Evaluation of various essential oils as repellents and insecticides against mosquitoes (*Diptera* : *Culicidae*)

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بسم الله الرحمن الرحيم

Allah schämt sich nicht, irgendein Gleichnis zu prägen, sei es auch nur mit einer Mücke. Diejenigen nun, die glauben, wissen, daß es die Wahrheit ist (und) von ihrem Herrn (kommt). Diejenigen aber, die ungläubig sind, sagen: "Was will denn Allah mit einem solchen Gleichnis?" Er führt damit viele irre. Aber er leitet damit (auch) viele recht. Und nur die Frevler führt er damit irre.

Heiliger Koran

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ABBREVIATIONS

A. D.	Anno domini
A2-II	Antennal sensilla trichodea type 2
ANOVA	Analysis of variances
B. C.	Before Christ
Bayrepel	(1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester)=**KBR 3023**
DEET	N,N-diethyl-m-toluamide
Df	Degree of freedom
e. g.	For example
EC_{50}	The effective concentration in 50% of exposure population. (scale for repellency)
EEE	Eastern Equine Encephalitis
F	Statistical test (f)
GC	Gas chromatography
Genapol	Oligoethylene glycol monoalkyl ether
GLC	Gas liquid chromatography
GOBP1	General Odorant-Binding Proteins 1
GOBP2	General Odorant-Binding Proteins 2
JE	Japanese Encephalitis
LC_{50}	The lethal concentration for 50% of the exposed population.
LC_{90}	The lethal concentration for 90% of the exposed population.
LCE	La Crosse encephalitis,
LSD	Least significant differences
M	Oil mixture
M. C.	Main constituent
M. W.	Molecular weight
MPG	Multiporous grooved sensillum
MPP	Multiporous pitted sensillum

MS/GC	Mass spectrometer / gas chromatograph analysis
MVE	Murray Valley encephalitis
OBPs	Odorant-binding proteins
P	Probability
PBPs	Pheromone-binding proteins
PC	Principal constituent
PEG	Polyethylene glycol
PP	The protection period
ppm	Part per million
R. T.	Retention time
RCBD	Randomized complete block design.
SEM	Scanning electron microscope
SLE	Louis Encephalitis
St	California Encephalitis
USDA	US Department of Agriculture
VEE	Venezuelan Equine Encephalitis
WEE	Western Equine Encephalitis
WHO	World Health Organization
%B	The percentage of biting mosquitoes
%L	The percentage of landing mosquitoes
%M	Percentage of mortality
%M. C.	Percentage of main constituent in the oil
%R	The percentage of repellency

1 . General introduction

There are about 100 trillion mosquitoes with at least 3,450 different species in the world (Ward,1992). They are found from the tropics to the arctic regions. All families of the mosquitoes belong to the order ***Diptera*** and are thus related to tabanids flies, gnats and midges. What makes mosquitoes different from all other diptera is the presence of a long, piercing mouthpart called proboscis, the scales on the hind margins and veins of their wings. Morphologically, mosquito males differ from females in that they have feathery antennae, long feathery palps and smaller mouthparts. A typical mosquito weights about 2.5 milligram, or about 20,000 mosquitoes per pound. Adult males do not feed on blood, they only feed on flower nectar, juice and sugar solutions, while females of most species require human and/or animal blood before oviposition, utilizing the protein in the blood to produce their eggs and to bring them to maturity. There are an estimated 10 trillion mosquitoes produced just in the U. S. each summer with about 170 species. To give an idea on the total amount of mosquitoes: there are available 41,000 mosquitoes for every human being (Tredten,2002).

1 . 1 Classification of mosquitoes

There are two groups dixid and chaoborid midges that in the past were placed together with the mosquitoes in the family ***Culicidae***, but now each group has family status (***Dixidae*** , ***Chaoboridea*** , ***Culicidae***). The family ***Culicidae*** now includes only mosquitoes (Service, 1993).

The family ***Culicidae*** is classified into three subfamilies, the largest and most diversified of which is divided into a number of tribes as (Table 1) :

Family ***Culicidae*** :

Subfamily ***Toxorhynchitinae***

Anophelinae

Culicinae

Table (1) Classification of the mosquitoes.

