. pharoensis	0.75 ± 0.06	19	59.6	0.008
	Culicines	0.54 ± 0.04	10	44.4	0.34
R.. challepensis	*An. arabiensis*	1.06 ± 0.01	42	78.9	0.0001
	An. pharoensis	0.60 ± 0.05	12	74.5	0.0001
	Culicines	0.15 ± 0.09	2	88.9	0.0001
Control	*An. arabiensis*	1.71 ± 0.01	199	-	-
	An. pharoensis	1.10 ±0.05	47	-	-
	Culicines	0.71 ± 0.09	18	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at $\alpha = 0.05$

4.2.2. Direct Burning of Leaves of *S.molle*, *M. azedarach* and *R. challepensis*.

Direct burning of the leaves of *R. challepensis* repelled *An. arabiensis* (27.7%), *An. pharoensis* (23.1%) and culicines (42.9%) (Table 2). The repellency of *S. molle* was greater than *R. challepensis* against *An. arabiensis* ($P<0.05$). *Melia azedarach* repelled more culicines (85.7%) although the difference with the control is statistically insignificant ($P> 0.05$). Leaves of *M. azedarach* repelled more than leaves of *S. molle* for *An. pharoensis*. No significant difference was obtained among all the treatment plant leaves when compared among themselves and the control against *An. pharoensis* and culicine mosquitoes ($P> 0.05$) and ($P> 0.05$), respectively.

Table 2 Percent repellency of direct burning of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicine

Test plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught	%Repellency	P Value**
S. molle	*An. arabiensis*	0.81 ± 0.16	27	75.0	0.01
	An. pharoensis	0.37 ± 0.15	7	46.2	0.35
	Culicines	0.20 ± 0.12	3	57.1	0.47
M. azedarach	*An. arabiensis*	1.05 ± 0.04	41	50.6	0.20
	An. pharoensis	0.27 ± 0.1	4	69.2	0.13
	Culicines	*0.07*±0.08	1	85.7	0.156
R. challepensis	*An. arabiensis*	1.18 ± 0.09	60	27.7	0.66
	An. pharoensis	0.52 ± 0.09	10	23.1	0.90
	Culicines	0.23 ± 0.14	4	42.9	0.60
Control	*An. arabiensis*	1.33 ± 0.04	83	-	-
	An. pharoensis	0.62 ±0.05	13	-	-
	Culicines	0.42 ± 0.07	7	-	-

SEM= standard error of the mean. *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentized range test at α= 0.05

4.2.3. Direct Burning of Leaves of *P. abyssinica*, *O. lamifolium* and *E. globulus*

On direct burning of the leaves of *P. abyssinica, O. lamifolium* and *E. globulus;* equally high and significant repellency was recorded against *An. arabiensis* for both *P. abyssinica* (%R=66; P<0.05) and *O. lamifolium* %R=67; P<0.05) (Table 3). The effect of *E. globulus* against *An. arabiensis* was moderate (50%) but not significant (P>0.05). Although the percentage repellency of *P. abyssinica, E. globulus* and *O. lamifolium* against *An. pharoensis* was high, there was no significant difference (P>0.05) as compared to the control. The same result was also obtained for the culicine mosquitoes.

Table 3. Percent repellency by direct burning of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines

Leaves of plant spp	Mosquito species	Mean ±SEM	No of mosquitoes caught	%Repellency	P-Value**
P. abyssinica	*An. arabiensis*	0.98 ± 0.3	39	66	0.02
	An. pharoensis	0.49 ± 0.17	11	70	0.11
	Culicines	0.20 ± 0.12	3	40	0.89
E. globulus	*An. arabiensis*	1.20 ± 0.09	59	50	0.22
	An. pharoensis	0.54 ± 0.16	13	64.9	0.17
	Culicines	0.15 ± 0.09	1	60	0.75
O. lamifolium	*An. arabiensis*	0.98 ± 0.13	39	67	0.02
	An. pharoensis	0.65 ± 0.14	12	54.1	0.37
	Culicines	0.15 ± 0.09	2	60	0.75
Control	*An. arabiensis*	1.5 ± 0.06	118	-	-
	An. pharoensis	0.99 ± 0.08	37	-	-
	Culicines	0.30 ± 0.2	5	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at $\alpha = 0.05$

4.2.4. Direct Burning of Leaves of flowers, and seeds of *L. camara*

Burning of the flower of *L. camara* resulted in 75.0% repellency against *An. arabiensis*, 81.3% against *An. pharoensis*, and 75.0% of culicine mosquitoes (Table 4). The seeds provided 62.9% repellency against *An. arabiensis*, 71.9% against *An. pharoensis* and 41.7% against culicines. Similarly, burning of the leaves of the plant provided 75.8%, 75.0%, and 58.3% repellency against the three mosquito species, in the same order. The results showed significant difference when compared to the negative control even though they were equally protective for both *An. arabiensis* and *An. pharoensis* ($P < 0.05$) respectively. However, all the plant parts showed no significant difference with the negative control against culicine mosquitoes ($F=0.99$, $p>0.05$).

Table 4. Percentage repellency of direct burning of different parts of *L. camara* against *An. arabiensis*, *An. pharoensis* and culicines.

