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STUDY ON MAIZE POLLEN VOLATILE COMPOUNDS AS POTENTIAL ATTRACTANTS TO FEMALE *Anopheles arabiensis* (Diptera: Culicidae) UNDER LABORATORY CONDITION.

By
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DECLARATION

I, the under signed, declare that this thesis is my original work; it has not been presented in other university, college or institutions, seeking for similar degree or other purposes. All sources of the materials used in the thesis have been duly acknowledged.

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List of Abbreviations

ANVER	African Network on Vector Resistance
BH660	Bako Hybrid-660
Bti	*Bacillus thuringiensis*
BG trap	BioGent trap
CDC	Center for Diseases Control
CEAG	Center for Environmental Advocacy and Governance
DDT	Dichlorodiphenyltrichloroethane
FID	Flame Ionization Detector
FMHO	Federal Ministry of Health
GC-EAD	Gas Chromatographic-Electroantennographic Detector
GC-MS	Gas Chromatographic Mass Spectrometer
IGR	Insect Growth Regulators
IPM	Integrated Pest Management
ITN	Insecticide treated net
IVM	Integrated Vector Management
MOH	Ministry of Health
OR	Olfactory receptor
ORN	Olfactory receptor neuron
RBM	Roll Back Malaria
WHO	World Health Organization
ZM-521	Melkassa-2

Abstract

This study on main Ethiopian vector of malaria, *Anopheles arabiensis* was designed to identify potential attractant volatile compounds from two differentially attractive strains of maize pollen, BH660 and ZM-521. The behavioral responses demonstrated by blood fed and unfed adult female *An. arabiensis* to odor extracts from BH660 and ZM-521 illustrated olfactory mediated attraction to both strains of maize pollen.

The mosquitoes were attracted to the odor extracts from both strains differently: blood fed mosquitoes were attracted to lower doses of BH660 than to ZM-521 volatile extracts; whereas unfed females were attracted to lower doses of ZM-521 than to BH660 volatile extracts. Odor collections from these maize varieties were analyzed with both the gas chromatograph (GC) coupled to the mass spectrometer (MS) and the GC coupled to electroantennal detection (GC-EAD). These analyses showed that the antennae of both blood fed and unfed female *An. arabiensis* respond to volatile compounds extracted from both BH660 and ZM-521 pollens. Compounds determined from BH660 pollen to be active by GC-EAD were putatively identified as tetrahydro-2, 5-dimethyl furan, nonane, decane, undecane, limonene, dodecane, 2-hexanol, ocyl acetate and decane, toluene, 3-hexanone, limonene, 2-hexanol, tridecane for blood fed and non blood fed respectively. Similarly, different compounds were identified from ZM-521 maize pollen, namely: nonane, 3-hexanone, dodecane, 2-hexanol, styrene, 2-heptanol, octyl acetate, 1-octen-3-ol, Zingiberene for blood fed and 3-hexanone, 2-hexanol, styrene, octyl acetate, 1-octen-3-ol, Zingiberene for blood unfed *An. arabiensis*. These results from the study showed that olfactory cues were the driving external factor that assisted both blood fed and nonblood fed *An. arabiensis* to inhabit areas near BH660 and ZM-521 maize pollen.

1. Introduction

Malaria is by far the most important disease transmitted by the most dangerous insect in the world. Malaria epidemics often affect vulnerable sub-populations with existing compromised health status and limited access to health care services (Kiszewski and Teklehaimanot, 2004). Today, the rate of susceptibility is higher than before the major global malaria eradication campaigns of the mid 20th century (Stratton *et al*., 2008). Annually, between 300-500 million malaria cases and 1-3 million deaths occur throughout the world (Breman *et al*., 2004). This disease can be transmitted to people of all age (WHO, 2008). Currently malaria is endemic on over 109 countries and territories in tropical and subtropical zones, spanning all continents of the world except Antarictica and Australia, that vary from very low to extremely high (WHO, 2008). Around the world, over 90 countries have only imported malaria and are considered non-endemic for malaria (WHO, 2008).

The situation of the disease is aggravated in poor countries, especially in sub-Saharan Africa, where > 90% of this disease death occurs and 5% of children die from the disease before reaching 5 years. In those countries, malaria infections during pregnancy cause an estimated 3 million newborns to suffer complications from low birth weight including death (CDC, 2004; Gwer *et al*., 2007: Phillips, 2001; RBM, 2003). This is because the majority of infections in Africa are caused by *Plasmodium falciparium*, the most dangerous and drug resistant of the four human malaria parasites (RBM, 2003).

In those endemic countries, poor population are at greatest risk 58% of the cases occur in the poorest 20% of the world population and these patients receive the worst care and have catastrophic economic consequences from their illness (Breman *et al*., 2004). Besides, as the plasmodium parasites become increasingly resistant to anti-malarial drugs, and insecticide resistant *Anopheles* mosquitoes become prevalent (White, 2004) a strong need to develop new control measures exists (Zhong *et al*., 2006). Consequently, malaria has a vast economic and medical importance. Therefore, malaria is currently one of the highest

public health priorities for international community (WHO, 2008). It is essential to develop new control methods with improved understanding of olfactory cues and behaviors of mosquitoes. As a result, malaria mosquito responses to olfactory cues are one of the main areas of research currently under exploration by researchers seeking effective and safe methods of vector control.

The odors that are involved in communication and that trigger specific behavioral responses are called semiochemicals (Nordlund, 1981). Under natural conditions, mosquitoes are exposed to a great number of volatiles released by plants or other organisms in their habitats (Nordlund, 1981). Some volatiles may contain useful, often essential, information to locate hosts or mates for example, whereas other volatiles may be of no survival value (Nordlund, 1981). This thesis is concerned with identifying those semiochemicals emitted from maize pollen involved in the previously reported observed attraction of malaria mosquitoes to regions of maize intensive agriculture (Kebede *et al*., 2005; Ye-ebiyo *et al*., 2000, 2003).

1.1. Malaria in Ethiopia

Reports place malaria as the primary health problem in Ethiopia, and as such it is a serious impediment to socio-economic development (FMOH, ANVER and WHO, 2007; Senay and Verdin, 2005). More than 65% of Ethiopia's population (70 million) is exposed to malaria and each year more than 5 million malaria cases are estimated to occur (Senay and Verdin, 2005; MOH, 2004). In 2002 and 2003, malaria accounted for 15.5% of out-patient consultations, 20.4% of hospital admissions and 27.0% of in-patient deaths (MOH, 2004). The transmission of malaria in Ethiopia is mostly dependent on season, altitude and rainfall (Endeshaw *et al*., 2008) and occurs following the main rainy season (June to August) and light rains (March to April). Thus, the peak of malaria transmission occurs during September to December (FMOH, ANVER and WHO, 2007).

The observed rise in the number of malaria cases in the past few years appear to be due, in part, to socio-economic migration, global market driven changes in agricultural practices and climate change. For example, reports indicate that there is a high influx of people with

low-level immunity into malaria endemic areas for social and economic reasons, such as resettlement and search for alternative income, as well as the expansion of agricultural and industrial developments in malarious areas of the country (Tulu *et al*., 1993). The recent development driven changes in agricultural practices, such as the increased cultivation of maize (Ye-ebiyo *et al*., 2000, 2003), have also been shown to be complicit in the rise in malaria in rural areas (Kebede *et al*., 2005).

Malaria transmission in Ethiopia depends substantially on the mosquito *An. arabiensis patton*, a member of *An. gambiae Giles* complex, in intermediate highlands in Ethiopia (FMOH, ANVER and WHO, 2007; MOH, 2002; Taye *et al*., 2006). *Anopheles funestus Giles* is the second most important malaria vector in Ethiopia. *Anopheles nili* is an important local malaria vector in the low land region of South West Ethiopia (FMOH, ANVER and WHO, 2007).

1.2. Malaria Parasites

Malaria infection is caused by a protozoan parasite of the genus *Plasmodium*, four species of which infect human beings *P. falciparium*, *P. vivax*, *P. ovale* and *P. malaria* (Service, 2000; CDC, 2004). Globally, the most common malarial parasite is *P. vivax* and most deadly is *P. falciparium* (CEAG, 2006; CDC, 2004). *Plasmodium falciparum* most likely originated in Africa, probably in the past 5,000–10,000 years, with the onset of agriculture (Coluzzi, 1999).

In Ethiopia, malaria is caused by infection with the parasites *Plasmodium falciparum* and *P. vivax* with relative frequency of 60% and 40% respectively (Deressa *et al*., 2006; MOH, 2004). Malaria caused by *P. malaria* (<1%) is found sporadically in some areas, while there has been no report about the occurrence of *P. ovale* induced malaria in the country (Deressa *et al*., 2006). These disease-agent proportions vary both spatially and temporally. Where malaria is epidemic, *P. falciparum* is the dominant parasite species that causes severe disease manifestations. Almost all deaths attributed to malaria happen due to infection by *P. falciparum* (MOH, 2004).

Thus, as a newly evolved parasite, *P. falciparium* is the predominant species responsible for over 120 million new cases, and all the malaria deaths, per year globally (CEAG, 2006). Rainfall-associated out breaks of *P. falciparium* malaria occasionally occur along the pacific coasts of Peru and Ecuador (Kiszewski and Teklehaimanot, 2004). *Plasmodium vivax* occurs more frequently in Asia than in Africa (Kiszewski and Teklehaimanot, 2004). In addition, other nations in the Americas that are repeatedly afflicted with outbreaks of *P. vivax* and *P. falciparium* malaria include Colombia, Venezuela, Peru, and Ecuador. Out breaks in Colombia and Venezuela often severely affect isolated Native American Communities (Kiszewski and Teklehaimanot, 2004). As a result of the intensive selective pressure exerted on this deadly parasite by the drugs aimed at protecting and curing those at risk, *P. falciparium* is responsible for the alarming drug-resistant strains now emerging in the most endemic areas.

1.3. Malaria Vectors

Human malaria is transmitted exclusively through bites of infected female *Anopheles* mosquitoes (CEAG, 2006). *Anopheles* mosquitoes have a worldwide distribution occurring not only in tropical areas but also in temperature regions. Although there are approximately 430 different species of *Anopheles*, only approximately 70 species are malaria vectors and of these only 40 species are epidemiologically important (CDC, 2004; Service, 2000; Takken and Knols, 1999). Malaria vectors are divided in to primary and secondary vectors, but this can be misleading because a species may be classified as primary vector in some areas but be only a secondary vector in others (Service, 2000).

Malaria vectors go through four stages in their life cycle: egg, larva, pupa and adult. The first three stages are aquatic and last 5-14 days, depending on the species and ambient temperature (CDC, 2004). Larval stage mosquitoes feed on algae, yeast, bacteria, protozoa and numerous other plant and animal microorganisms found in the water (Service, 2000). Adult male *Anopheles* mosquitoes feed exclusively on sugar sources, for example nectar and honeydew, and therefore do not transmit the disease. Female mosquitoes also feed on sugar from different sources, but need blood for the development of their eggs. Depending

on species, female mosquitoes may lay 100-300 eggs at a time and may lay an average of 1000-3000 at a single breeding site. The average life span of the female mosquito is 3 to 100 days; while the males survive 10 to 20 days (Yoseph, 2007). Most mosquitoes remain within 1 mile of the breeding site from which they emerged. A few species may range up to 20 miles or more (CDC, 2004; Yoseph, 2007).

Anopheles larvae thrive in many different types of large, more or less permanent, habitats ranging from fresh- and salt-water marshes, mangrove swamps, grassy ditches, rice fields, edges of streams and rivers to ponds and borrow pits (Service, 2000). The larval stages of the mosquitoes that comprise the *An. gambiae* species complex frequently dwell in transient bodies of water in which suspended soil particles are abundant (Minakawa *et al*., 1999), particularly toward the end of rainy season when these puddles begin to contract (Ye-ebiyo *et al*., 2003). These mosquitoes exploit turbid water as frequently as clear water (Ye-ebiyo *et al*., 2003). In contrast, many other kinds of mosquitoes appear to develop more readily in clear water than in turbid water (Savage *et al*., 1990).

Those most important malaria vectors in sub-Saharan Africa are members of *An. gambiae* species complex (Onyabe and Conn, 2001; Service, 2000). The complex consists of seven species *An. gambiae*, *An. arabiensis*, *An. melas*, *An. merus*, *An. quadriannulatus*, *An. bwambae* and *An. quadriannulatus* species B, a newly described species from Ethiopia, that vary in their ability to transmit malaria (Service, 2000; Toure *et al*., 2004). Two species of the *An. gambiae* species complex, *An. gambiae* and *An. arabiensis* (Onyabe and Conn, 2001) in conjunction with *An. funestus* (Gary, 2005) are the most broadly distributed and the most efficient vectors of malaria in sub-Sahara Africa (Kameu, 2007; Takken and Knols, 1999).