Subfamily *TXORHYNCHITINAE*	Subfamily *ANOPHELINAE*	Subfamily *CULICINAE*		Alternative placement
		Tribe *SABETHINI*	Tribe *CULICINI*	
Toxorhynchites (3)(mainly cosmotropical)	***Anopheles***(6) (cosmopolitan)	***Sabethes***(4)(neotropical)	***Aedeomyia***(2)(mainly cosmotropical, australasian)	*Aedeomyiini*
	Bironella(3) (australasian)	***Wyeomyia***(10) (New World, mainly neotropical)	***Aedes***(43)(cosmopolitan)	*Aedini*
	Chagasia (neotropical)	***Phoniomyia*** (neotropical)	***Armigeres***(2)(oriental, australasian)	
		Limatus(neotropical)	***Eretmapodites***(afrotropical)	
		Trichoprosopon(neotropical)	***Haemagogus***(2)(neotropical)	
		Tripteroides(5)(oriental, mainly australasian)	***Heizmannia***(2)(oriental)	
		Topomyia(2)(mainly oriental, australasian)	***Opifex***(New Zealand)	
		Maorigoeldia(New Zealand)	***Psorophora***(3)(New World, mainly neotropical)	
		Malaya(afrotropical, oriental, australasian)	***Udaya***(oriental)	
		Johnbelkinia(neotropical)	***Zeugnomyia***(oriental)	
		Runchomyia(3)(neotropical)	***Culex***(22)(cosmopolitan)	*Culicini*
		Shannoniana(neotropical)	***Deinocerites***(New World, mainly neotropical)	
			Galindomyia(Colombia)	
			Culiseta(7)(cosmopolitan, mainly temperate old World)	*Culisetini*
			Ficalbia(afrotropical, oriental, australasian)	*Ficalbiini*
			Mimomyia(3)(afrotropical, australasian, oriental)	
			Hodgesia(afrotropical, oriental, australasian)	*Hodgesiini*
			Coquillettidia(3)(cosmopolitan)	*Mansoniini*
			Mansonia(2)(mainly cosmotropical)	
			Orthopodomyia(cosmopolitan)	*Orthopodomyiini*
			Uranotaenia(2)(cosmopolitan)	*Uranotaeniini*

From (Service,1993)

1 . 2 Importance of mosquitoes as vectors

Mosquitoes seriously harm large numbers of people worldwide by transmitting pathogenic organisms that cause diseases and death. Especially in tropical areas heartworm, malaria, yellow fever, dengue and filariasis and many types of encephalitis viruses are transmitted. Malaria is a constant threat even in many parts of world, where known vectors exist. Malaria, among all insect-borne diseases, has been the most deadly in modern history. During last century alone it has killed between 100-300 million people, mostly babies and small kids, and it infects and debilitates hundreds of millions of others. Each year about 700,000,000 men are infected by diseases carried by mosquitoes. In comparison, only 21 million people died in combat in World War I, World War II and the Korean War combined. Over 60 species of ***Anopheles*** mosquitoes are known to be capable of transmitting malaria. Travelers returning from abroad can constantly introduce the causal agents of malaria, which are microscopic protozoa in the genus ***Plasmodium***. On average, one person dies every 10 seconds as a result of a little mosquito "bite". Our primary reason for controlling mosquitoes usually is only to lessen the annoyance caused by their bites and then only secondarily to reduce the transmission of agents of diseases to human and animals by several mosquito. The annoyance caused by mosquito feeding can include the itching, restlessness, loss of sleep and nervous irritation in all people, pets and domestic animals that suffer from their attacks. Mosquitoes do not really "bite", but they penetrate their victim's hide or skin with their proboscis or hollow, flexible snout. The female has a pump in her head which she uses to suck blood. The average meal takes about 1 millionth of a gallon per bite. Their saliva leads to the host itching. Usually this minor annoyance cannot be documented in terms of economic loss, but, obviously, there may be some major economic losses, for example decreased recreation income and lower milk and beef production due to blood loss and irritation. Occasionally extremely large numbers of mosquitoes can actually cause the death of domestic animals through blood loss and anaphylactic shock from reactions to mass injections of mosquito saliva.(Tredten,2002).