Plant parts of *L. camara*	Mosquito species	Mean ±SEM	No of mosquitoes caught	%Repellency	P-Value**
Flowers	*An. arabiensis*	0.94 ± 0.03	31	75	0.006
	An. pharoensis	0.39 ± 0.05	6	81	0.009
	Culicines	0.20 ± 0.12	3	75	0.47
Seeds	*An. arabiensis*	1.10 ± 0.06	46	62.9	0.04
	An. pharoensis	0.44 ± 0.16	9	71.9	0.01
	Culicines	0.31 ± 0.19	7	41.7	0.77
leaves	*An. arabiensis*	0.91 ± 0.08	30	75.8	0.004
	An. pharoensis	0.44 ± 0.08	8	75	0.02
	Culicines	0.20 ± 0.19	5	58.3	0.47
Control	*An. arabiensis*	1.45 ± 0.133	124	-	-
	An. pharoensis	0.94 ± 0.07	32	-	-
	Culicines	0.54 0.14	12	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at $\alpha = 0.05$

4.2.5 Thermal Expulsion of Seeds of *S. molle, M. azedarach* and *R. challepensis*

Thermal expulsion of the seeds of *S. molle* provided 53.8%, 28.6% and 18.9% repellency against *An. arabiensis*, *An. pharoensis* and culicine mosquitoes, respectively. Against the same species, *M. azedarach* on the other hand exhibited 49.4%, 35.7%, and 25% respectively, where as *R challepensis* gave 70.3%, 53.6% and 75.0% repellency respectively (Table 5). None of these protection were significant except for the seeds of *R. challepensis* against *An.arabiensis* which was found to be highly protective as compared to the control than the other two (P 0.05). Similarly, with regard to *Anopheles pharoensis* and culicine mosquitoes, no significant repellency were obtained among all the treatment plants as compared to the control ($P > 0.05$).

Table 5 Percent repellency of thermal expulsion of volatiles of plant seeds against *An. arabiensis*, *An. pharoensis* and culicines

Test plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
S. molle	*An. arabiensis*	1.27 ± 0.06	73	53.8	0.06
	An. pharoensis	0.75 ± 0.09	20	28.6	0.55
	Culicines	0.59 ± 0.11	13	18.8	0.98
M. azedarach	*An. arabiensis*	1.31 ± 0.06	80	49.4	0.10
	An. pharoensis	0.73 ± 0.06	18	35.7	0.2
	Culicines	0.52 ± 0.18	12	25.0	0.89
R. challepensis	*An. arabiensis*	1.07 ± 0.098	47	70.3	0.00
	An. pharoensis	0.60 ± 0.09	13	53.6	0.07
	Culicines	0.24 ± 0.14	4	75.0	0.19
Control	*An. arabiensis*	1.59 ± 0.08	158	-	-
	An. pharoensis	0.89 ± 0.06	28	-	-
	Culicines	0.66 ± 0.12	16	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.6. Thermal Expulsion of the Leaves of *S. molle, M. azedarach* and *R. chllepensis*

Thermal expulsion of the leaves of *S. molle*, *M. azedarach* and *R. chllepensis* resulted in more than 70% repellency to the three groups of mosquitoes (Table 6). Both *Anopheles arabiensis* and *An. pharoensis* significantly repelled by the leaves of all plant species ($P<0.05$) as compared to the negative control. However, the protection against the culicines was not significant for both the leaves of *M. azedarach* and *R. challepensis*, though the percent repellency was high (>60% repellency; Table 6). However, leaves of *S. molle* exhibited significant repellency effect to culicine mosquitoes.

Table 6. Percent repellency of thermal expulsion of volatiles from leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines.

Test plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
S. molle	*An. arabiensis*	0.87 ± 0.07	27	80.9	0.00
	An. pharoensis	0.54 ± 0.04	10	75.0	0.06
	Culicines	0.15 ± 0.09	2	85.7	0.049
M. azedarach	*An. arabiensis*	1.04 ± 0.07	41	70.9	0.005
	An. pharoensis	0.40 ± 0.16	8	80.0	0.01
	Culicines	0.23 ± 0.08	3	78.6	0.12
R. challepensis	*An. arabiensis*	1.11 ± 0.13	54	61.7	0.01
	An. pharoensis	0.42 ± 0.07	7	82.5	0.02
	Culicines	0.25 ± 0.099	4	71.4	0.19
Control	*An. arabiensis*	1.55 ± 0.04	141	-	-
	An. pharoensis	0.99 ± 0.13	40	-	-
	Culicines	0.58 ± 0.14	14	-	-

SEM= standard error of the mean. *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.7. Thermal Expulsion of leaves of *P. abyssinica*, *E. globulus* and *O. amifolium*

Thermal expulsion of *P. abyssinica*, *E. globules* and O. *lamifolium* gave almost more than 50% repellency to *An. arabiensis*, *An. pharoensis* and culicines (Table 7). However, none of their repellency were significantly effective against all the mosquitoes (P>0.05).

Table 7. Percent repellency of thermal expulsion of volatiles of leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicine.