Some *Anopheles* species are zoophilic, feeding on animals, and others are anthrophophilic, feeding on humans, while still others exhibit both feeding behaviors (Service, 2000; Takken and Knols, 1999). *Anopheles gambiae senso stricto* is considered the most efficient malaria vector because of it's highly anthropophilic character, its preference for feeding and resting indoors and its high susceptibility to infection with plasmodium parasite (Pitts

and Zwiebel, 2006; Service, 2000; Takken and Knols, 1999). The remaining members of the *An. gambiae* complex are not so host specific. *An. arabiensis*, notably, is known to vary from being anthropophilic to largely zoophilic depending on geographical location.

Anopheles nili and *An. moucheti* are both associated with running rivers, as their larvae find a suitable environment along riverbanks. *Anopheles nili* lives near fast running water in the west and central African equatorial areas, mainly in forest but occasionally in Sahel savannah, and is more abundant during the rainy season. There are both anthropophilic and zoophilic forms. *Anopheles moucheti* occurs in the central African equatorial forest and the neighboring areas, in more slowly running water with dense vegetation on riverbanks (Janssens and Wery, 1987).

Anopheles melas breeds in coastal salt waters such as mangrove swamps; it is restricted to West Africa (Service, 2000). *Anopheles merus* lives along the East Coast counterpart (Janssens and Wery, 1987) and in East and southern Africa, but can be found breeding in inland salt-water habitats. Adults of both species bite humans and rest indoors and outdoors; they are both regarded as secondary malaria vectors (Service, 2000).

Thus, the severity of malaria transmission by its *Anopheles* vectors varies depending on the feeding, resting site, the distribution and many other factors.

1.4. Relationship between Maize pollen and Malaria

Maize (*Zea mays* L.) is a member of grass family Gramineae (Bizuayehu, 2007) and is the second largest food commodity crop in the world after wheat, although in the developing countries of Latin America and Africa, it ranks first (Gudu *et al*., 2004).

Maize grows over a wider geographical and environmental range than any other cereal. It is grown at latitudes varying from the equator to slightly north and south of 50°, from sea level to over 3000 meters elevation, under heavy rainfall and semi- arid conditions cool and very hot climates (Abdelmola and Sable, 2007). Thus, millions of people depend on maize for their daily food in the world especially in sub-Saharan Africa. In Ethiopia, maize is the

major staple food and one of the main sources of calories in maize producing regions (Seboksa *et al*., 2001).

Maize pollen is produced in large quantities by numerous florets, for example single anthers produce in the order of 2000 (Miller, 1985) to 7500 grains with a volume of 700x10.9 cm^3 and a weight approximating 247x10.9g (Goss, 1968; Miller, 1985). As a result of this small size, air currents can disperse maize pollen. Therefore, the estimated number of pollen grains produced by an average-sized plant ranges from 14 million to about 50 million (Miller, 1985).

Maize cultivation and intensified agricultural activity in general, contribute to the production of vector mosquitoes in diverse ways (Kebede *et al*., 2005). Previous studies conducted in Ethiopia, especially in the Bure (Kebede *et al*., 2005) and Waktola districts (Yoseph, 2007), indicated that the presence of maize agriculture increased mosquito populations and directly enhanced the cycles of human biting and malaria transmission. As a result, maize grown in the immediate vicinity of households becomes an important source of food for the larvae of *An. arabiensis* (Ye-ebiyo *et al*., 2000), the main vector of malaria in Ethiopia. In addition, the pollen release was within late August-September, when temperature and moisture is ideal for mosquito breeding coinciding with the most dangerous period in the year for contracting malaria (Kebede *et al*., 2005).

The present study uses pollen from the maize variety BH660 that has been identified by previous researchers in connection with increased incidence of malaria (Kebede *et al*., 2005; Yoseph, 2007) and ZM-521 maize variety to know the relationship between the pollen and mosquito incidence. BH660 is a high yielding and disease tolerant (Worku *et al*., 2001) three way cross-hybrid from the parental lines of A-7033, F7215 and 142.1e (Keneni and Wolde, 1999). It is adapted to areas with altitudinal range of 1600-2200m and annual rainfall of 100-1500mm (Worku *et al*., 2001). The second maize variety is ZM-521, a variety released in 2004. It is also disease tolerant and adapted to areas with an altitudinal range of 1200-1700m and yearly rainfall 600-800mm (http: //www.eiar.gov.et/center.htm).

More mosquitoes survive to adulthood and develop more quickly into larger and longer-lived vectors, as a result of getting nutriment from pollen source close to breeding sites. Thus play a great role in increasing vectoral capacity of the mosquitoes (Ye- ebiyo *et al*., 2003; Kebede *et al*., 2005). It is known that mosquito larvae seek their food from micro-organism like algae, though the resulting adults are much smaller than those having access to maize pollen (Ye-ebiyo *et al*., 2003). Thus, reserve sugar deposits carried over from larval stage serve as sources of energy for flight, reproductive fitness, survival, longevity and increases body size for male and female *Anopheles* mosquito (Foster, 1995; Yoseph, 2007), while biting frequency increases when sugar is unavailable (Gray, 2005). At the same time, these plants may reduce human to mosquito contact by reducing the female's need for energy (Gary and Foster, 2001). Either way, these plants have the potential to affect the density, behavior and age structure of the adult vector populations and therefore the inoculation rate of malaria (Gary, 2005). The association between mosquitoes and maize is dependent on the nutrient content of the maize pollen as shown by Bizuayehu (2007).

1.5. Olfactory cues in mosquito's life

Olfactory cues are undoubtedly the most important group of external stimuli affecting mosquito behaviors. Whereas the type of response to these cues is mostly genetically determined, there is a certain plasticity in these that is governed by physiological condition and external stimuli (Takken and Knols, 1999). Besides, mosquito's response to behavioral cues depend on physiological status determined by age, size and physiological status with regard to nutrition, digestion and gonotrophic stage (Klowden, 1996). Thus, an olfactory cue used is essential to locate host, oviposition sites, sugar source and mate partner (Takken and Knols, 1999).

Semiochemicals are chemicals that mediate interactions between organisms (Law and Rangier, 1971). For insects, these chemical cues are important in the selection of suitable host for feeding, egg laying, mate selection, courtship, resource allocation and other behaviors (Birch and Haynes, 1982). Chemical communication involves production and release of specific chemicals (semiochemical) by the emitter and the detection and

olfactory processing of these signals leading to appropriate behavioral response by the receiver (Wolde-Hawariat, 2008). Two groups of semiochemical exist: pheromones mediate interactions between individuals of the same species (intraspecific) and allelochemicals mediate interactions between individuals of different species (interspecific). Allelochemicals are grouped as: allemones, where only emitters of the chemicals benefits: kairmones, only recipient benefit: synomones, both emitter and recipient benefits and apneumones, are emissions by non-living materials and benefits the recipient (Kabeh, 2007).

The receptors for semiochemicals are located on the antennae, maxillary palpi (Takken and Knols, 1999) and proboscis (labellum) (Pitts and Zwiebel, 2006). These appendages bear numerous hairs like structure called sensilla. Chemoreceptor neurons in the sensilla on insect's antennae mediate detection and processing of chemical stimuli (Puri *et al*., 2006). Those sensilla house olfactory receptor neurons (ORNs) responsible for behavioral responses to volatile cues including host finding by female mosquitoes, are critical components of vector capacity on far ability of mosquitoes to transmit diseases (Takken and Knols, 1999). Olfactory receptor neurons have been shown to encode odor quality, quantity and temporal change in odor concentration (Mustaparta, 2002). This is due to a highly specific interaction between the odor molecules and odorant receptor proteins embedded on the dendrites of ORNs (Hansson, 1999). In *An. gambiae*, at least 79 ORs have been identified, along with a highly conserved receptor *AgOr7* that is expressed both in antennae and palps (Pitts *et al*., 2004).

It is known that female mosquitoes depend on olfactory cues for sugar feeding, host seeking and oviposition site selection, while male mosquitoes mostly respond to odors utilized for sugar feeding (Bentley and Day, 1989; Luntz, 2003; Takken and Knols, 1999). Mosquitoes of three genera, *Culex, Anopheles* and *Aedes,* have been proven to feed on plant tissues as a source of regular diet (Muller and Schlein, 2005). As a result, sugar provides an important food for mosquitoes (Gary, 2005) by giving immediate energy demand (Foster, 1995). Flower extracts and synthetic plant odours were shown to attract mosquitoes (Foster and Hancock, 1994; Healy and Jepson, 1988). Information on the

compounds in floral fragrances attractive to mosquitoes is scarce. The major compounds of floral fragrances include: aldehydes, alcohols, carboxylic acids, aliphatic esters, terpenes and their ketones, and aromatics and phenols (Qiu, 2006). Thus, when the larval diet was reduced, the resulting adult mosquitoes landed more often on the plant source compared with adults developed from well-nourished larvae (Foster and Takken, 2004; Takken and Knols, 1999). Besides, unique role of nectar-related odours have been reported in *An. gambae* where both in males and female showed stronger responses to nectar-related odours than human odours soon after emergent and that this sensitivity was maintained throughout the males life and that females gradually (5 days on) develop preference for human odours (Foster and Takken, 2004).

With a few exceptions, female mosquitoes must bite a host and take a blood meal to obtain the necessary nutrients for the development of eggs in ovaries (Service, 2000). In haemtophagous insects those kairomones are used in the process of searching and locating the host for blood feeding. Human skin emitts 300-400 chemicals, however, only a few chemicals such as lactic acid, octanol, and CO_2 have been found to be attractive to mosquitoes (Fradin, 1998; Puri *et al*., 2006). In the case of anthropophilic mosquitoes, such as *Culex quinquefasciatus* and *Anopheles gambiae*, emanations other than CO_2 must play an important role during host seeking to distinguish humans from other CO_2 emitting vertebrates (Spitzen *et al*., 2008).

Consquently, taking of blood meal alters the internal state of the female mosquito leading to a behavioral switch from host seeking to search for an oviposition site (Tilak *et al*., 2005). Correct choice of the oviposition site by blood fed female mosquito is the most critical factor for the survival of their progeny (Takken and Knols, 1999; Rejmankova *et al*., 2005), since mosquitoes are the one with no parental care. In addition, it is the principal factor responsible for the distribution of the mosquitoes in breeding sites and their subsequent dispersion in different geographical areas (Tilak *et al.,* 2005). Several studies reported that oviposition pheromones and various kairomones are responsible for attractivness of oviposition sites of culicine mosquitoes (McCall and Cameron, 1995; Takken and Knols, 1999). Information on specific oviposition attractants is available for

several species of the genera *Culex* and *Aedes*, but much less information is available for anopheline mosquitoes (Rejmankova *et al*., 2005).

1.6. Malaria vector control

Mosquito control is the task of managing the population to reduce their damage to human health, economic and enjoyment of mosquito-ridden areas. As a result, of malaria control efforts across the world, 80 countries are now in the phase of malaria control; 12 countries are making the program transition to elimination; 11 countries are operating malaria elimination program; and 6 countries are actively engaged in preventing re-introduction of malaria (WHO, 2008). Even though malaria continues to cause disease and death in million of persons living in areas of the world where it is endemic, despite 4 decades of research on vaccines and new drug methods of control. As the plasmodium parasite becomes increasingly resistant to anti-malarial drugs, and insecticide-resistant *Anopheles* mosquito become increasingly prevalent (White, 2004; Zhong *et al*., 2006) a strong need to develop new control measure is sought. Various methods have been used to combat malaria vectors. These include biological control, physical control, chemical control, genetic control, personal protection and IPM (Service, 2000).

1.6.1. Biological control

Biological control has the advantage of target specificity with little effect on non-target organisms and is regarded as environmentally safe. Especially, using either the incidence of predators, parasite or pathogens in habitats must be greatly increased to obtain worthwhile control, or they have to be introduced into the habitats from which they originally are absent (Service, 2000). However, the high cost and difficulties encountered in mass production preclude their wide use, especially in tropical countries.

Larvivorous fish are the most commonly used biological control agents, among the most commonly used is *Tilapia nilotica* (CDC, 2008) and *Gambusa affinis* (CEAG, 2006; Service, 2000). These two species have been used in North America and also eliminated malaria from Palestine, Israel and Italy (CEAG, 2006). Among the bacteria, *Bacillus*

thuringiensis H-14 (Bti) and *Bacillus sphaericus* have shown high potency for mosquito control without adversely affecting non-target organisms and the environment (Kanzok and Jacobs-Lorena, 2006; Lardeux *et al*., 2002). From fungi the imperfect fungi of the genera *Culicinomyces*, *Tolypocladium*, *Metarhizium* and *Beauveria* can be readily cultured on artificial media to yield infective stages of mosquito control (Kanzok and Jacobs-Lorena, 2006). On the other side, two most known species of protozoa are *Vavraia culicis*, which parasitiszed mainly on culicines, and *Nosemia algerae* which appeared to be most effective against *Anopheles* (Yap, 1985).