Malaria : at least 1 of 4 species of the ***Plasmodium*** parasite that infect humans, ***Plasmodium vivax, P. falciparum, P. malariae and P. ovale,*** were found living in the blood of nearly 300 million people. Malaria is transferred to humans only via mosquitoes and now affects 300 - 500 million new people per year and kills 1.5 to 2.7 million people per year. Many drugs such as chloroquine no longer can control malaria - the disease has developed resistance to it. Malaria is found in at least 102 countries. In 395 A.D. 330,000 acres of farmland in Rome's Compania region were abandoned due to a malaria epidemic - Rome fell 81 years later (Tredten,2002). Currently about 2,100 million people live under the threat of malaria in 103 countries, and about 445 million of these are in areas without control. Malaria parasites are transmitted only by ***Anopheles*** mosquitoes. Although there are some 422 ***Anopheles*** species, only 70 are malaria vectors and among these probably only about 40 are important. Malaria vectors are often divided into primary and secondary vectors, but this is rather unsatisfactory, because a species can be a so-called primary vector in one area and a secondary vector or even a non-vector in another. Likewise the ability of ***Anopheles*** species to transmit malaria depends much on their physiological susceptibility (Service,1993) .

Elephantiasis : This disease is caused by three nematode species belonging to the family ***Onchocercidea*** **:** ***Wuchereria bancrofti*** ; ***Brugia malayi*** and ***Brugia timori*** . The life cycles of these three species are the same: microfilariae in the host's blood are ingested by the vector, and in some mosquitoes such as some ***Anopheles*** species, which have a well-developed pharyngeal armature, many are physically destroyed during their passage to the mid-gut, others may be excreted through the anus. Surviving microfilariae commence exsheathment within a few minutes of entering the stomach and penetrate its wall to pass into the haemocoel. From here they migrate to the thoracic flight muscles, where the same larvae become more or less inactive, grow stumpier and after two days have developed into sausage-shaped forms. These moult twice and the resultant third stage larvae migrate through the head and reach the fleshy labium of the proboscis after ten days or more. If very high numbers of microfilariae are ingested their development can cause high mosquito mortality or reduce flight capability. When an infective mosquito feeds a few infective parasite larvae rupture the labella and are deposited in a drop of haemolymph onto the skin, many die, but a few enter the skin through the mosquito's bite or abrasions. This method appears inefficient to transmit the nematode. Southgate (1984) estimated that we need a large number of infective bites to produce a patent microfilaraemia. In the human body the parasite passes to the lymphatic system. After 8-12 months adult female release thousands of microfilariae which migrate to the blood and become able to produce microfilariae over the next 15-18 years. At the end, they obstruct the lymph system and this results in the legs and scrotums swelling to grotesque proportions, a disease known as tropical elephantiasis. These species are transmitted by several mosquito species such as by the malaria vector ***Anopheles barbirostris*** or by ***Culex quinquefasciatus***. Furthermore various ***Aedes*** species also are vectors. There are about 751 million people at risk of lymphatic filariasis in 76 countries, and some 79 million people are actually infected (Service,1993).

Dog heartworm : This is a filarial parasitic disease caused by the nematode ***Dirofilaria immitis*** belonging to the family ***Onchocercidea*** . It occurs mainly in the tropics and subtropics, but also extends into other areas. In addition to dogs the worm infects other rarely cats and very occasionally man. This nematode is different from ***Wuchereria*** and ***Brugia***, since it undergoes development in the mosquito's Malpighian tubules. It is transmitted by a number of different mosquitoes, at least 72 species of ***Anopheles***, ***Culex***, ***Aedes*** and ***Mansonia*** species are susceptible to this parasite. The nematodes, which lodge and grow in the heart of the vertebrate hosts, can be fatal if left untreated.