Test plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
P. abyssinica	*An. arabiensis*	1.05 ± 0.25	58	51.7	.648
	An. pharoensis	0.27 ± 0.18	6	62.5	.350
	Culicines	0.33 ± 0.21	8	60.0	.613
E. globulus	*An. arabiensis*	1.02 ± 0.25	55	54.2	.594
	An. pharoensis	0.30 ± 0.17	6	62.5	.350
	Culicines	0.30 ± 0.17	6	70.0	.545
O. lamifolium	*An. arabiensis*	1.12 ± 0.27	72	40.0	.768
	An. pharoensis	0.42 ± 0.14	8	50.0	.721
	Culicines	0.48 ± 0.10	16	55.0	.910
Control	*An. arabiensis*	1.44 ± 0.14	120	-	-
	An. pharoensis	0.66 ± 0.12	16	-	-
	Culicines	0.64 ± 0.20	18	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.8. Thermal Expulsion of Leaves, Flowers and Seeds of *L. camara*

The thermal expulsion of leaves, flowers and seeds of *L. camara*, all provided high significant protection against *An. arabiensis* varying from 58% to 69.2% ($P<0.05$) (Table 8). The protection against *An. pharoensis*, although the percentage repellency is high for all of them (62.5% to 75%) was not statistically significant ($P>0.05$); similarly high but insignificant repellency (above 51.9% was obtained) against culicines except for the seeds of *L. camara* (22.2%) (Table 8).

Table 8. Percent repellency of thermal expulsion of volatiles of flowers, leaves and seeds of *L. camara* against *An. arabiensis*, *An. pharoensis* and culicines mosquitoes.

Plant parts of *L. camara*	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
Flower	*An. arabiensis*	1.18 ± 0.06	58	69.2	.002
	An. pharoensis	0.42 ± 0.14	8	75.0	.240
	Culicines	0.53 ± 0.19	13	51.9	.535
Seeds	*An. arabiensis*	1.28 ± 0.10	79	58.0	.014
	An. pharoensis	0.51 ± 0.19	12	62.5	.068
	Culicines	0.68 ± 0.19	21	22.2	.890
Leaves	*An. arabiensis*	1.20 ± 0.04	61	67.6	.004
	An. pharoensis	0.38 ± 0.22	10	68.8	.195
	Culicines	0.35 ± 0.13	6	77.8	.186
Control	*An. arabiensis*	1.66 ± 0.07	188	-	-
	An. pharoensis	0.91 ± 0.12	32	-	-
	Culicines	0.84 ± 0.12	27	-	-

SEM= standard error of the mean. *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.9. Effect of Fresh Leaves of *S. molle, M. azedarach* and *R. challepensis*

The results of the effect of fresh leaves of *S. molle, M. azedarach,* and *R. challepensis* against the mosquitoes are summarized in Table 9. The repellency effect against *An. arabiensis* varied from 36.4% ($P>0.05$) for *S. molle* to 59.1% for *R. challepensis*, but the protection is highly significant only in the latter.

However, the repellent effect of the fresh leaves of these plants were more effective against *An. pharoensis,* varying from 77.3% for *M. azedarach,* to 81.8% for *S. molle;* the protection being significantly different from the control ($P<0.05$).
Against the culicines, protections ranging from 33. 3% by *R. challepensis* to a high protection (89.32%) by *M. azedarach* was provided, although none of them were significant ($P>0.05$).

Table 9. Percent repellency of fresh leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines.

Fresh leaves of plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	% Repellency	P-Value**
S. molle	*An. arabiensis*	1.33 ± 0.05	84	36.4	.277
	An. pharoensis	0.39 ± 0.16	8	81.8	.005
	Culicines	0.30 ± 0.12	5	58.3	.856
M. azedarach	*An. arabiensis*	1.30 ± 0.05	77	41.7	.157
	An. pharoensis	0.52 ± 0.09	10	77.3	.020
	Culicines	0.15 ± 0.09	2	83.3	.445
R. challepensis	*An. arabiensis*	1.13 ± 0.099	54	59.1	.008
	An. pharoensis	0.51 ± 0.03	9	79.5	.018
	Culicines	0.42 ± 0.14	8	33.3	.996
Control	*An. arabiensis*	1.52 ± 0.06	132	-	-
	An. pharoensis	1.04 ± 0.10	44	-	-
	Culicines	0.46 ± 0.2	12	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.10. Effect of Fresh Leaves of *P. abyssinica, E. globulus* and *O. lamifolium*

The effect of fresh leaves of *P. abyssinica, E. globulus* and *O. lamifolium* against the mosquitoes are shown in Table 10. The effect against *An. arabiensis* resulted from 50% protection due to *E. globulus* to 72.5% protection due to *O. lamifolium*, but none was a significant protection. The protection against *An. pharoensis* resulted from 50% due to *E. globules* to 70.3% due to *O. lamifolium*; the effect of both *P. abyssinica* and *O. lamifolium* was significant ($P<0.05$). Against culicines, repellency ranged from 55.3% due to *E. globulus* to 66% due to *P. abyssinica*; none was however, significant ($P>0.05$).