1.6.2. Environmental modification

Environmental modification is any physical transformation that is permanent or long-lasting of land, water and vegetation, aimed at preventing, eliminating or reducing the habitats of vectors without causing adverse effects on the quality of human environment (Service, 2000; Yohannes *et al*., 2005). Yohannes *et al.* (2005) reported that source reduction was carried out by community resulting in 49% relative reduction in *An. arabiensis* adults in the dam village in Tigray compared with pre-intervention period. However, environmental changes from road, dam or pipeline construction, deforestation, and irrigation can generate larval breeding sites (Knight *et al*., 2003). Therefore, these control practices can mostly be used in urban and peri-urban areas and mostly require community participation and intersectional collaboration.

1.6.3. Personal protection

Personal protection measures are based on insecticide-impregnated material such as bed nets and curtains mainly, but clearly we are facing implementation problems. These measures include air-conditioning, screens, bed nets, clothing covering exposed parts of the body during biting periods of *Anopheles* malaria vectors, repellents and insecticides (Schoepke *et al*., 1998). ITNs are one form of personal protection that has repeatedly been shown to reduce severe disease and mortality due to malaria in endemic regions (CDC, 2008). Currently, only pyrethroid insecticides are approved for use on ITNs (CDC, 2008).

In Ethiopia, the use of ITNs was introduced in selected areas as one of the important malaria control measures in 1997/98 on cost recovery basis (MOH, 2000). But there are disadvantages: in hot climates, nets may be uncomfortable to use due to poor ventilation, cost of nets precludes wide use in many rural poor communities, frequent washing of the nets reduces the effectiveness of the treated nets. If people are not used to them, it might require behavioral change and people need to be taught how best to use the nets (MOH, 2002; Kolaczinski and Graham, 2004). Another problem to the use of ITNs is that it is used at bed time, but most people are bitten outdoors before bedtime.

On the contrary, it has been demonstrated that if they are properly applied they can provide a 30 to 60% reduction in malaria morbidity and can be useful in terms of preventing drug resistance. Further more, in community wide trials in several Africa settings, ITNs have been shown to reduce all cause mortality by about 20% (CDC, 2008).

1.6.4. Chemical control

Chemical control used for rapid control of large area, although misuse of those insecticide leads to resistance, resurgence of pest and finely replacement of insecticide which is unaffordable in developing country. The initial idea of eradicating malaria became more difficult due to the DDT-resistance of anopheline mosquitoes and chloroquine resistant-strains of *P. falciparum.* Due to their persistence in the environment and accumulation in food-chains DDT, thus it is suggested that organochlorine should not be used as larvicides and sprayed inside houses (Curtis, 1996; Service, 2000).

There are over 125 mosquito species with documented resistance to one or more insecticides (CDC, 2004). Recent evidence from Africa indicates that pyrethroids and DDT resistance is more widespread than anticipated (WHO, 2007). Due to the fact insecticides are generally neurotoxins and, as such, can pose significant health risks to humans and other animals (Justice *et al*., 2003).

1.6.5. Genetic control

The main principles of genetic control are based on propagation of sterility or other desirable genetic factors in successive generations. The general goal is birth control (rather than death) through reduction or replacement of a population following release(s) of a desirable genotype (Toure *et al*., 2004). The aim is to introduce large numbers of healthy, but sterile, insectary-reared males into field population that will compete with natural fertile males for female mates. So resulting in large numbers of infertile inseminations. In El Salvador the release in the 1970s, of some 4.36 million chemosterilized male *An. albimanus* (an important malaria vector that had developed resistance to must insecticides) over 4.5 months in an isolated coastal region of about 15km^2 caused a more than 97% reduction in the biting population (Service, 2000).

Genetically modified mosquitoes are a novel approach that is also being investigated (Justice *et al*., 2003). A transgenic mosquito engineered to be resistant to infection by the Plasmodium parasite could theoretically eradicate malaria, (Moreira *et al*., 2002) but replacing a wild population of mosquitoes with transgenic animals faces large technical and public relations hurdles (Scott *et al*., 2002).

1.6.6. Integrated Vector Management

Insects that feed on the blood of vertebrate are difficult to control and many previous efforts have been unsuccessful. This is becoming an ever-increasing issue, not only in developing countries, but also in developed countries (Logan *et al*., 2007). Investigating the ways in which biting insects interact with each other, their environment and their hosts in providing valuable knowledge that will lead to the development of improved control technologies; more interestingly the integration of one or more control methods presently known as in IVM become more fruitful. As result, unilateral use of any control method can have unwanted and unintended side effects. Integrated vector management is the use of a cost effective combination of vector control measures that are appropriate to local conditions and priorities and relatively safe for human health and the environment (CEAG, 2006).

Recent advances in chemical ecology research have led to the identification of new semio chemicals that show great potential as control agents against biting insects (Logan *et al*., 2007). Natural chemical attractants, mating stimulants or repellents are often effective in incredibly small concentration. The effectiveness of these chemicals and ease of synthesis favor their use as lures (Kabeh, 2007). All these chemical signals are effective in the eradication of several vector-species, when enhanced by traps, insecticides, chemo sterilants, sex repellent and attractants and lastly when synergized (Kabeh, 2007). There fore, incorporation of semiochemical into the IVM system would prove highly beneficial, since it is environmentally safe and cost effective control measure.

1.6.6.1. Monitoring

The use of pheromones for monitoring arthropods began in the 1970s, and so predates their use for population reduction (Norris *et al*., 2003). Sex and aggregation pheromones are species-specific attractants, and traps baited with these can be used as specific monitoring tools for the target insect species.

Trapping, as a means to monitor mosquito populations, is an integral component of surveillance efforts, yet standard techniques for interpreting the results are lacking (Jensen *et al*., 1994). Importantly, trap design (e.g., counterflow, downdraft), placement (e.g., height above ground, time of day), location (local environment, habitat specificity of mosquito), and use of attractants (e.g., light, CO_2, octenol) influence mosquito abundance estimates (Kline, 1999). These factors affect the species, number, and reproductive status (parity) of mosquitoes captured (Reisen *et al*., 1999; Mboera *et al*., 2000). Interestingly, it is essential to have knowledge about trap biases when deciding what traps to use, where they are to be deployed, and how to interpret the results (Brown *et al*., 2008).

Various chemicals in the environment, serve as oviposition attractants for mosquitoes even when present in relatively small quantities (Beehler and Mulla, 1993). Interestingly, traps containing feeding attractants are used, for instance oviposition traps and graved traps mimic these natural oviposition sites through the use of crude infusion lures. Mostly,

ovitraps are the preferred surveillance tools for monitoring *Aedes* activity, which can be made more attractive to the gravid female by the addition of an attractant. These ovitraps can also be provided with an insecticide like IGRs for effectively controlling the breeding (Tilak *et al*., 2005).

1.6.6.2. Lure and Kill and Mass Trapping

The aim of "Lure and kill" or "Mass trap" approach for insect control is to reduce the vector population by attracting vectors with pheromones and then either trapping or killing responding individuals (Norris *et al*., 2003). The advantage of lure and kill over mass trapping is that many attractive sources can be employed (Campion *et al*., 1989). So far perhaps the most successful use of lure and kill devices is the use of odor baited traps to control testes fly (Torr *et al*., 1997).

The development of trapping systems that utilize semiochemicals as lures depends upon the identification of behaviorally active compounds (Logan and Birkett, 2007). Thus olfactory cues play important role in life of mosquitoes mediating oviposition sites, sugar and blood feeding and mate finding. These chemical attractants are used as lure to attracts and trap mosquitoes and kill with integrated conventional methods. Among odors that are not human-specific, Takken and Knols (1999) found that mosquitoes are attracted to the scents of 1-octen-3-ol and acetone. In fact, products are already sold in the USA based on one of these compounds, 1-octen-3-ol (Flowtron Mosquito Attractant, Flowtron Outdoor Products, Melrose, MA, USA), which is used to attract mosquitoes to electric traps or ''bug zappers'' (Justice *et al*., 2003). In addition, mosquitoes strongly react to odours such as CO_2 and L-lactic acid (Kline, 1999).

The main olfactory cues used for trapping mosquitoes, CO_2 and its importance have been shown in several malaria vector species. Thus CDC-type traps that incorporate carbon dioxide as bait are routinely used in mosquito surveillance programs in many areas of United States (Service, 1993 cited in Caltrans, 1998), including California. Many mosquito trapping devices are based on generating, CO_2 to lure the mosquitoes to the device (Rutledge, 2008). The distance at which a female particular mosquito species responds to

carbon dioxide is influenced by factors affecting the carbon dioxide plume such as release rate, environmental factors such as wind speed, and by innate host preference (Caltrans, 1998).

At present, the best options for trapping *Ae.aegypti* are backpack aspirators, sticky ovitraps, and the BG trap (Morrison *et al*., 2008). The development of new, less expensive designs could extend their use into developing country settings (Morrison *et al*., 2008). For instance, MosquiTRAP™ is a sticky trap specifically designed to capture graved females of *Aedes aegypti* (L.) and allows the identification of the mosquito in the field during the inspection of the trap (Gama *et al*., 2007). On the other side, BG trap is an attractant trap that requires electricity and it appears to be comparable in efficacy to backpack aspiration collections of *Ae. aegypti* adults (Willams *et al*., 2007).

1.6.6.3. Push-Pull

Push-pull strategies use a combination of behavior-modifying stimuli to manipulate the distribution and abundance of vector and beneficial insects for vector management (Cook *et al*., 2007).

Consequently, push-pull strategies may control disease transmitting flies of medical importance, such as mosquitoes, by exploiting natural differential attractiveness within a host species (Costantini *et al*., 2001) or using botanical repellents as push stimuli and attracticide based on host odors or attractive pheromone (Blackwell *et al*., 1994) as pull stimuli.

2. Objectives

2.1. General objective

Investigation of volatile compounds from ZM-521 and BH660 maize varieties pollen as potential attractants to adult blood fed and unfed female *Anopheles arabiensis* mosquitoes under laboratory conditions to see the effect maize production on prevalence of malaria vector.

2.2. Specific objectives

- To study behavioral response to attractive volatile compounds
- To investigate and compare the response of blood fed and unfed female *Anopheles arabiensis* antennae to volatile compounds from ZM-521 and BH660 pollens.
- To identify volatile compounds from the pollens of ZM-521 and BH660.

3. Materials and Methods

The study was conducted in two parts. The first part was carried out in Ethiopia focusing on behavioral responses of *Anopheles arabiensis*, while the second part was carried out in Sweden dealing with electrophysiological responses of *Anopheles arabiensis* to odors of BH660 and ZM-521 pollens.

3.1. Rearing of *Anopheles arabiensis* mosquitoes

In Ethiopia, *An. arabiensis* was reared in Nazareth Mosquito Rearing Center (Oromia Regional State, Ethiopia) and used for behavioural bioassy. The larvae were fed daily on fafa and subjected to natural ambient light. The colony were maintained at 27+1^{o}c, 85+5% RH and 13:11 (L: D). On pupation, the pupae were transferred into standard 30 ×30×30 cm nettings cages. After emergence, adult mosquitoes were held in cages and provided with sugar and distilled water via moistened cotton wick placed on the top of the cages. The female *Anopheles arabinesis* mosquitoes were fed on rabbit twice for 3 hours. The cages were kept in the insectary at ambient tropical temperature and under artificial light provided by fluorescent tubes. Relative humidity was maintained at ambient by placing water basins placed in the insectary (Gerberg, 1979).

The colony was maintained under standard insectary conditions (27 °C, 75% R.H., L: D 12:12h). The eggs, larvae and pupae were kept in demineralized water; larvae were fed baby fish food (Flakes). Adult mosquitoes were held in plastic cages with mesh lids (20 cm diameter by 30 cm height). Adults were supplied with access to 10% sucrose. For this experiment, 5-9 days old females that had been and had not been blood-fed were used. Female were allowed to feed on a human volunteer arm for 30min.

3.2. Headspace volatile collection

3.2.1. Collection of volatile from of ZM-521 and BH660 pollens

Sample plants of ZM-521 and BH660 were grown starting July 8 and 16, 2008, respectively. The maize varieties were grown at Melkassa Agricultural Research Center 117 km from the capital, Addis Ababa ($8^{o}24'$, $38^{o}07'$1550 m.a.s.l) in East Oromia region (http://www.eiar.gov.et/centers.htm). The odors from ZM-521 and BH660 pollens were collected using head space technique before pollen shedding. Thus, odors of ZM-521 pollen were collected from September 13 to September 20, 2008.