Yellow fever : Yellow fever is one of important mosquito-borne disease, an outbreak in Ethiopia in 1960 – 1962 resulted in an estimated 115,000 deaths. Also in 1986 – 1987 serious outbreaks occurred in West African cities (Clements,1992). Originally it is a zoonotic disease for forest monkeys, but it can easily spread to man via ***Aedes*** mosquitoes especially by ***Aedes aegypti***. Yellow fever is caused by viruses found in the mosquito salivary gland after they took a blood meal from infected man or animal 12-15 days before.

Dengue : The dengue fever and the dengue haemorrhagic fever, are caused by dengue viruses. The vectors for man are species of ***Aedes aegypti*** , ***A. albopictus*** , ***A. scutellaris*** and ***A. polynesiensis* ,** which breed efficiently in urban environments. Dengue was once largely restricted to India, South-east Asia and the Southern Pacific, but its range increased dramatically with tropical urbanization, and it is now present in Africa and the Americas. Since its first description from the Philippines in 1953, dengue haemorrhagic fever has become one of the leading causes of childhood illness and mortality in Southeast Asia too. And it has been reported increasingly in the Americans over the past decade (Clements,1992).

Encephalitis : Many types of mosquito-transmitted encephalitis occur in the world. These are Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), California encephalitis, St. Louis encephalitis (SLE), Venezuelan equine encephalitis(VEE), La Crosse encephalitis, Japanese encephalitis (JE), and Murray Valley encephalitis (MVE). Each type is caused by a different virus or virus complex affecting the central nervous system. Symptoms of EEE in horses include fever, impaired vision, irregular gait, reduced reflexes, inability to swallow, convulsion and death. These viruses are normally transmitted by mosquitoes from birds or small mammals. Occasionally horses or humans are infected. Despite the small number of people infected annually by eastern equine encephalitis, it is considered a serious disease because it is often fatal. Vaccinating horses properly will prevent them from contracting Eastern equine encephalitis (Service,1993 ; Tredten,2002).

The given examples were some important mosquito-borne diseases, but in fact there are many more parasites transmitted by mosquitoes for example about 200 recognized arboviruses are known of being mosquito-borne and about 100 of them infect humans (Service,1993). And many kind of bacteria, fungi and worms are also mosquito-transmitted.

1. 3 Regulation of the behavioral aspects in mosquitoes

The road is still long to reach a complete understanding of host-mosquito relationship, specially that associated with preference between hosts, attraction to special host than others and the sensory nervous management mechanism in this behavior. However we should not omit the last efforts, that provide the minimum of knowledge in this important field.

For review these works we will approach the following :

1. 3. 1 The nervous system of mosquitoes

The nervous system of insects is divided into three systems connected together, these systems have integrative work to serve the detection of external and internal effectors (sensation), analysis these effectors and produce the suitable reactions for them (response). These systems are:

- Central nervous system consisting of brain, subesophageal ganglion and dorsal nerve cord, being employed to analyze of nerve signals imported from other body organs.

- Sympathetic nervous system include groups of nerve ganglions spread in the body organs.

- Peripheral nervous system composed of a net of nerve fibers dispersed on the body surface under the cuticle layer in the whole of the body regions such as antennas, mouthparts, legs, back and others. This system recognizes senses of the external effectors such as mechanical effectors, temperature, humidity and miscellaneous odors, thereafter translate them to nerve signals pass through nerve connectors to central nervous system .

Davis (1984) suggested that the peripheral sensory organs may play two roles in the mediation of mosquito behavior. First, they detect the presence and intensity of airborne chemical signals that permit a female mosquito to find a host. Second, the alterations in receptor sensitivity brought about by physiological changes associated with certain events, e.g. neurosecretory activity associated with blood feeding, may act to switch the female into or out of a host-seeking behavioral mode.

1. 3. 2 Host seeking behavior

The study of insect behavior is one of the difficult studies, more complex and shows large differences between researchers. So the behavioral studies were less numerous in the insect studies field. Since the medical and veterinary important mosquitoes are carrier of many dangerous pathogens, they attracted more insect behavioral studies, especially those associated with host seeking behavior, feeding and egg laying. Furthermore, the transmission of disease by mosquitoes depends on the ability of the female to find a potential host organism and to take a blood meal from the host. This behavior is not expressed immediately following adult emergence. Blood-feeding has been reported to be initiated between 24 and 72 hours after a female mosquito had emerged (Seaton and lumsden 1941; Bishop and Gilchrist 1946; laarman1955.)