Table 10. Percent repellency of fresh leaves of different test plants against *An. arabiensis*, *An. pharoensis* and culicines

Test of plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
P. abyssinica	*An. arabiensis*	1.27 ± 0.14	82	58.2	0.21
	An. pharoensis	0.80 ± 0.14	25	66.2	0.04
	Culicines	0.62 ± 0.15	16	66.0	0.17
E. globulus	*An. arabiensis*	1.36 ± 0.12	98	50.0	0.42
	An. pharoensis	0.98 ± 0.09	37	50.0	0.27
	Culicines	0.70 ± 0.16	21	55.3	0.30
O. lamifolium	*An. arabiensis*	1.13 ± 0.09	54	72.5	0.06
	An. pharoensis	0.77 ± 0.12	22	70.3	0.03
	Culicines	0.70 ± 0.16	19	59.6	0.26
Control	*An. arabiensis*	1.64 ± 0.14	196	-	-
	An. pharoensis	1.27 ± 0.08	74	-	-
	Culicines	1.07 ± 0.098	47	-	-

SEM= standard error of the mean. *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.11. Effect of Fresh Seeds of *S. molle, M. azedarach* and *R. challepensis*

The evaluation results of fresh seeds of *S. molle, M. azedarach* and *R. challepensis* against the mosquitoes are summarized in Table 11. The effect against *An. arabiensis* varied from51.9% repellency by *S. molle* to 59. 4% by *R. challepensis*, but none was significant. On the other hand, the repellency against *An. pharoensis* varied from 44.2% by *R. challepensis* to 72.1% by *M. azedarach*; the latter being the only significant ($P<0.05$). Repellency against culicines ranged from 26.7% by *S. molle* to 73.3% by *R. challepensis*, none was significant. No plant was consistently as being a strong or weak repellent for all mosquito species.

Table 11. Percent repellency of fresh seeds of different test plants against *An. arabiensis*, *An. pharoensis* and culicine.

Test plant spp.	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
S. molle	*An. arabiensis*	1.25 ± 0.13	77	51.9	0.12
	An. pharoensis	0.71 ± 0.06	17	60.5	0.26
	Culicines	0.54 ± 0.09	11	26.7	0.85
M. azedarach	*An. arabiensis*	1.22 ± 0.09	67	58.1	0.08
	An. pharoensis	0.50 ± 0.19	12	72.1	0.04
	Culicines	0.35 ± 0.13	6	60.0	0.21
R. challepensis	*An . arabiensis*	1.22 ± 0.07	65	59.4	0.08
	An. pharoensis	0.80 ± 0.01	24	44.2	0.49
	Culicines	0.23 ± 0.14	4	73.3	0.06
Control	*An. arabiensis*	1.59 ± 0.09	160	-	-
	An. pharoensis	1.05 ± 0.08	43	-	-
	Culicines	0.67 ±0.04	15	-	-

SEM= standard error of the mean, *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.2.12. Effect of Fresh Leaves Flowers and Seeds of *L. camara*

The effects of fresh parts of *L. camara* against the various mosquitoes are summarized in Table 12. Against *An. arabiensis,* repellency varied from 38.5% by the leaves to 43.4% by the seeds of the plant. However, the effects against *An. pharoensis* was higher, resulting in 65% by the leaves to 100% protection by the fresh seeds; but this was only significant both in the flower and seeds ($P<0.05$). Nevertheless, the effect against both species was lower in the leaves as higher in the seeds.

On the other hand all the parts were found to be less effective against the culicines ($P>0.05$).

Table 12. Percent repellency of fresh parts of *L. camara* against *An. arabiensis*, *An. pharoensis* and culicine mosquitoes

Fresh parts of *L. camara*	Mosquito species	Mean ±SEM	No of mosquitoes caught*	%Repellency	P-Value**
Flowers	An. *arabiensis*	1.20 ± 0.16	73	40.2	0.34
	An. pharoensis	0.27 ± 0.18	6	70.0	0.05
	Culicines	0.93 ± 0.14	36	53.2	0.99
Seeds	An. *arabiensis*	1.16 ± 0.17	69	43.4	0.25
	An. pharoensis	0.00 ± 0.00	0	100	0.003
	Culicines	1.02 ± 0.09	40	48	1.00
Leaves	An. *arabiensis*	1.30 ± 0.04	75	38.5	0.63
	An. pharoensis	0.43 ± 0.04	7	65	0.28
	Culicines	0.78 ± 0.18	26	66.2	0.87
Control	An. *arabiensis*	1.50 ± 0.04	122	-	-
	An. pharoensis	0.73 ± 0.12	20	-	-
	Culicines	0.99 ±0.31	77	-	-

SEM= standard error of the mean. *= Total number of mosquitoes collected during the four nights

**= P-value obtained from Tukey's studentised range test at α= 0.05

4.3 Comparison of Direct Burning, Thermal Expulsion and Fresh Plant Parts.

Figures 1, 2 and 3 depicted the results obtained from comparison of the three methods. The direct burning of the seeds of *R. challepensis* was found to be more effective against culicine mosquitoes followed by direct burning the leaves of *M azedarach* . In addition, direct burning of the flowers and leaves of *L. camara* and the seeds of *R. challepensis* and *L. camara* provided greater than 70% repellency against A*n. arabiensis and An. pharoensis*.

The thermal expulsion of the seeds and leaves of *R. challepensis* leaves of *S. molle* and *Melia azedarach*, flowers and leaves of *L. camara* provided above 65% repellency against *An. arabiensis*. Application of fresh plant parts indicated that almost all the plant parts repel <60% except for *O. lamifolium* which seemed to exhibit 72.5% repellency against *An. arabiensis*. Leaves of *S. molle* and *R. challepensis* gave 80% repellency against *An. pharoensis*. almost 100% of *An. pharoensis* were repelled by placing fresh seeds of *L. camara*.