On the other hand, odors of BH660 pollen were collected from October 3, to October 10, 2008. The collections of odors were performed by randomly selecting ZM-521 maize pollen border which were grown with other maize varieties. Besides, collection of odors from BH660 pollen variety was preformed by randomly selecting the pollen throughout the cultivation. These were done by enclosing the pollen part in a Teflon bag (Meny, Toppits, 35-43 cm) (Wold-Hawariat, 2008; Fig. 1.) and air was pumped into the system through an activated charcoal filter. A glass cartridge filled with 0.0350 mg of Super Q (super Q, 25 mg mesh 50-80 supelco) used for trapping the odors. At the end point, a pump that pushes air into the bag were attached as well as a filter Super Q with polypropylene wool and nylon stoppers in both ends (Birgersson and Bergström, 1989 cited in Wold-Hawariat, 2008). Volatile compounds absorbed on the super Q were rinsed with 300µl hexane glass vial. All sample vials with extracts, were stored at -20^{o}c until used in behavioral and gas chromatography experiments (Wei and Kang, 2006; Wold-Hawariat, 2008). The pollen collected from maize plant is also kept in the same condition.

The volatile collections were preformed from these maize pollen varieties that have not been excised and were growing under natural conditions (Jones and Poppy, 2006).

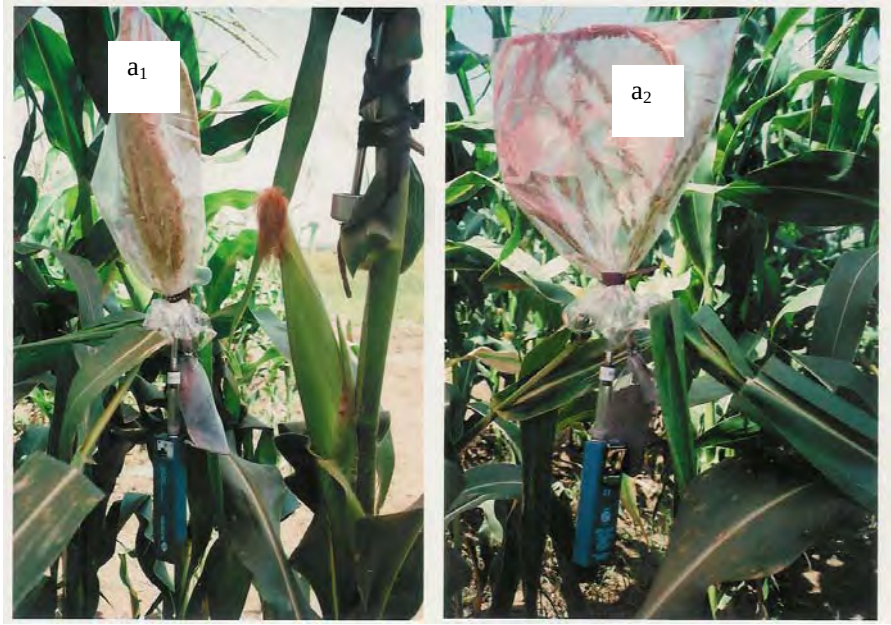

Fig. 1. Head space collection of odors from ZM-521 pollen.
a_1 & a_2 volatiles being collected from pollens enclosed by Teflon bags.

3.2.2. Collection of volatile from breeding sites

Odors were collected from mosquitoes breeding sites using Teflon bag. For an open-air circulating system, air was pumped through an activated charcoal filter. The volatile compounds absorbed in super Q for 2 hours and eluted with 300µl hexane. Then the extracts were kept at -20°C until used in electrophysiological methods.

3.3. Behavioral responses

3.3.1. Oviposition bioassay

This oviposition bioassay was done using BH660 pollen and the other ovipostion assay trial was preformed using extract of ZM-521 pollen. The bioassays consisted of two 3.7cm diameter with 4.5cm depth polypropylene cups (test and control) that were placed in a small bucket (18.5cm top and 12.5cm bottom diameters, 17.5cm depth; Fig. 2.). Ten blood fed female *An. arabinesis* mosquitoes were released into each bucket, which then was covered by nylon mesh (Fig. 2). In each cup, 15ml of water taken from the breeding site of the mosquitoes were placed at opposite sides of the bucket. A known amount of BH660 pollen was placed in the test cup (0.14g, 0.16g and 0.18g) and the other cup was used as a

control; this bioassay was repeated three times for each amount of maize pollen. Then the small buckets were placed in condition maintained at 27±1°C, 85 ± 5% RH and 13:11(L: D) (Nazareth Mosquitoes Rearing Center). In addition, to restrict visual cues presented by yellowish colored pollen infusions, the small buckets were placed in a dark area and calm area to avoid oviposition disturbance. Oviposition response was scored by counting the number of eggs on the test cup and control cup after 54 hours.

Fig. 2. Oviposition bioassay conducted at Nazareth Mosquitoes Rearing Center using BH660 and ZM-521 pollen extracts.

The second ovipostion assay was performed using the other maize variety. In this cause extracts of ZM-521 pollen volatile put into 15ml water collected from breeding site in one of the cup and hexane on the other cup. Doses including 20µl, 40µl and 60µl were used for extract of ZM-521 pollen and the solvent hexane. Except the above condition the trial was conducted and maintained under similar condition as the first trial (Fig. 2.).

3.3.2. Landing bioassay using BH660 pollen extract

Bioassays were conducted in rectangular plastic bowl (21.5cm × 29.5cm × 12cm; L ; D; H) covered by mesh on the top for mosquitoes entrance and for air movement (Fig. 3.). On opposite sides, 29.5cm apart and 5.5cm depth from the bottom surface cylindrical shaped polypropylene were placed (Fig. 3.). Cylindrical shaped polypropylene were used to place (test and control) using dispensers one in each side. This bioassay was

performed by changing the doses (10µl, 20µl, 40µl, 60µl and 80µl) of extracts of both BH660 maize pollen variety (test) and hexane (control). Ten adult female *An. arabinesis* mosquitoes that were sugar fed were used for the first trial and the second trial was preformed using blood fed *An. arabinesis*. Five and seven day's old blood unfed and blood fed *An. arabinesis* mosquitoes were placed in a rectangular plastic bowl for 25 min. To determine an optimal time-course of assay, observations were made. Assays were conducted under dark conditions and maintained at 27±1°C, 85 ± 5% RH. In the bioassay, tests and controls were presented at the same time. Care was taken during counting to not disturb landed *An. arabinesis* mosquitoes.

Fig. 3. Landing bioassay conducted at Nazareth Mosquitoes Rearing Center using BH660 and ZM-521 pollen extracts.

3.3.3. Landing response of blood fed *An. arabinesis* to extract of ZM-521 pollen

The extracts which were collected from headspace ZM-521 pollen were used for this bioassay. Bioassays for the first and the second experiments were conducted in rectangular plastic bowl similar to those described above (Fig. 3.). The one test and one control were placed 29.5cm (with three replicates) apart away from the entrance and left for 25 min in

each time of experiments. The tests were alternated in every experiment by varying the doses (10µl, 20µl, 40µl, 60µl and 80µl) varying the doses of ZM-521 pollen and hexane extracts. Ten female *Anopheles arabinesis* mosquitoes fed on a rabbit's bloods for 8 hours and aged 7 days were placed in each rectangular plastic bowl. The bioassay experiment was maintained at 27±1°C, 85 ± 5% RH and under dark conditions. Then the landed mosquitoes were counted to determine the attractiveness and the dose responses of female *Anopheles arabinesis* mosquitoes to ZM-521 extracts.

The second experiment was done by changing the control (hexane) to extracts obtained from breeding sites of mosquitoes. The test extract was ZM-521pollen variety and the doses (10µl, 20µl, 40µl, 60µl and 80µl) were altered every time for both treatments. Besides, all other conditions were the same as the above (first) experiments.

3.3.4. Landing response of blood unfed *An. arabiensis* to extract of ZM-521 pollen

This landing bioassay was performed using extracts of ZM-521 pollen (test) and hexane (control). In this case, the concentrations (10µl, 20µl, 40µl, 60µl and 80µl) of the extracts were changed after each trial. Only ten sugar fed *An. arabiensis* and aged 7 day were introduced into rectangular plastic bowl. The experiment was preformed at the time when *An. arabinesis* were active at dawn and dusk, maintained under 27±1°C, 85 ± 5% RH conditions. Thus each bioassay was repeated three times.

3.4. Electrophysiological responses

3.4.1. Gas-Chromatographic-Electroantennographic detector (GC-EAD)

The antennae of *Anopheles arabiensis* mosquitoes were prepared as described by Tesfaye and Hailemariam (unpublished) with a few modifications. The head part of the *Anopheles arabiensis* mosquitoes which were 5-9 days old, and blood fed and unfed respectively were

excised. Then the antennae were fixed in a micropipette (1.5mm O.D. X 1.17 mm I.D.) filled with Ringer solution (0.75g NaCl, 0.035g KCl , 0.021g $CaCl_2$ and 100ml deionized H_2O). Thus, these were done by seizing the mouthpart using microincissors to prevent the antennae damage. The distal tip of one antenna was cutoff and fixed into the second electrode filled with the same Ringer solution. The essences of cutting the distal parts were to complete the electrical circuit and improve the recording. To have stable electrical contact, movement of the head and antennae was restrained. The life of the prepared excised antenna was extended by a continuous flow of humidified air with a speed of 0.5L/min was passed through a glass tube over the antenna.

In order to use the insect antennae as a GC detector, the effluent from the column was directed to the antennal preparation (Syntech). The column effluent was splited, half being directed to the GC detector and the other half passing simultaneously over the antennal preparation outside the GC oven via a suitable transfer lines (Bruce *et al.,* 2005; syntech). To prevent condensation of fractions in the transfer lie, this was needed to be heated up to the maximum temperature. The odor samples were analyzed on a gas chromatograph (US3223314H) on a DB-wax column (30m x 0.25mm ID) with average velocity of 45cm/sec. The oven temperature was set at 30°C for 3 min, increased to 225°C for 8°C/min, which was maintained for 8 min. The carrier gas was hydrogen. Furthermore, 2µl sample of volatiles were injected onto the GC column, which were separated it into its components (Bruce *et al*., 2005). Three successful GC–EAD recordings with different female antennae were performed for each extract.

However, only a response to fractions containing compounds was shown, which activated the receptor cells on the antenna. The signal from the antenna was monitored and recorded simultaneously with the signal from the FID, and both signals were synchronized in time (Syntech; Wold-Hawariat, 2008).

3.4.2. GC-MS analysis

In order to identify volatiles compounds from the extracts (odors) the same type of the column and is programme was used as in the GC-EAD analysis, and also the same extracts was used as GC-EAD. Thus, is capable of giving important information regarding the structure of the compounds with a nanogram or less of a compound both in electron impact and chemical ionization mode. In this case, the carrier gas was helium. In each run, 2µl of the odor extract was injected in the GC. Peaks were identified by comparing them with the spectra from a custom made library (Goran. Pers. Com.).

3.5. Data analysis

All data generated from the ovipostion and landing bioassays were calculated using a choice index (Sharon. Pers. Com.). In the case of ovipostion assay, the choice indices were calculated by taking the ratio between each value of experimental (test) and control *An. arabiensis* to total number of egg laid during the bioassay period. On the other hand, data from landing bioassays were analyzed separately using choice index i.e calculated differently from the data of the ovipostion assay. The mean number calculated from the choice index of both the oviposition and landing behaviors experiments of the treated and control *An. arabiensis* were used to distinguish the responses between the two. The final data (results) following the choice index calculation were subjected to comparison using Student-t-test.

4. Results

4.1. Oviposition assays on blood fed *An. arabiensis*

Oviposition site selections by female mosquito play an important role for mosquito progeny. In this first trial of oviposition assays, blood-fed *An. arabiensis* laid more eggs in breeding site water with BH660 pollen within 54 hours than water collected from known mosquito breeding sites of mosquitoes alone. A significant difference (P<0.01; Fig. 4.) between the test and control were found for all doses of BH660 pollen tested.

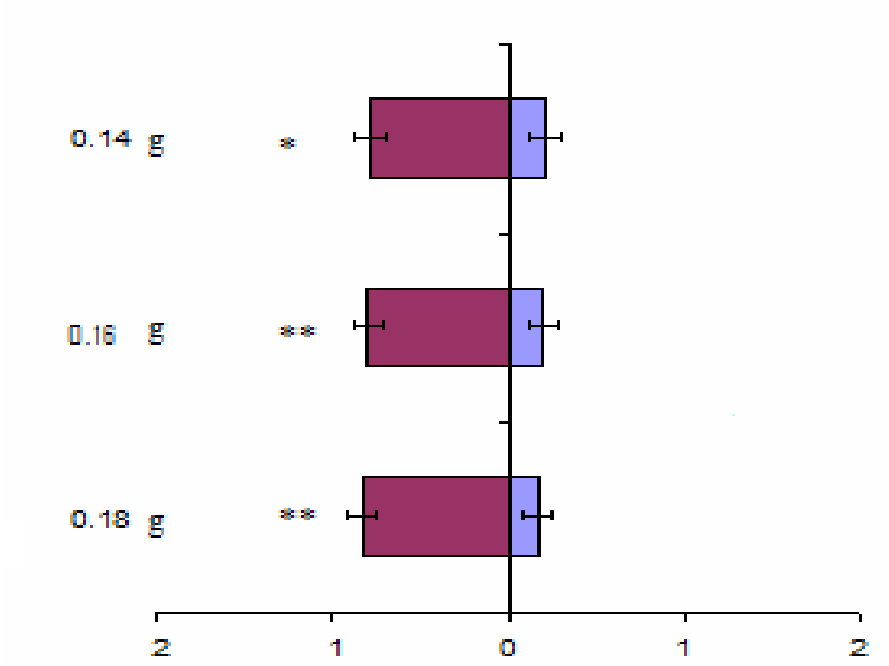

Fig. 4. Oviposition choices of blood-fed female *An. arabiensis* mosquitoes between water from a known breeding site and BH660 pollen in water from breeding sites at different doses. Error bars represent standard errors of the mean; *; P<0.05; **; P<0.01. Three replicates of 10 mosquitoes each.