A similar period of maturation appears to be required before the peripheral sensory organs are fully responsive. In electrophysiological studies of the chemosensory neurons associated with host-seeking behavior, the female required at least 3 to 4 days post-emergence until we could obtain measures of receptor specificity and sensitivity (Davis 1984). He also suggested that the peripheral sensory organs may play two roles in the mediation of mosquito behavior: First, they detect the presence and intensity of airborne chemical signals that permit a female mosquito to find a host. Second, the alterations in receptor sensitivity brought about by physiological changes associated with certain events such as neurosecretory activity associated with blood feeding may act switch the female into or out of a host-seeking behavioral mode. In addition he describe of the temporal relationship between the development of sensitivity in the receptor for the host-attractant lactic acid, and the onset of host-seeking behavior. This gives more support to the hypothesis that the peripheral sensory organs have a primary role in the selection of host-seeking behavior. Moreover, in the preliminary study of the depression of lactic acid-receptor sensitivity accompanying the inhibition of host-seeking behavior 48 hours after a blood meal (Davis and Takahashi 1980).

In the studies conducted at California State University on 525 virgin female ***Ae. aegypti*** mosquitoes whose ages ranged from 12 to 360 hours. Davis (1984) reported, that host-seeking behavior was not observed before 18 to 24 hours post emergence, but at 30 hours, about 10% of the females tested began to exhibit host-seeking behavior. This reached the 50% response level by about 66 hours: After 102 hours post emergence, 90% of the female in each trial would actively seek a host. Those females between 30 and 102 hours post emergence were in a transitional condition during which their host-seeking behavior was clearly age-dependent. The host-seeking behavior of virgin females of ages greater than 108 hours post emergence showed a consistent response rate of 94% for as long as 15 days post emergence. These females were considered to be mature adults and formed the basis for comparisons with the younger age groups. Finally, he assured, that the development of activity in the chemosensory afferent neurons associated with the antennal grooved-peg sensilla and development of host-seeking behavior in newly emerged virgin adult female ***Ae. aegypti*** mosquitoes is age-dependent. Furthermore, the presence of high lactic acid-sensitivity and specificity showed a 1:1 correlation with the presence of host-seeking behavior.

In the previous study Davis and Takahashi (1980) reported that the sensitivity of lactic acid-excited afferent neurons to lactic acid became depressed in female mosquitoes about 30 to 48 hours after they had obtained a blood meal and that this change in receptor sensitivity was coincident with the depression of host-seeking behavior reported by Klowden and Lea (1979). As well as they noticed, that such changes in the sensitivity of receptors that detect host-attractant stimuli could account for the presence or absence of host-seeking behavior. This final notice means that, the final decision of the female to become a host-seeker depends on many factors and is regulated by the central nervous system.

1. 3. 3 The role of olfaction in host-seeking behavior

Many studies attempted to explain the role of odor that is released from the host in mosquitoes host-seeking behavior, but the diversity of host-odors and the numerous organic compounds that are associated with hosts sweat or sebum, make the result of most studies were unclear. Accordingly it is apparent, that lactic acid is the only compound whose role as a kairomone for blood-seeking mosquitoes has been confirmed (Davies et al 1987), although several other skin products such as amino acids as well as steroids have also been shown to be attractive. Bowen (1991) said, that a meaningful analysis of odor-mediated host-seeking behavior requires knowledge of the specific chemical components of host-seeking and the amount released by the odor source per unit time, (b) the configuration of the stimulus in time and space, and (c) the contribution of each host odor to the individual responses that make up the composite behavior of host-seeking.