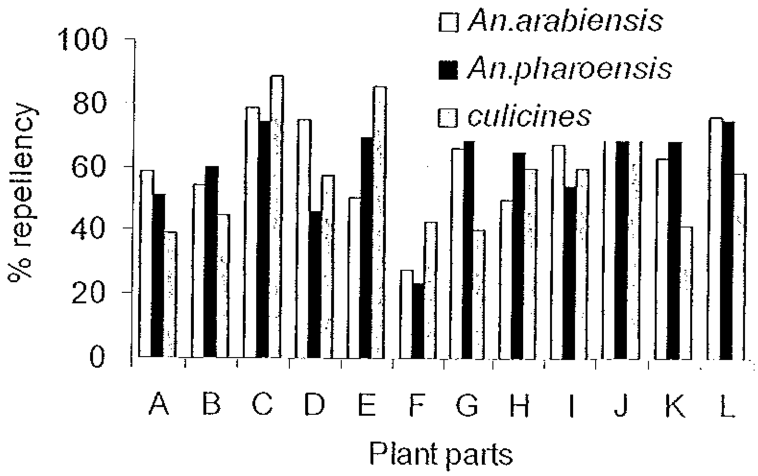

Figure 1 Repellency by direct burning of plant parts

Key:

A= seeds of *Schinus molle*

B=seeds of *Melia azedarach*

C= seeds of *Ruta challepensis*

D= Leaves of *Schinmus molle*

E= Leaves of *Melia azedarach*

F=Leaves of *Ruta challepensis*

G=Leaves of *Pychnostachys abyssinica*

H=Leaves of *Eucalyptus globulus*

I= Leaves of *Ocimum lamifolium*

J= Flowers of *Lantana camara*

K= Seeds of *Lantana camara*

L= Leaves of *Lantana camara*

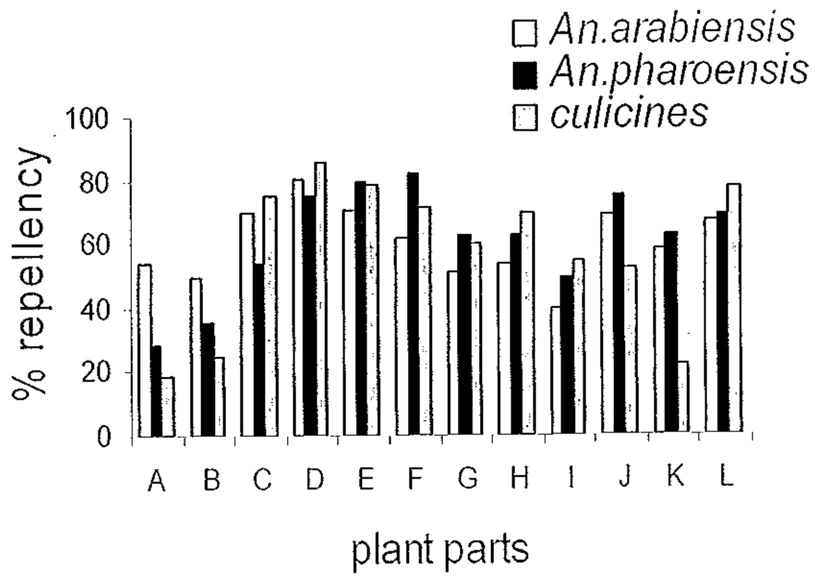

Figure 2 Repellency rate of thermal expulsion of plant parts

Key:

A= seeds of *Schinus molle*

B=seeds of *Melia azedarach*

C= seeds of *Ruta challepensis*

D= Leaves of *Schinnus molle*

E= Leaves of *Melia azedarach*

F=Leaves of *Ruta challepensis*

G=Leaves of *Pychnostachys abyssinica*

H=Leaves of *Eucalyptus globulus*

I= Leaves of *Ocimum lamifolium*

J= Flowers of *Lantana camara*

K= Seeds of *Lantana camara*

L= Leaves of *Lantana camara*

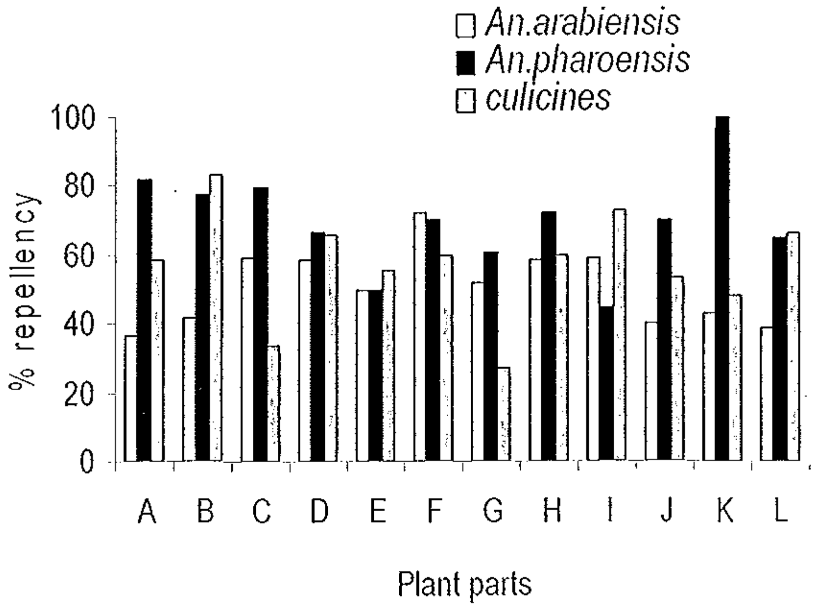

Figure 3 Repellency rate of fresh parts of plants

Key:

A= seeds of *Schinus molle*

B=seeds of *Melia azedarach*

C= seeds of *Ruta challepensis*

D= Leaves of *Schinmus molle*

E= Leaves of *Melia azedarach*

F=Leaves of *Ruta challepensis*

G=Leaves of *Pychnostachys abyssinica*

H=Leaves of *Eucalyptus globulus*

I= Leaves of *Ocimum lamifolium*

J= Flowers of *Lantana camara*

K= Seeds of *Lantana camara*

L= Leaves of *Lantana camara*

5. DISCUSSION

This study was concerned to evaluate the repellency of some local plants by employing traditional and other modified methodologies applied in other studies. The seven plants and their parts showed different repellency effects to *An. arabiensis*, *An. pharoensis* and culicines.

Some of the plants exhibit remarkable repellency effect while the others not. Similarly most of the plants show differences among their parts as well as with the methods of application they were applied. This is to mean that the repellency effect of the plants varies with the method of application. More over, certain plants repel better for a particular mosquito than equally for all. This can indicate that the mode of action of different repellents differs with the types of mosquito species.

Of all the treatments, burning the seed of *R. challepensis* was the most important and significantly more protective than the other plants. Similar result was obtained from the thermal expulsion of the seeds and leaves but the leaves produced more smoke consistently than the seeds. Longer protection from burning of the leaves couldn't be achieved, as the leaves tend to exhaust shortly. Fresh leaves of *R. challepensis* also effectively repelled *An. arabiensis* and *An. pharoensis* than the culicines.

The other plant that was evaluated was *S. molle*. Direct burning of the seeds of the plant exhibited profound repellency effect against *An. arabiensis* and *An. pharoensis*, but it provided little repellency effect for the culicines. Similarly, burning of the leaves exhibited good repellency for *An. arabiensis* only. Thermal expulsion of the seeds of *S. molle* on the other hand showed little repellency for all the mosquito species. Leaves of the plant exhibited remarkable repellency effect for *An. arabiensis* and the culicines. In addition *An. pharoensis* were effectively protected by placing the fresh leaves, but little effect has been obtained by placing fresh seeds.

In many parts of the country the fresh leaves and seeds are used to repel house flies (*Musca domestica*) by putting its fresh part on dining tables in restaurants (Pers. com. Dr. Zemede

Asfaw). The plant is also traditionally used to repel insects (Bekele *et al.*, 1993). More over the Essential oil of this plant tree has been used to control for different disease that could exist by different parasites. It is also provided effective anti-feedant as well as repellent property for House flies (*Musca domestica*) in Ethiopia.

Direct burning of the seed of *M. azedarach* found to repel *An. arabiensis* and *An. pharoensis* but it was less effective to the culicine mosquitoes. Thermal expulsion of the seeds as well as the fresh parts other than the seed exhibited less repellency to *An. arabiensis*, *An. pharoensis* and culicine mosquitoes. Fresh seeds gave remarkable protection. Thermal expulsion of the leaves showed more promising results than their direct burning and the effect was more pronounced to *An. arabiensis* and *An.pharoensis*. Fresh leaves repelled sufficiently only *An. pharoensis*.

Of all the treatments of *O. lamifolium*, the fresh leaves only showed effective repellency to *An. pharoensis* suggesting the limitation of the other methods of application as well as the non-repellency effect to *An. arabiensis* and culicines.

Other studies indicated the potential repellency of a number of its relative species to malaria vectors elsewhere. Live potted *O. americium* was reported to maintain repellency against *An. gambiae* s.s in a Kenyan trial (Seyoum *et al*; 2003). However, the same application of *O. kilimandscharicum* and *O. suave* under the same situation were found to be less effective.

In other experiments, Seyoum *et al.* (2002 b and 2003) reported that burning the leaves of the two plants repelled *An. gambiae* s.l *and An. funestus*. Thermal expulsion and periodic direct burning of the leaves of the two plants and *O. americanum* in a semi-field experimental hut in two villages in western Kenya provided satisfactory results against *An. gambiae* s.l and *An. funestus* (Seyoum *et al.*, 2002a). In Guinea- Bissau it was experimentally shown that the fresh leaves of *O. canum* significantly repelled *An. gambiae* s.l, *An. pharoensis* and other culicine mosquitoes (Palsson and Jaenson, 1999). The oil of *O. suave* is also known to repel and kill all active stages of the tick, *Rhipihicephalus appendiculatus* (Mwangi *et al.*, 1995). It has also been shown that *O. selloi* is an effective repellent in Brazil against *Anopheles brazililiensis* (Padilha de Paula *et al.*, 2003).