On the other hand, in the second trial of oviposition bioassay, blood-fed *An. arabiensis* laid their eggs on either hexane laced water or on ZM-521 maize pollen headspace extract laced water. After 54 hours, more eggs were counted on the water containing ZM-521 maize extract than water from breeding sites of mosquitoes (Fig. 5). Thus significant differences between the treatments were found at 40µl (P<0.05; Fig. 5.) and 60 µl (P<0.01; Fig. 5.)

doses. According to these results, blood-fed female *An. arabiensis* appear behaviorally attracted to both varieties of pollen for oviposition.

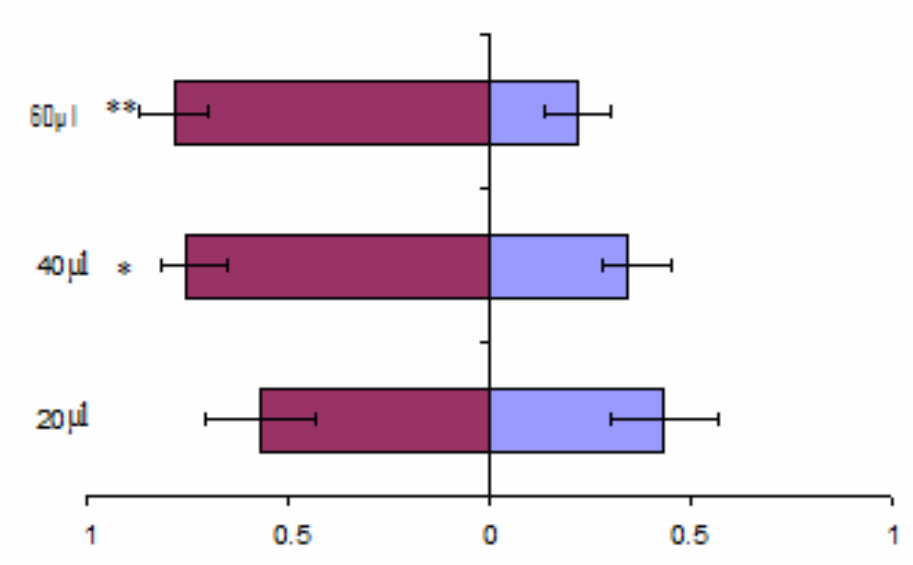

Fig. 5. Oviposition choice of blood-fed *An. arabiensis* mosquitoes between extract of ZM-521 pollen. Error bars represent standard errors of the mean; *; P<0.05; **; P<0.01. Three replicates of 10 mosquitoes each.

4.2. Bioassay using extracts of BH660 and ZM-521 pollen

4.2.1. Landing response of nonblood-fed and blood fed *An. arabiensis* mosquitoes using extract of BH660 ZM-521 pollens

The extracts from the headspace of BH660 pollen in the organic solvent hexane were used as the test and control volatiles in this set of landing assays, respectively. Thus more landing responses of nonblood-fed and blood fed *An. arabiensis* mosquitoes were found on the test than the control. Consequently, statistically significant differences were found between control and test at the following doses: 40µl (P<0.05; Fig. 6. a, c); 60µl and 80µl (P<0.01; Fig. 6. a, c).

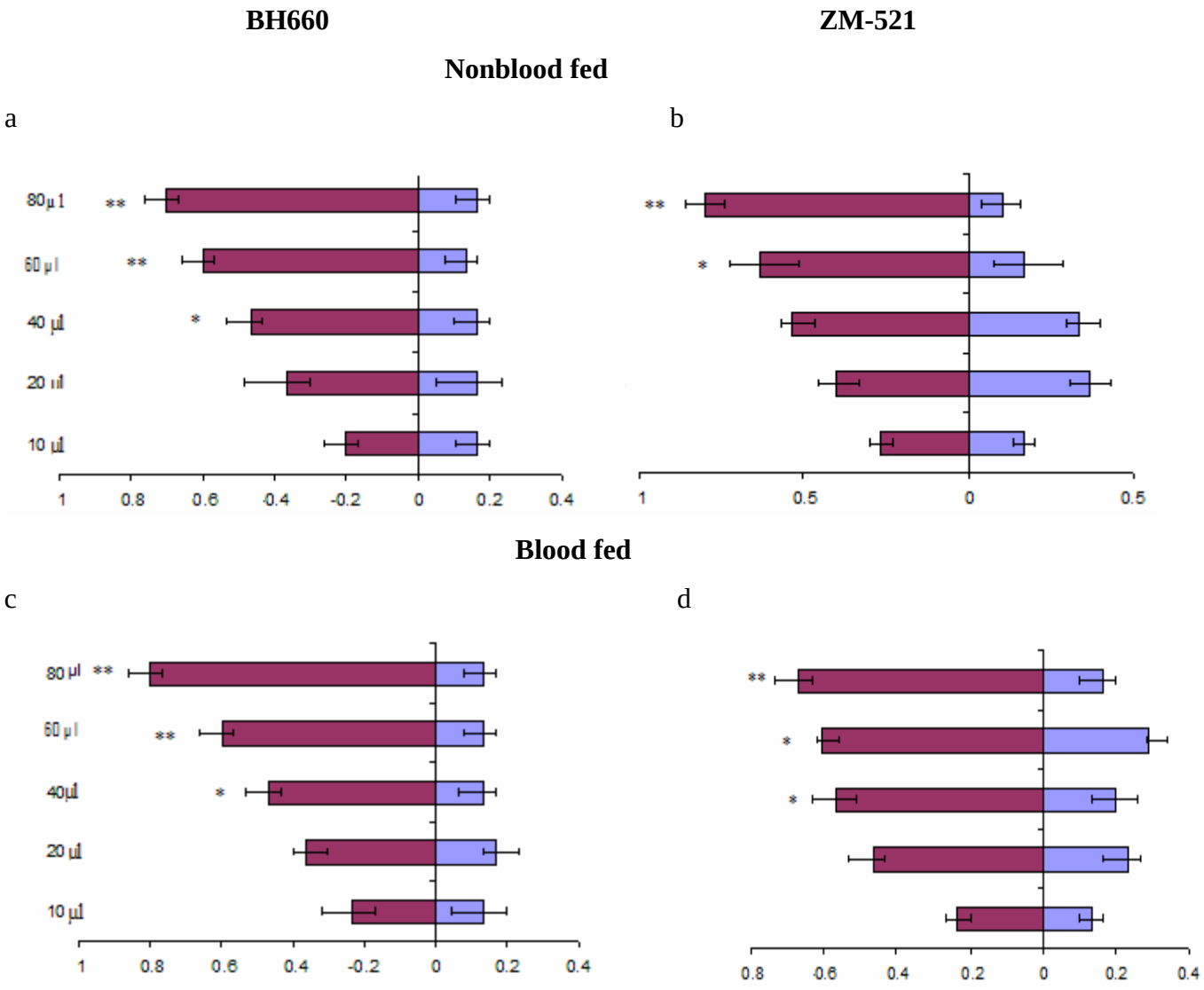

Fig. 6. Landing response to extracts of BH600 and ZM-521 pollens. a. nonblood fed *An. arabiensis* response to BH660 b. nonblood fed *An. arabiensis* response to ZM-521 pollen. c. blood fed *An. arabiensis* response to BH660 maize pollen. d. blood fed *An. arabiensis* response to ZM-521 maize pollen. Error bars represent standard errors of the mean; *; $P<0.05$; **; $P<0.01$.

Results from the landing bioassay illustrate that a significantly larger number of nonblood-fed and blood-fed *An. arabiensis* landed on the ZM-521 pollen extract than on the control. In addition, the number of *An. arabiensis* observed to land near the dispenser increased as

doses of the pollen extract increased. Similarly, nonblood-fed *An. arabiensis* were more attracted to ZM-521 pollen extracts and showed significant difference at 60µl ($P<0.05$; Fig. 6. b) and 80µl ($P<0.01$; Fig. 6. b.). Similarly, blood-fed *An. arabiensis* females showed significant difference at 40µl, 60µl ($P<0.05$; Fig. 6. d) and 80µl (ttest; $P<0.01$; Fig. 6. d.).

The above bioassay illustrated that blood fed *An. arabiensis* were more attracted than nonblood fed on both extracts of maize pollens. Though blood fed and nonblood fed *An. arabiensis* were founded to be more attracted to BH660 pollen.

4.2.2. Landing bioassay using blood-fed *An. arabiensis*

Landing responses of blood-fed female *An. arabiensis* to extracts collected from breeding sites and ZM-521 pollen showed non-significant difference at doses 10µl and 20µl ($P>0.05$; Fig. 7). Significant differences were found at doses 60µl, 40 µl ($P>0.05$; Fig. 7) and 80µl ($P>0.01$; Fig. 7). These illustrate that more blood-fed *An. arabiensis* mosquitoes were attracted and choices these sites. Thus olfactory cues may play a significant role in orienting blood-fed mosquitoes to these sites.

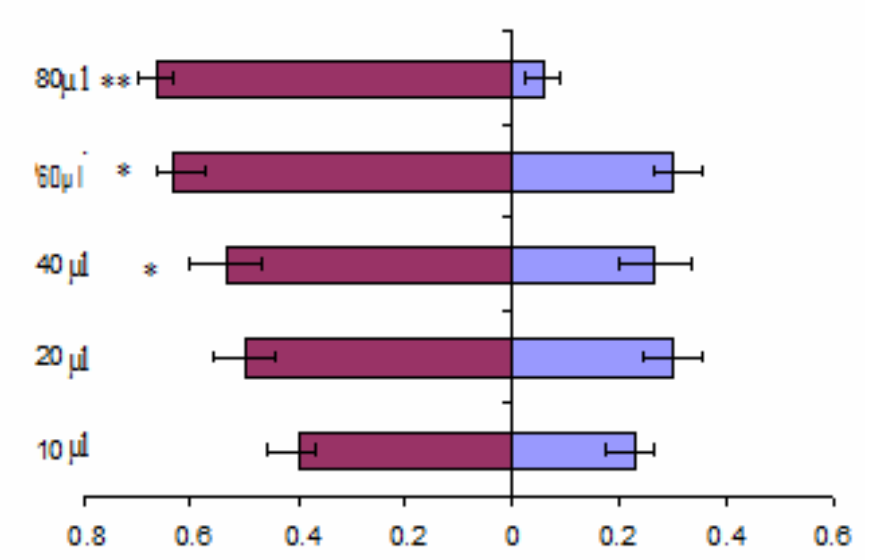

Fig. 7. Blood-fed *An. arabiensis* mosquitoes landing response to extracts from ZM-521 pollen and breeding sites of mosquitoes. Error bars represent standard errors of the mean; *; $P<0.05$; **; $P<0.01$.

4.3. GC-EAD analysis and identification of volatile compounds

Representative GC-EAD chromatograms of mosquito antennae responses to headspace collection volatiles from, BH660 and ZM-521 pollens are presented. Antennae from blood-fed and nonblood-fed *An. arabiensis* were found to respond to compounds in the headspace of pollens of both varieties (Figs 8 and 9). The volatiles from BH660 and ZM-521 pollens that elicited consistent electrophysiological responses to blood-fed and non blood-fed are shown below (Fig. 8 and 9; Table 1).