Based on a combination of detailed ultrastructural information and careful counts of sensillar types on the antennae of ***Ae. aegypti*** and ***An. stephensi***, Mclver (1982) calculated, that 93% and 85%respectively of neurons in the flagellar nerves of these species carry information on odors. This speaks to the importance of odor information to the mosquito and its speaks to the great variety of odor information available in the mosquito's environment. But the role of olfaction is less well understood. Although there is no doubt that blood feeding arthropods make use of airborne chemicals for orientation and host finding, remarkably little is known about the identity of the volatiles (kairomones) that cause this behavior (Sutcliffe 1987). Furthermore, it is generally assumed that host-seeking behavior is elicited by odor complexes rather than by a single compound (Takken 1991). Carbon dioxide is an important element in these complexes, since almost all haematophagous insect species respond to it (Clements 1963; Gillies 1980; Nicholas and Sillans 1989).

In mosquitoes only the females take blood meals which are required for egg production. Blood is taken from a wide variety of vertebrate hosts. In most species a definite host preference is present, that is determined genetically. For instance many ***Culex spp***. are ornithophilic, whereas most ***Anopheles spp***. feed on higher mammals such as bovids, suidae and man. Indeed some species feed on one kind of host only. Since host-seeking is activated by an olfactory stimulus, the odor complex must therefore contain host specific chemicals to which only certain mosquito species respond. Host-finding on mosquitoes usually involves a flight from a distance to the host. It can be described by a series of behavioral steps, which begin when a receptive insect is activated by a chemical produced by the host and which end when the insect alights on the host. In the vicinity of the host, mosquitoes respond to non-olfactory cues such as convection heat and body moisture (Parker 1952; Laarman 1958; Daykin et al. 1965; Takken 1991). This short-distance attraction is influenced by CO_2, which was found to significantly increase the response of ***Ae.aegypti*** and ***An. atroparvus*** to body heat and moisture when offered as an additional stimulus (Khan et al 1966; Laarman 1955). Body heat and moisture can only be observed at short distances from the host and although it should not be ruled out that olfactory cues are involved at short distance as well, kairomones are thought to primarily play a role in long distance host-orientation and attraction of mosquitoes.

Long-distance attraction is determined by a combination of visual and chemical stimuli, which the mosquitoes uses to orient itself in its host-finding drive(Takken 1991).

Laarman (1955) stated that, mosquitoes are attracted to air-borne stimuli produced by a host and that smell is a strongly determinative factor in locating the blood supplier. Thompson and Brown (1955) showed that, mosquitoes were attracted to human sweat and (Roessler 1961) mentions several volatile substances emanated by human skin as mosquito attractants. Clements (1963) reviewed the subject and concluded that mosquitoes are attracted from a distance to a number of chemicals produced by natural hosts. At that time, however, there were many conflicting results and much of the evidence was inconclusive. It was however clear that, host-odors play an important role in host-orientation of blood seeking mosquitoes.

Carbon dioxide : was first reported as a mosquito attractant by (Rudolfs 1922).

Reeves (1951) demonstrated its attractancy for female mosquitoes in field studies. Since then many studies about the responses of mosquitoes to CO_2 have been published, which were reviewed by (Clements 1963), who concluded that under laboratory conditions carbon dioxide clearly causes activation, orientation and alighting of mosquitoes, but that it remained to be determined whether these effects would also appear in the field. In a series of field experiments in the Gambia the role of CO_2 as field attractant was confirmed (Gillies 1974). Removal of up to 95% of CO_2 from expired human air resulted in a significant reduction of mosquitoes attracted, but did not lead to a reduction in mosquitoes attempting to feed once in close proximity to the host (Snow 1970). According to (Gillies 1980) the effect of CO_2 on mosquito behavior can be described in two ways : (1) it activates and induces upwind flight and (2) it acts as true kairomone. Although there is sufficient evidence to support the activating role of CO_2 (Laarman 1958; Clements 1963), direct evidence for its role as a kairomone has not been published todate. In the absence of other host factors, sustained flight takes place only in response to intermittent pulses of CO_2 (Omer 1979; Gillies 1980). This in fact what happens in outdoor air stream, where the natural turbulence of the air causes any volatile substance to appear as short pulses rather than a continuous flow. As a kairomone, CO_2 acts as a synergist. For instance the attractant effect of lactic acid is only apparent in the presence of CO_2 (Acree et al. 1968; Price et al. 1979). In spite of the crucial role of CO_2 in mosquito host-orientation, the physiology of CO2 receptivity and the subsequent behavioral response is not known (Takken 1991). Carbon dioxide is commonly used to attract mosquitoes to traps used for mosquito surveillance and in field experiments. It is being released as dry ice or from gas cylinders. In the last case, the release rate can be controlled to simulate the CO_2 concentration expired by natural hosts. At rate of 500 -700 ml/min it was found to attract mosquitoes over a distsnce of 15 m (Gillies and Wilkes 1972). This rate is roughly the equivalent output of CO_2 from two calves.