Extracts of essential oils of *O. kenyense, O. suave and O. kilimandscharicum* were evaluated for their efficacy against stored product insects to protect maize and sorghum and satisfactory results were obtained (Jembere *et al.*, 1995; Bekele *et al.*, 1996, 1997; Obeng-Oferi and Reichmuth, 1997; Obeng-Oferi *et al.*, 1997).

Direct burning of the leaves, flowers and seeds of *L. camara* produced effective repellency for both *An. arabiensis* and *An. pharoensis* but were less effective to repel culicine mosquitoes. Thermal expulsion of the plant parts provided satisfactory effect only for *An. arabiensis*. The fresh parts were effective repellents only to *An. pharoensis*.

Other studies revealed that methanol extracts of the flowers provided 94.5% protection against *Ae. albopictus* for two hours (Dua, *et al.*, 2001). In western Kenya, people commonly apply direct burning of the plant parts to repel mosquitoes (Seyoum *et al.*, 2002a), and experimental trials showed that thermal expulsion and direct burning of the seeds and leaves were found to be effective repellents for *An. gambiae* s.l (Seyaum *et al.*, 2002a; 2002b). Similarly Dua *et al.* (2001) reported that different fraction of the plant flower by using chromatographic methods provided about 75.8% repellency for *Aedes* mosquitoes when evaluated on human voleenters.

Of all the plants tested, *E. globulus* maintained the least repellency for *An. arabiensis*, *An. pharoensis* and culicine mosquitoes in all the three applications, suggesting its uselessness for practical application. Contrary to this, similar experiments conducted on the fresh leaves and burning the leaves of *E. citrodora* in Kenya and Guinea-Bissau appear to be effective repellent to *An. gambiae (*Seyoum *et al.*, 2002a; Palsson and Jaenson, 1999).

Eucalyptus-based products have been found effective as mosquito repellents in various studies (Trigg, 1996; Moore *et al.*, 1996 and Schreck *et al.*, 1991). Extracts of the lemon eucalyptus, quwenling was found to be effective against *Ae.albopictus* and *An. quadrimaculatus* (Scherek and Leonhandt, 1991). However, it showed less effectiveness to *Ae.agypti* in its duration of protection than deet. As a topical mosquito repellent, quwenling had a shorter duration of repellency than deet. On the other hand, quwenling is more repellent to *An. darlingi* than deet

(Moore *et al.*, 2002). It provides complete protection for 6-7 hours against *An. gambiae* and *An.funestus* (Trigg, 1996).

Direct burning of the leaves of *P. abyssinica* provided significant repellency against *An. arabiensis* On the other hand it provided less repellency effect for *An. pharoensis* and culicines. Only *An. pharoensis* has been found to be effectively repelled by placing fresh leaves. However, thermal expulsion of the plant leaves resulted in low protection of all the mosquitoes. People in the southern and southwestern Ethiopia use the plant's fresh leaves for repelling ants (Z. Asfaw and T. Gebre-Michael, pers. Comm.). Moreover, in Wondo-Genet, the fresh leaves are placed on faces for the purpose of repelling houseflies (pers. Obs.).

6. CONCLUSIONS AND RECOMMENDATIONS

1) Though many studies on traditional mosquito repellent plants are conducted in different countries there are limitations of such investigations in Ethiopia.

2) Of the three mosquito species collected from the human volunteers *An. arabiensis* were found dominant followed by *An. pharoensis* and culicines respectively.

3) Some of the plants exhibit remarkable repellency effect while others not and most of the plants showed differences among their parts as well as with the methods of application they were subjected. This indicates that mode of action of the different plants differ with type of mosquito species.

4) Of all the plant treatments tested burning as well as thermal expulsion of seeds of *R. challepensis* was found highly repellent.

5) Leaves of *E. globulus* showed little or no repellency in all applications of the three methods.

6) Thermal expulsion of leaves were found better than the others, because the leaves smoke slowly, and the smoke remain longer than direct burning.

8) Traditional methods of using local plants for mosquito control is accessible, affordable and easy to handle by local communities. Therefore, it is important to incorporate with the other available vector control options.

9) Conducting further study is required to evaluate the repellency of oils of the plants and the out door status of repellency of same plants.

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APPENDICES

Appendix 1. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant seeds of (*S. molle, M. azedarach*, and *R. challapensis*) using direct burning

Mosquito spp.	Sum of squares	df	Mean square	F-value	P-value
An. arabiensis	.84	3	.28	60.13	0.000
An. pharoensis	.53	3	.19	11.6	0.001
culucines	.69	3	.23	11.7	0.001

Appendix 2. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant seeds of (*S. molle, M. azedarach*, and *R. challapensis*) using thermal expulsion.

Mosquito spp.	Sum of squares	df	Mean square	F-value	P-value
An. arabiensis	.54	3	.18	7.5	0.004
An. pharoensis	.17	3	.06	2.6	0.102
culucines	.41	3	.14	1.8	0.21

Appendix 3. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves of (*S. molle, M. azedarach*, and *R. challapensis*) using direct burning.