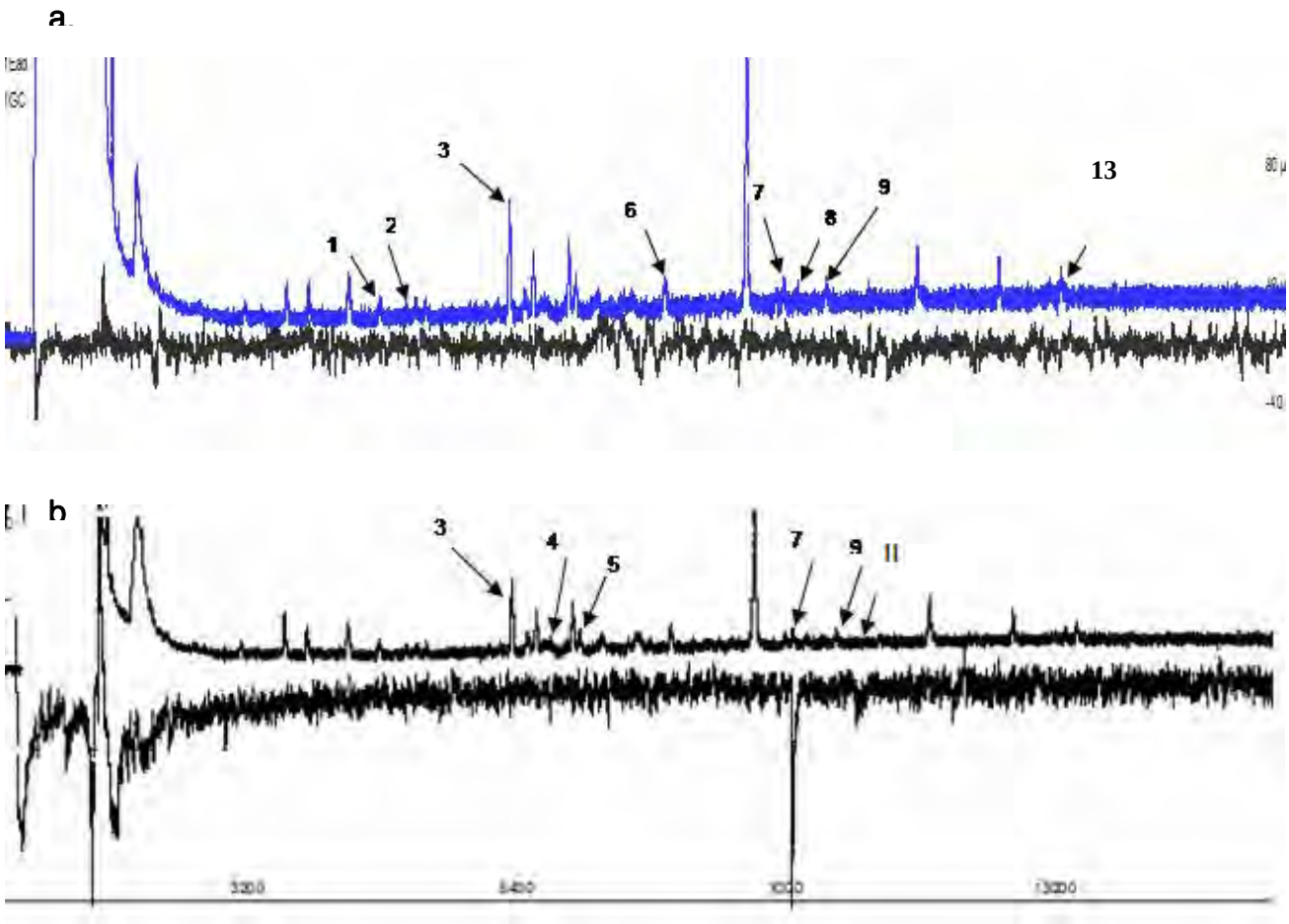

Fig. 8. Responses of GC-EAD using female *An. arabiensis* antennae to headspace volatile compounds from BH660 pollens. **a.** Blood-fed and **b.** nonblood-fed *An. arabiensis* mosquitoes. The lower trace indicates the EAD and the upper trace indicates GC response.

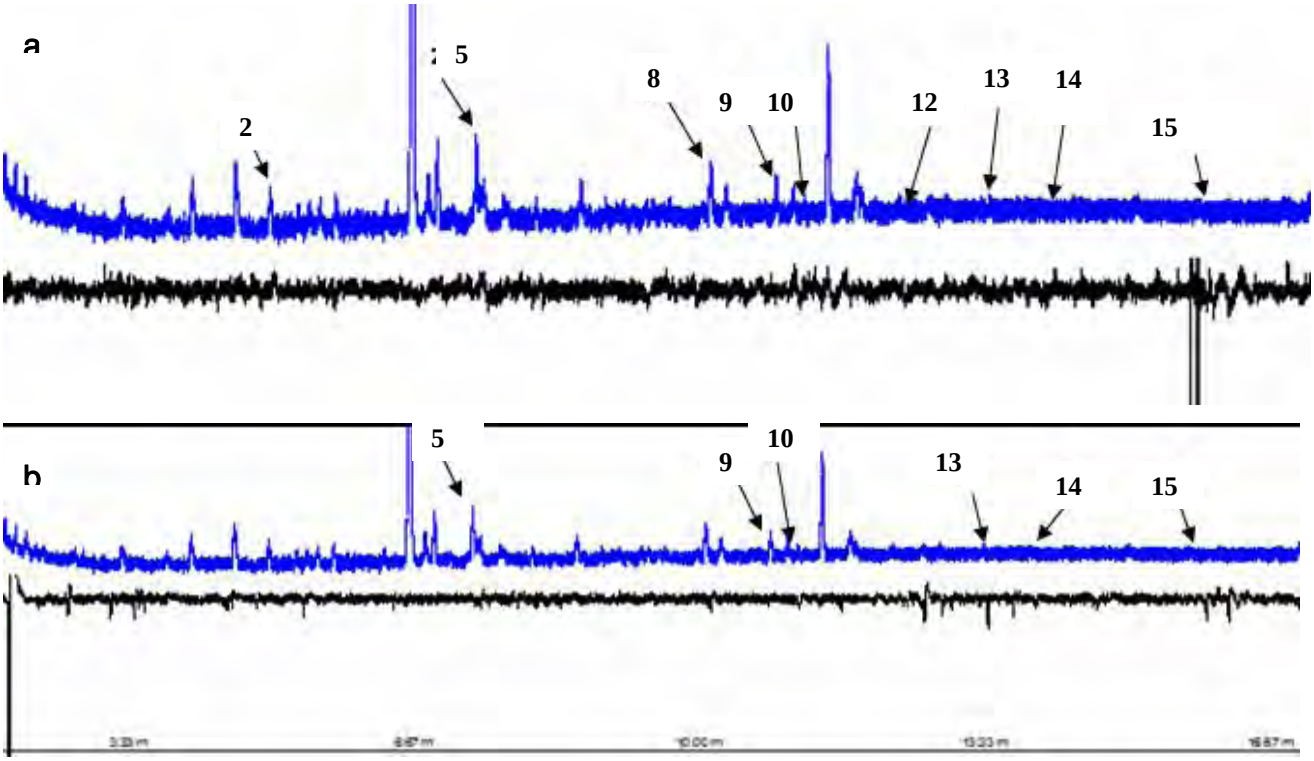

Fig. 9. GC-EAD analysis of ZM-521 pollens headspace volatiles using **a.** blood fed and **b.** unfed female *Anopheles arabiensis.* The lower trace indicates the EAG and the upper trace indicates GC response.

Table. 1. Volatile compounds eliciting electrophysiological responses during GC-EAD recording from *An. arabiensis* antennae exposed to headspace collection of BH660 and ZM-521 pollens.

Peak	Retention Time (GC-MS)	Compound	Blood-fed BH660	Blood-fed ZM-521	Nonblood-fed BH660	Nonblood-fed ZM-521
1	4.57	Tetrahydro-2,5-dimethyl furan	+			
2	4.87	Nonane	+	+		
3	6.53	Decane	+		+	
4	7.14	Toluene			+	
5	7.4	3-Hexanone		+	+	+
6	8.46	Undecane	+			
7	10.22	Limonene	+		+	
8	10.32	Dodecane	+	+		
9	10.68	2-Hexanol	+	+	+	+
10	11.26	Styrene		+		+
11	12.16	Tridecane			+	
12	12.48	2-heptanol		+		
13	13.59	Octyl acetate	+	+		+
14	14.69	1-octen-3-ol		+		+
15	16.45	Zingiberene		+		+

According to the results from GC-EAD, several compounds have been putatively identified from BH660 and ZM-521 pollens using GC-MS (Figs 8 and 9; Table 1). Only blood-fed *An. arabiensis* responded to nonane and dodecane in both varieties pollens, whereas all females responded to 2-hexanol (Table 1). Only decane and limonene from BH660 and styrene, 1-octen-3-ol and Zingiberene elicited EAG responses in both blood-fed and nonblood-fed mosquitoes (Table 1).

5. Discussion

The life of a female mosquito comprises several major activities: foraging (sugar and blood), mating and ovipositing, which are all regulated by endo- and exogenous factors. During these activities semiochemicals- assist the female mosquitoes to find their sugar sources, blood-hosts, mating partners or oviposition sites (Takken and Knols, 1999). Based on this general fact (hypothesis), the present studies dealt with the role of olfactory cues that mediate female *An. arabiensis,* which is the main vector of malaria in Ethiopia, in finding maize pollen. Thus study as indicated in the results section clearly showed the role of olfactory cues in locating laboratory reared female *An. arabiensis* to maize cultivation.

Maize pollen, in particular constitutes an important source of nutriment for *An. arabiensis* in Ethiopia (Ye-ebiyo et al., 2003). In the present work, both nonblood fed and bloods fed female *An. arabiensis* were attracted to maize pollen which is a good source of starch and sucrose. As a result, sugar provides an important food for female mosquitoes (Gary, 2005) by giving immediate energy demand (Foster, 1995). The reserve deposit of sugar serves as source of energy for flight, reproduction fitness, survival, longevity and increases body size for female Anopheles mosquitoes (Foster, 1995; Gary, 2005).

Different maize varieties may have different adaptation areas, so, they may show difference in specific outcomes due to genetic and environmental interactions (Bizuayehu, 2007). These maize varieties BH660 and ZM-521 which were used in the present study showed different attraction to blood fed and nonblood *An. arabiensis*. Interestingly, BH660 maize pollen was more attractant than ZM-521 maize pollen. This probably is attributed to difference in nutritional content in the pollens of two maize varieties.

According to the results, behavioral responses of blood-fed and blood starved female *An. arabiensis* to oviposition and landing bioassays showed the presence of potential attractants in pollen as well as the extracts. In the oviposition bioassay, blood-fed *An. arabiensis* deposited more eggs on a substrate with BH660 pollen compared to water collected from their natural breeding site alone. This is in agreement with previous works which revealed that oviposition behavior of many species of mosquitoes uses tactile, chemotactile,

olfactory or visual cues to asses breeding sites (Bentley and Day, 1989; Takken and Knols, 1999). In this case, it is however ambiguous to conclude as the only external cues is olfactory; it is also possible that they may used tactile to determine the suitability of host (maize pollen). In contrary, the oviposition assay using extract of ZM-521 pollen showed similar results; more eggs were recorded on cups containing ZM-521 pollen plus water from breeding sites than hexane plus water from breeding sites. In this case, it is to assume that olfactory cues were the only responsible factor because all cues where restricted from interfering.

Attraction to various flower extracts has already been shown in *An. arabienesis* (Healy and Jepson, 1988). Similiarly, potential attractants in both extracts of BH660 and ZM-521 pollens were founded from behavioral response to female *An. arabiensis* in the present work. There is the possibility that mosquitoes identify and trace nectar- rich flowers based on olfactory detection and discrimination of volatile profile of plant and flowers (Syed and Leal, 2007). Thus mosquitoes use olfactory appendages including antennae, maxillary palps (Syed and Leal, 2007; Takken and Knols, 1999) and proboscis (Pitts and Zwieble, 2006). Among there antennae are the most important multimodal sensory organs for the mosquitoes (Hansson, 1999). Consequently, both blood fed and nonblood fed *An. arabiensis* antennae were responded to extracts from BH660 and ZM-521 pollens. Thus kairomones nonane, 3-hexanone, dodecane, ocyl acetate and 2-hexanol compounds have been identified from both maize varieties. Volatile compounds from BH660 pollen including tetrahydro-2, 5-dimethyl furan, nonane, limonene, decane, undecane, 2-hexanol, ocyl acetate caused elicite to blood-fed *An. arabiensis* and nonblood-fed *An. arabiensis* responded to decane, toluene, 3-hexanone, 2-hexanol, tridecane and limonene. On the other side, volatile compounds from ZM-521 maize pollen that caused response to blood-fed *An. arabiensis* were nonane, 3-hexanone, dodecane, 2-hexanol, styrene, 2-heptanol, ocyl acetate, 1-octen-3-ol, Zingiberen and nonblood-fed *An. arabiensis* responded to all volatile compounds except nonane, 2-heptanol and dodecane.

Female's tendency to engage in host-seeking changes in concert with variations in physiological state such as age, reproductive status, and diapause (Klowden, 1888).The

physiological status with regard to nutrition, digestion and gonotrophic stage affect mosquitoes response to behavioral cues (Klowden, 1996). Thus, after taking blood meal the behavior of *An. arabiensis* changed. As a result, olfactory receptors in mosquito undergo alteration sensitivity that correlated with these changes in host-seeking behavior (Davis, 1984). Consequently, blood fed female *An. arabiensis* search suitable environments to lay their eggs.