Vickery et al. (1966) report a synergistic response of CO_2 when released in combination with a chicken as bait. Stryker and Young (1970) did not find a synergistic response caused by the release of CO_2 and lactic acid together, except for ***Ae. vexans,*** which was attracted in greater numbers than with the combined catches of either bait. Takken and Kline (1989) demonstrated the synergistic role of CO_2 released at a rate of 200 ml/min in combination with 1-octen-3-ol.

The concentration of CO_2 in atmospheric air 0.03 – 0.04% and in human breath 4.5%, Excretion from the total human skin surface is about 0.3 – 1.5% of that expired from the lungs. Local atmospheric levels can very considerably, depending on time of day and density of vegetation, so the CO_2 differential between atmospheric levels and biologically relevant objectives is considerable. Mosquitoes are electrophysiologically sensitive to changes in CO_2 levels as low as 0.01%. unnaturally high CO_2 levels can have anomalous effects on behavior and physiology, because CO_2 induces and maintains flight, mosquitoes may be reluctant to terminate flight and land under such conditions, particularly in the absence of other odors (Bowen 1991).

Skin emanations : Volatile substances produced by mammalian skin have been incriminated as mosquito attractants. The best known odorous products are those present in human sweat. Skin emanations are produced (1) by eccrine subdoriferous glands which are distributed over the entire body surface, but most abundant on the palms of the hands, the soles of the feet and the forehead, (2) by apocrine subdoriferous glands, most numerous in armpit regions, inguinal areas and around body apertures, and (3) by sebaceous glands, most abundant about the scalp and face and none on the palms of the hands and soles of the feet (Takken 1991). In other mammals sebaceous glands are more equally distributed over the entire body surface (Sokolov 1982). Sweat is a watery solution of sodium chloride from the sudoriferous glands, containing traces of the non-colloidal constituents of blood plasma. Sebum is an oily material containing lower fatty acids, higher primary alcohol esters and cholesterol, in addition to albumins and inorganic salts (Thompson and Brown 1955). Sweat usually is a mixture of sudor and sebum.

(Parker 1948) was the first to describe the attractive effect of sweat for ***Ae. aegypti***. (Brown et al. 1951) found attraction for sweat at low concentrations, but repellency at high concentrations. Furthermore, human sweat was attractant to mosquitoes (Roessler 1961). As well as Skinner et al. 1965; Maibach et al. (1966) said that, sweat was found to contain attractive substances for ***Ae. aegypti***. Which could be extracted with ether and ethyl alcohol. Mayer and James (1969) found attractive substances present on the human arm which could be rinsed off with water and acetone. Moreover attraction effect to armpit sweat was found, but no attraction or slight repellency to sweat collected from a human trunk and leg (Muller 1968), also he mentioned the attractiveness of several organic acids including lactic acid. Whereas the attractancy of lactic acid has been confirmed by several authors (Smith et al. 1970; Carlson et al. 1973; Bar-Zeev et al. 1977). The ability or inability of lactic acid to elicit host-seeking behavior must be evaluated in light of two considerations (1) the probability that a multi-component chemical stimulus is required possibly involving as yet unidentified host odors as well as CO_2. and (2) the effect of the physiological state of the female mosquito on odor reception (Bowen 1991). In the study on the some material isolated from human hand, Schreck et al. (1981) noticed that, there is material attractive to ***Ae. aegypti*** and ***An. quadrimaculatus*** in addition to or other than lactic acid, the compound responsible for the attraction has not been chemically identified. Finally, (Price et al 1979) investigated the role of humidity, CO_2 and temperature in conjunction with human skin emanations in attractiveness for female ***An. quadrimaculatus*** . it was concluded that mosquitoes were primarily attracted by chemical emanations other than CO_2 and water.