Mosquito spp.	Sum of squares	df	Mean square	F-value	P-value
An. arabiensis	.60	3	.20	5.4	0.014
An. pharoensis	.30	3	.10	2.3	0.13
culucines	.25	3	.09	1.8	0.2

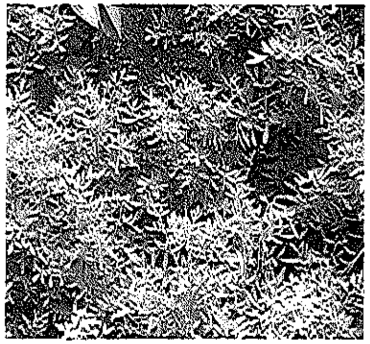
Appendix 4. Summary table for analysis of variance (ANOVA) for repellency of mosquitoes by plant leaves of (*S. molle, M. azedarach*, and *R. challapensis*) using thermal expulsion

Mosquito spp.	Sum of squares	df	Mean square	F-value	P-value
An. arabiensis	1.02	3	.34	11.76	0.001
An. pharoensis	.90	3	.30	6.2	0.009
culucines	.44	3	.15	3.5	0.051

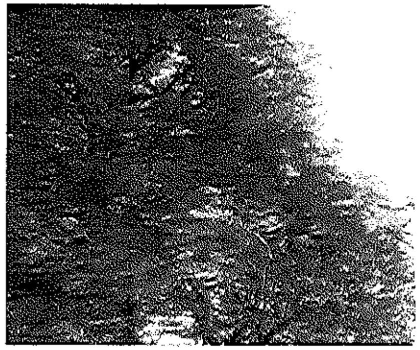
Appendix 13 : Test plants

Lantana camara

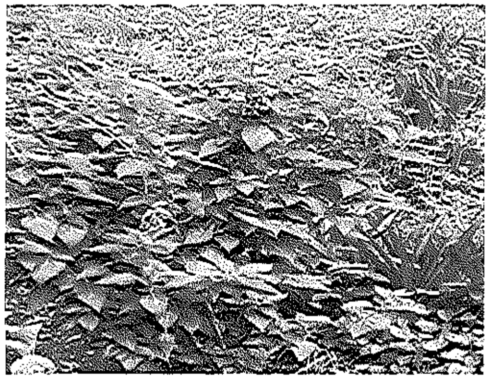
Pychnostachys abyssinica



Ruta challepensis



Schinus molle

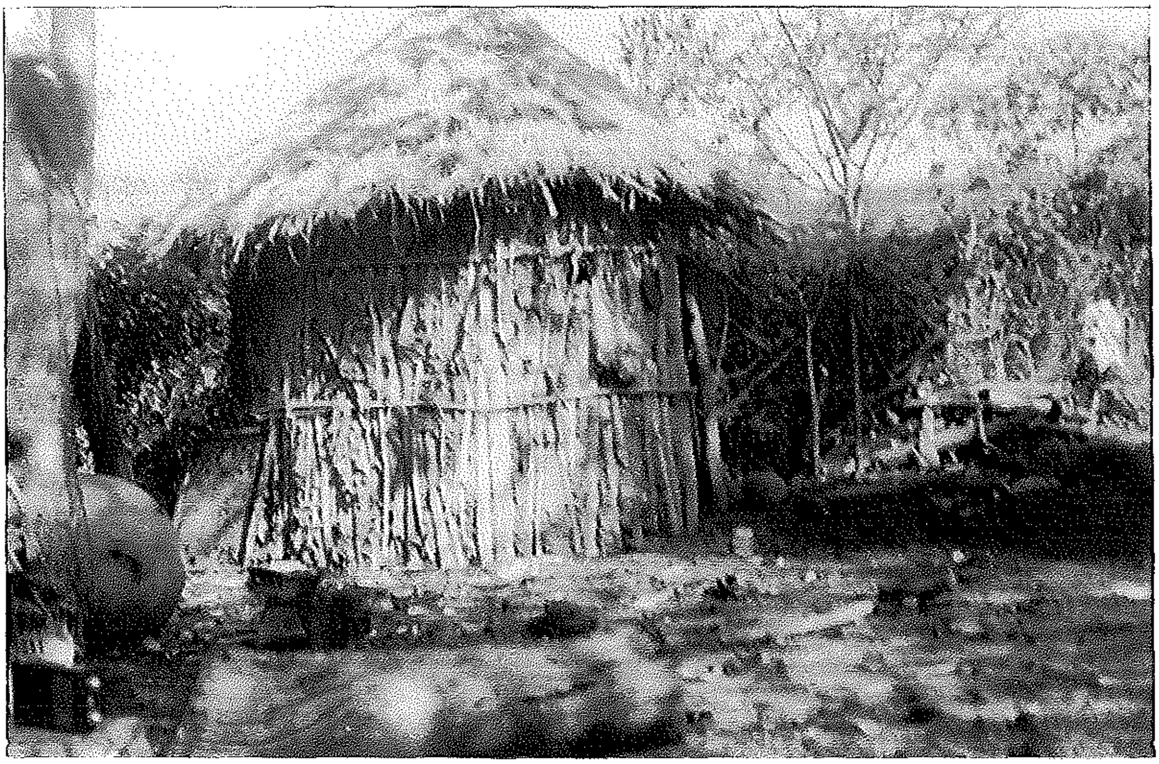


Ocimum lamifolium

Eucalyptus globulus

Melia azedarach

Appendix 14: Experimental test tukuls



Appendix 15 : Human baits

Direct burning

Thermal Expulsion

Fresh parts