Correct choice of the oviposition site by blood-fed female mosquito is the most critical factor for the survival of their progeny (Takken and Knols, 1999), since mosquitoes are the one with no parental care. Tilak *et al.* (2005) stated that blood fed female mosquitoes is the principal factor responsible for the distribution of the mosquitoes in breeding sites and their subsequent dispersion in different geographical areas. In Ethiopia the geographic distribution of malaria coincides closely with the distribution of maize culture (Ye-ebiyo *et al.,* 2000). Thus it is possible to conclude that blood fed female *An. arabiensis* were responsible for selection of BH660 and ZM-521 pollens as a breeding sites for their young using olfactory cues emitted from these maize varieties.

Consequently, from the study it made apparent that olfactory cues play an important role in assisting blood-fed and non blood-fed *An. arabiensis* near to maize cultivation. Interestingly, maize pollen shed coincides to the period near the end of rainy season when mosquito-breeding sites are most stable and abundant, optimizing its effect on larvae (Kebede et al., 2005). Adult mosquito that feed on maize pollen as larvae are larger, and larger mosquitoes generally live longer, thus increasing the force of malaria transmission.

6. Conclusions

The present study attempted to find out the role of olfactory cues in mediating blood-fed and unfed *An. arabiensis* to maize pollen. The following points were concluded from the study.

- *Anpheles arabiensis* were attracted to BH660 and ZM-521 pollens and chemical cue is one of the most external factors which play a great role in orienting female *An. arabiensis* to that pollens maize.
- The behavioral and electrophysiological responses studies clearly showed that BH660 pollen was more attractant and also have more potential attractant compounds than ZM-521 pollen.
- Oviposition bioassay and GC-EAD have now shown blood-fed female *An. arabiensis* are more attracted to those maize pollens.
- Olfactory cues assist blood-fed *An. arabiensis* to inhabit this maize cultivation. In fact the female is the one that is responsible for distribution of her young in different geographical areas. As a result, mosquitoes are the one with no parental care. Thus olfactory cues were proved to play a critical role in orienting blood-fed and unfed *An. arabiensis* to BH660 and ZM-521 pollens.

7. Recommendations

Based on the results from this study, the following could further enrich the present findings:

- Behavioral bioassay using synthetic compounds that were identified from BH660 and ZM-521 pollens.
- Further works on BH660, ZM-521 and other pollens varieties should be done to strength the present.
- A plethora of chemical originating from skin, breath, plant /nectar and oviposition sites are detected by those ORN. ORNs have been shown to encode odor quality, quantity and temporal change in odor concentration. It is essential to perform single cell sensillum recording, to know which ORN in *An. arabiensis* sensillium responded to the identified compounds.
- The transmission of malaria in Ethiopia might have a relation to the intensified maize crop cultivation and it would of interest to study more about external cues responsible for *An. arabiensis* to inhabit around maize cultivation.

- Signaling chemicals play an essential role in mosquito life cycles. They provide the means where by, mates, host and oviposition sites are located and recognized. Knowledge about mosquitos' behaviors and chemical ecology, and how these affect in transmission of diseases is still marginal in Ethiopia and need due attention.
- Thus, further works is needed at great magnitudes on *An. arabiensis* as it is the main vector of malaria in Ethiopia. As a result, improved knowledge of these chemical cues on *An. arabiensis* would lead to the application of semiochemicals as environmentally friendly mosquito vector management strategy.

8. Reference

Abdelmula, A.A. and Sabiel, S.A.I. (2007). Genotypic and Differential Responses of Growth and Yield of some Maize (*Zea mays* L.) Genotypes to Drought Stress. Tropentag 2007 University of Kassel-Witzenhausen and University of Göttingen, October 9-11, 2007 Conference on International Agricultural Research for Development.

Beehler, J.W. and Mulla, M.S. (1993). Effect of the insect growth regulator methoprene on the ovipositional behavior of *Aedes aegypti* and *Culex quinquefasciatus*. *J. Am. Mosq. Control*. Assoc. 9:13–16.

Bently, M.D. and Day, J.D. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Ann. Rev. Entomol*. **34**:401-21.

Birch, M.C. and Haynes, K.F., (1982). *Insect Pheromo*

nes (Studies of Biology No. 147). Edward Arnold Limited, London.

Bizuayehu, E. (2007). Comparison among maize (*Zea mays*) varies in relation to pollen reduction and nutrient composition. MSc. Thesis. Addis Ababa University, Addis Ababa.

Blackwell, A., Dyer, C., Mordue (Luntz), A.J., Wadhams, L.J. and Mordue, W., (1994). Field and laboratory evidence for a volatile pheromone produced by parous females of the Scottish biting midges, *Culicoides impunctatus. Physiol. Entomol.* **19**:251-57.

Breman, J.G., Alilio, M.S. and Mills, A. (2004). Conquering the intolerable burden of malaria: What`s new,What`s needed: A summary. *Am. J. Trop. Med. Hyg*. **71**:1-15.

Brown, H. E., Paladini, M., Cook, R.A., Kline, D., Barnard, D. and Fish, D. (2008). Effectivness of Mosquito Trap in Measuring Species Abundance and Compostion. *J. Med. Entomol.* **45**(3): 517-521.

Bruce, T.J.A, Wadhams, L.J and Woodcock, C.M. (2005). Insect host location volatile situation. *Trends plant Sci*. **10**:269-274.

Caltrans (1998). Vector Control Background Monitoring Plan (Mosquitoes and Midges) for Caltrans Retrofit BMP Pilot Project Sites, Caltrans District 11. 1-17Pp. 1-24Pp.

Campion, D.G., Critchley, B.R. and McVeigh, L.J. (1989). Mating disruption. In: Insect Pheromone in Plant Protection, Jutsum, A.R. and Gordon, R.F.S. (eds). 89-116. John Wiley and Sons, Chichester, UK.

CDC, **(2004)**. Centers for disease control and prevention. Department of Health and Human Services. http://www.cdc.gov/malaria/biology/.

CEAG, **(2006).** Approaches to Effective Malaria Control that Avoid DDT in Kenya: Use of *Bacillus thuringiensis* israelensis (BTi) African. African Center for Environmental Advocacy and Governance (CEAG Africa). International POPs Elimination Project – IPEP www.ipen.org.

Coluzzi, M. (1999). The clay feet of the malaria giant and its African roots: hypotheses and African roots: hypotheses and inferences about origin spread and control of *Plasmodium falciparum. Parassitologia.* **41**: 277–283.

Cook, S.M., Khan, Z.R. and Pickett, J.A., (2007). The use of push-pull strategies in Integrated Pest Management. *Annu. Rev. Entomol*. **52**:375-400.

Costantini, C., Birkett, M.A., Gibson, G., Ziesmann, J., Sagnon, N.F., Mohammed, H.A., Coluzzi, M. and Pickett, J.A. (2001). Electroantennogram and behavioural responses of malaria vector Anopheles gambiae to human-specific sweat components. *Med. Vet. Entomol*. **15**:259-66.

Curtis, C.F. (1996). Control of Malaria Vectors in Africa and Asia. Unversity of Minnesota, Minnesota.

Davis, E. E. (1984). Regulation of sensitivity in the peripheral chemoreceptor systems for host-seeking behaviour by a haemolymph-bornef actor in *Aedes aegypti. J. Insect Physiol.* **30**:179-83.

Deressa, W. Ali, A. and Berhane, Y., (2005). Review of the interplay between population dynamics and malaria transmission in Ethiopia. *Ethiop. J. Health. Dev.* **20**(3):137-144.

Endeshaw,T., Gebre, T., Ngondi, J., Graves, P.M., Shargie, E.B., Yeshewamebrat Ejigsemahu, Y. Ayele, B., Yohannes, G. Teferi, T., Messele, A., Zerihun, M., Genet, A., Mosher, A.W., Emerson, P.M., and Richards, F.O. (2008). Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. *Malar. J*. **7**: 118.

FMOH, ANVER and WHO (2007). Entomological profile of malaria in Ethiopia. Cdc (2008). Malaria: Vector Control. Division of Parasitic Diseases. ZVED.

Fradin, M.D.M.S. (1998). Mosquitoes and Mosquito Repellents: A Clinician's Guide. *Ann. Intern. Med.* **128**:931-940.

Foster, W.A (1995). Mosquito sugar feeding and reproduction energetics. *Ann. Rev. Entomal*. **40**:443-477.

Foster, W.A. and Hancock, R.G. (1994). Nectar-related olfactory and visual attractants for mosquitoes. *J. Am. Mosq. Control. Assoc*. **10**:288-96.

Foster, W. A. and W. Takken. (2004). Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bull. Entomol. Res.* **94**: 145-157.

Gama, R.A., Silva, E.M., Silva, I. M., Resende, M.C. and Eiras, A.E. (2007). Evaluation of the Sticky MosquiTRAP™ for Detecting Aedes (Stegomyia) aegypti(L.) (Diptera: Culicidae) during the Dry Season in Belo Horizonte, Minas Gerais, Brazil. *Neotrop. Entomol*. **36**(2):294-302.

Gary, R.E, Jr (2005). Biology of malaria vector *Anopheles gamblae* Behavioral and Reproductive Components of Sugar feeding. PhD. Thesis. Ohio State University, Ohio.

Gary, R. E. Jr. and W. A. Foster. (2001). Effects of available sugar on the reproductive fitness and vectorial capacity of the malaria vector Anopheles gambiae (Diptera: Culicidae). *J. Med. Entomol*. **38**: 22-28**.**

Gerberg, E.J. (1979). Manual for mosquito rearing and experimental Techniques. *American Mosquito Control Association. Inc. California*. (5): 1-11.

Goss, J. A. (1968). Development, physiology, and biochemistry of corn and wheat pollen. *Bot. Rev*. **34**: 333-358.

Gudu, S., Maina, S.M., Onkware, A.O., Ombakho, G. and Ligeyo, D.O. (2001). Screening of Kenyan maize germplasm for tolerance to low PH and aluminium for use in acid soils of Kenya. Seventh Easter and Southern Africa Regional Maize Conference 11th-15th February, pp.216-221.

Gwer, S., Newton. , C.R.J.C and Berkley, J.A. (2007). Over-Diagnosis and Co-Morbidity of severe malaria in African children: A guide for clinicians. *Am. J. Trop. Med. Hyg*. **77**:6-13.

Hansson, B.S. (1999). *Insect olfaction.* Springer Verlag, Berlin, Germany.

Healy, T.P. and Jepson, P.C. (1988). The location of floral nectar sources by mosquitoes: the long-range responses of *Anopheles arabiensis* Patton (Diptera: Culicidae) to *Achillea millefolium* flowers and isolated floral odour. *Bull. Entomol. Res.* **78**:651–57.

Janssens, P.G. and Wery, M. (1987). Malaria in Africa south of Sahara. *Ann. Trop. Med. Parasitol*. **81**:487-498.

Jensen, T., O. R. Willis, T. Fukuda, and D. R. Barnard. (1994). Comparison of Bidirectional Fay, Omnidirectional, CDC, and Duplex Cone Traps for sampling adult *Aedes albopictus* and *Aedes aegypti* in north Florida. *J. Am. Mosq. Control Assoc*. **10**: 74-78.

Jones, S.A and Poppy, G.M (2006). Comparison of glass and plastic bags for enclosing living plant parts for headspace analysis. *J. Chem. Ecol*. **32**:845-64.

Justice, R.W., Biessmann, H., Walter, M.F., Dimitratos, S.D. and Woods, D.F. (2003). Genomics spawns novel approaches to mosquito control. *BioEssays*. **25**:1011–1020.

Kabeh, J.D. (2007). Review: Influence of Semio-chemicals on group Behaviors and Application in Insect Pest and Vector Management. *Int. J. Agri. Biol*. **9** (6):937-940.

Kamau, L., Agai, D., Matoke, D., Wachira, L., Gikandi, G. and Vulule, J.M. (2007). Status of insecticide susceptibility in *Anopheles gambiae* sensu lato and *Anopheles funestus* mosquitoes from Western Kenya. *J. Inst. Sci*. **8**:11, available online: insectscience.org/8.11

Kanzok, S.M. and Jacobs-Lorena, M. (2006). Entomopathogenic fungi as biological insecticide to control malaria. *TRENDS in parasitology*. **22**:50-51.

Kebede, A., McCann, J.C., Kiszewski, A.E., and Ye-ebiyo, Y. (2005). New evidence of the effect of agro-ecological Change on malaria transmission. *Am. J. Trop. Med. Hyg*. **73**:676-680.

Keneni, G. and Wolde, L. (1999). *A guide for maize seed producers*, (Kirub, A. ed). Ethiopian Agricultural Research Organization (EARO).

Kiszewski,A. and Teklehaimanot, A. (2004).A review of the clinical and epidemiologic burdens of epidemic malaria. *Am. J. Trop. Med. Hyg.* **71**: 128–135.

Kline, D.L. (1999). Comparison of two american biophysics mosquito traps: The professional and a new counterflow geometry trap. *J. Am. Mosq. Control. Assoc*. **15**:276–282.

Klowden, M. J. (1988). Factors influencing multiple host contacts by mosquitoes during a single gonotrophic cycle. *Entomol. Soc. Am. Misc. Publ.* **68**:29-36.

Klowden, M.J. (1996). Endogenous factors regulating mosquito host-seeking behaviour. In: Olfaction in Mosquito Interactions, Wiley, Chichester, pp. 212–225 CIBA Foundation Symposium 200.

Knight, R.L., Walton, W.E., O'Meara, G.F., Reisen, K.W and Wass, R. (2003). Review: Strategies for effective mosquito control in constructed treatment wetlands. *Ecolo. Engineering*. **21**:211-232.

Kolaczinski, J. and Graham, K. (2004). Considerations for the use of insecticide-treated mosquito nets in complex emergencies. http://www.bayercropscience.com/.

Lardeux, F., Riveire, F., Sechan, Y. and Loucke, S. (2002). Control of the Aedes vectors of the Dengue viruses and Wuchereria bancrofti: the French Polynesian experience. *Ann. Trop. Med. Parasitol*, **96**: 105-116.

Law, J.H. and Regnier, F.E. (1971). Pheromones. *Ann. Rev. Biochem*: **40**: 533-548.

Logan, J.G., Birkett, M.A., Clark, S.J., Powers, S., Seal, N.J., Wadhams, L.J. Mordue (Luntz), J.A. and Pickett, J.A. (2007). Identification of Human-Derived Volatile Chemicals that Interfere with Attraction of *Aedes aegypti* Mosquitoes. *J. Chem. Ecol*. **34**:308-322.

Logan, J.G. and Birkett, M.A. (2007). Review: Semiochemicals for biting fly control: their identification and exploitation. *Pest Manag Sci*. **63**:647–657.

Luntz, A.J.M. (2003). Arthropod semiochemiacls:mosquitoes midges and selice. *Biochem. Soc.Trans*. **31**:128-133.

Mboera, L.E.G., B.G.J. Knols, M.A.H. Braks, and W. Takken. (2000). Comparison of carbon dioxide-baited trapping systems for sampling outdoor mosquito populations in Tanzania. *Med. Vet. Entomol*. **14**: 257-263.

McCall, P.J. and Cameron, M.M. (1995). Oviposition pheromones in insect vectors. *Parasitology Today*. **11**:352-55.

Miller, P. (1985). *Maize pollen: collection and enzymology*. **In**: Maize for biological research, (Sheridan, W.F., ed.). Plant Molecular Biology Assoc.Charkottesville.VA. 279-285Pp.

Minakawa. N., Mutero, C.M., Githure, J.I., Beier, J.C. and Yan, G. (1999). Spatial distribution and habitat characterization of anopheline mosquito larvae in Western Kenya. *Am J Trop Med Hyg.* **61**:1010–1016.

MOH, (2000). Malaria profile. Commercial printing press. Addis Ababa, Ethiopia. 12 pp.

MOH, (2002). Guideline for malaria vector control in Ethiopia. Addis Ababa Ethiopia. 14pp.

MOH, (2004). Malaria: diagnosis and treatment guidelines for health workers in Ethiopia. Addis Ababa. pp.1-3.

Moreira, L.A, Ito, J., Ghosh, A., Devenport, M., Zieler, H., Abraham, E.G., Crisanti, A, Nolan, T., Catteruccia, F. and Jacobs-Lorena, M. (2002). Bee venom phospholipase inhibits malaria parasite development in transgenic mosquitoes. *J. Biol. Chem*. **277**:40839–40843.

Morrison, A.C., Zielinski-Gutierrez, E., Scott, T.W., and Rosenberg, R., (2008). Defining Challenges and Proposing Solutions for Control of the Virus Vector *Aedes aegypti*. *PLoS. Med*. **5**(3): 68.

Muller, G. and Schlein, Y. (2005). Plant tissues: the frugal diet of mosquitoes in adverse conditions. *Med. Vet. Entomol*. **19**:413–422.

Mustaparta, H. (2002). Encoding of plant odour information in insects: peripheral and central mechanisms. *Entomol. Exp. Appl*. **104:**1-13.

Nordlund, D.A. (1981). Semiochemicals: A review of the terminology. In: Nordlund, D.A., Jones, R.L. and Lewis, W.J. (eds.). *Semiochemicals:* Their role in pest management. Wiley, New York. 13-28.

Norris, R.F., Caswell-Chen, E.P. and Kogan, M. (2003). *Concepts in Intergrated Pest Management*. Prentice-Hall,Inc., New Jersey. 373-387.

Onyabe, D.Y. and Conn, J.E. (2001). The distribution of two major malaria vectors, *Anopheles gambiae* and *Anopheles arabiensis*, in Nigeria. **96:**1081-1084.

Phillips, R.S. (2001). Current status of malaria and potential for control. *American Society for Microbiology*. **14**:208-226.

Pitts, R. J., Fox, A. N. and Zwiebel, L. J. (2004). A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*. *Proc. Natl. Acad. Sci*. USA. **101**: 5058–5063.

Pitts, R.J. and Zwiebel, L.J. (2006). Antennal sensilla of female Anopheline sibling species with differing host ranges. *Malar. J*. **5**:26.

Puri, S.N., Mendki, M.J, Sukumaran, D., Ganesan, K., Prakash, S. and Sekhar, K. (2006). Electroantennogram and Behavioral Response of *Culex quinquefasciatus* (Diptera: Culicidae) Females to Chemicals Found in Human Skin Emanations. *J. Med. Entomol*. **43** (2):207-213.

Qiu, Y. T., Smallegange, R. C., Van Loon, J. J. A., Ter Braak, C. J. F. and Takken, W. (2006). Interindividual variation in the attractiveness of human odours to the malaria mosquito *Anopheles gambiae s.s. Med. Vet. Entomol.* **20**: 280–287. (doi:10.1111/j.1365-2915.2006.00627.x).

RBM, (2003). The burden of malaria in Africa. Africa Malaria report 2003.

Reisen, W. K., K. Boyce, R. C., Cummings, O., Delgado, A., Gutierrez, R. P., Meyer, and Scott. (1999). Comparative effectiveness of three adult mosquito sampling methods in habitats representative of four different biomes of California. *J. Am. Mosq. Control. Assoc.* **15**: 24-31.

Rejmankova, E, Higashi, R., Grieco, J., Achiee, N. and Roberts, D. (2005). Volatile substances from larval habitats mediate species-specific oviposition in *Anopheles* mosquitoes. *J. Med. Entomol*. ***42:*** 95–103.

Rutledge, C.R. (2003). Mosquito control device and services for Florida homeowners, University of Florida. http://edis.ifs.ufl.edu/.

Savage, H.M., Rejmankova, E., Arredondo-Jim'enez, J.I., Roberts, D.R., Rodr'iguez, M.H., D.R. and Rodr'iguez, M.H. (1990). Limnological and botanical

characterization of larval habitats for two primary malarial vectors, *Anopheles albimanus* and *Anopheles pseudopunctipennis*, in coastal areas of Chiapas State, Mexico. *J. Am. Mosq. Control. Assoc.* **6***:* 612–620.

Schoepke, A., Steffen, R. and Gratz, N. (1998). Effectiveness of personal protection measures against mosquito bites for malaria prophylaxis in travelers. *J. Travel. Med*. **5**:188-192.

Scott, T.W., Takken, W., Knols, B.G. and Boete, C. (2002). The ecology of genetically modified mosquitoes. *Science*. **298**:117–119.

Seboksa, G., Nigussie, M., and Bogale, G. (2001). Stability of drought tolerant maize genotypes in the drought stressed areas of Ethiopia. Seventh Easter and Southern Africa Regional Maize Conference 11th-15th February. pp.301-304

Senay, G. and Verdin, J. (2005). Developing a malaria early warning system for Ethiopia. San Diego.California.UC2409.

Service, M.W. (2000). *Medical Entomology for Students*. 2nd edition, Cambridge University Press, Cambridge. 12-47 pp.

Spitzen, J., Smallegance, R. and Takken, W. (2008). Effect of human odours and positioning of CO2 release point on trap catches of the malaria mosquito *Anopheles gambiae sensu stricto* in an olfactometer. *Physiol. Entomol*. **33**:116-122.

Stratton, L., O'Neill, S., Kruk, E. and Bell, L., (2008). The persistent problem of malaria: Addressing the fundamental causes of a global killer. *Social Science and Medicine.* **67**:854–862.

Takken, W. and Knols, B.G.J. (1999). Odour mediated behavior of Afro tropical malaria mosquitoes. *Annv.Rev. Entomol*. **44:**131:57.

Taye, A., Hadis, M., Adugna, N., Tilahun, D. and Wirtz, R.A. (2006). Biting behavior and *Plasmodium* infection rates of *Anopheles arabiensis* from Sille, Ethiopia. *Acta Tropica*. **97:** 50–54.

Tilak, R., Gupta, M.V., Suryam, M.V., Yadav, M.J.D. and Gupta B.K.K.D. (2005). A Laboratory Investigation into Oviposition Responses Reesponses of Aedes aegypti to Some Common Household Substances and Water from Conspecific Larvae. *MJAFI.* **61**:227-229.

Torr , S.J. , Hall , D.R. , Phelps , R.J. & Vale , G.A . (1997). Methods for dispensing odour attractants for tsetse flies (Diptera: Glossinidae). *Bull. Entomol. Resea.* **87:**299-311.

Toure, Y.T., Oduola, A.M.J. and Morel, C.M. (2004). The *Anopheles gambiae* genome: next steps for malaria vector control. *TRENDS in parasitology*. **20**:143-149.

Tulu, A. (1993). Malaria. In: Zein Ahmed Zein and H. Kloos, (eds.). *The Ecology of Health and Disease in Ethiopia.* Westview Press, Colarado. Pp. 341-352.

Wei, J.N. and Kang, L. (2006). Electrophysiological and behavioral response of a parasitic wasp to plant volatiles induced by two leaf miner species. *Chem. Senses*. **31**:467-477.

White, N.J. (2004). Anti-malarial drug resistance. *J. Clin. Invest*. **113**:1084-1092.

WHO, (2007). Malaria Elimination: A field manual for low and moderate endemic countries. 20 Avenue Appia, 1211 Geneva 27, Switzerland.

WHO, (2008). Global malaria control and elimination report of a technical review. Geneva, Swizerland. 8pp.

Williams, C.R., Long, SA; Webb, CE; Bitzhenner, M; Geier, M, et al. (2007). Aedes aegypti population sampling using BG-Sentinel traps in north Queensland Australia: Statistical considerations for trap deployment and sampling strategy. *J. Med. Entomol*. **44:**345–50.

Wolde-Hawariat, Y. (2008). Electrophysiological and behavioral responses of Sorghum chafer, *Pachnoda interrupta* (Coleoptera: Scarabaeiae) to host plant volatile compounds. Ph.D. Thesis. Addis Ababa University, Addis Ababa.

Worku, M., **Tuna, H., Nigussie, M. and Deressa, A. (2001).** Maize production trends and research in Ethiopia. In: Enhancing the contribution of maize to food security in Ethiopia: *Proceedings of the second national maize workshop of Ethiopia, 12-16 November 2001,* (Nigussie, M., Tanner, D., and Twumasi-Afriyie, S., eds). Ethiopian Agricultural Research Organization (EARO) and International Maize and Wheat Improvement Center (CIMMYT). Addis Ababa, Ethiopia.

Yap, H.H. (1985). Biological control of mosquitoes, especially malaria vectors, Anopheles species. *Southeast Asian Journal of Tropical Medicine and Public Health*. **16:** 163-172.

Ye-ebiyo, Y., Pollack, R.J. and Spielman, A. (2000). Enhanced development in nature of larval Anopheles arabiensis mosquitoes feeding on maize pollen. *Am. J. Trop. Med. Hyg*. **63:**90-93.

Ye-ebiyo, Y., Pollack, R.J, Kiszweski, A.F and Spielman, A (2003). Enhancement of development of larval Anopheles arabiensis by proximity to flowering maize (*Zea mays* L.) in turbid water and when crowded. *Am. J. Trop. Med. Hyg*. **68:**748-752.

Yohannes, M., Haile, M., Ghebreyesus, T.A., Witten, K.H., Getachew, A., Byass, P. and Lindsay, S.W. (2005). Can source reduction of mosquito larval habitat reduce malaria transmission in Tigray, Ethiopia. *Trop. Med. Int. Health*, **10:** 1274-1285.

Yoseph, S. (2007). Studies on the influence of maize pollen on the body size of Anopheles mosquitoes and the effect of number of cattle people on the indoor density of *Anopheles* mosquitoes in relation to malaria transmission. MSc. Thesis. Addis Ababa University, Addis Ababa.

Zhong, D., Temu, E.A, Guda, T., Gouagna, L., Menge, D., Pai, A., Githure, J., Beier, J.C and Yan, G. (2006). Dynamics of Gene introgression in the African malaria vector *Anopheles Gambia*. *Genetics*. **172:**2359-2365.