Exhaled air : Air leaving the lungs contains numerous host-seeking chemicals to which mosquitoes may be attracted or repelled. The combined human expired air comprises at least 102 various organic compounds of endogenous and exogenous origin. The mean concentrations of 97% of these compounds fall within trace level range of 0.06 to 9.5

nanogram per liter. Acetone, isoprene and acetonitrile, account for 51% of the mean organic contents (Krotoszynski et al 1977). These data were obtained from a group of 28 carefully selected healthy individuals who were required to follow specific dietary and behavioral rules before being admitted to the test and are thought to represent valuable baseline information for studies that depend on the use of human expired air (Takken 1991). It should be realized that considerable variation in the chemical composition of human expired air may exist depending on sex, age, status of health and type of diet. Apart from the organic compounds, expired air contains several gases. Carbon dioxide, playing an important role in mosquito host-orientation is present at 4.5% and expired air is the principal source of this gas in mammals, the skin producing only very small quantities (Gillies 1980).

In the higher vertebrates, most gaseous exchanges between the body and the environment occur in the lungs. During this process, volatile chemicals in the blood other than CO_2 may be given off to the air being expired, for this reason the attractiveness of blood for haematophagous insects has been studied (Takken 1991).

Schaerffenberg and Kupka (1951) reported the attractiveness of blood for ***Stomoxys*** and ***Culex*** . Laarman (1955) demonstrated the attraction of ***An. maculipennis atroparvus*** to human breath as well as to the air led over a rabbit. He also reported the attractiveness of fresh rabbit blood for mosquitoes and suggested that odorous substances from blood play a role in mosquito host-orientation via expired air. Roessler (1961) reported the attractive effect of bovine and serum for ***Ae. aegypti*** . In the same study the attractive effect of phenols and steroids, which might be present in exhaled air, is described. Roessler and Brown (1964) give a possible explanation for the difference in attractiveness of men and women to mosquitoes, which they thought was based on different quantities of hormones in the expired air. Muller (1968) found an attractive response in ***Ae. aegypti*** for pig blood. With exception of the above mentioned study by Laarman (1955), no data on tests with expired air only for the purpose of studying mosquito host-seeking behavior have been published to-date. In contrast exhaled air from bovids was studied by workers investigating the behavior of tsetse flies in laboratory and field studies. This led to the discovery of acetone and 1-octen-3-ol as kairomones (Vale and Hall 1985). Furthermore, the attractive role of 1-octen-3-ol for mosquitoes and biting midges has been described (Takken and Kline 1989). It was found that the effect of octenol could be greatly enhanced by CO_2 which acted as a synergist. 1-octen-3-ol is a chemical present in numerous leguminous plants and has been identified in cow breath, probably being released from food plants during rumination (Hall et al. 1984). It may therefore not be surprising that octenol is also attractive for several species of mosquitoes that feed on animals such as cattle and deer. Other kairomones are likely to be present in exhaled air.

Urine : urine contains one or more compounds to which mosquitoes are attracted (Muller 1968). In the other study Roessler (1961) showed that, mosquitoes responded positively in a windtunnel where air led over urine fractions containing diphenols and steroids was being released.

1. 3. 4 Mosquitoes food preference in natural hosts

Food preference is a very clear phenomenon in mosquitoes species and varieties. Some species feed only on one kind or one group of hosts , therefore, others species prefer to feed on specific hosts but under certain conditions they may feed also on any host without distinction. There are many studies conducted in many mosquito species. Van Thiel (1939) studied the degree of zoophily and anthropophily in individuals of the species ***An. maculipennis*** in Europe, by allowing mosquitoes to choose between the odors of man and pig, he found that, ***An. atroparvus*** was zoophilic and ***An. maculipennis*** var. ***labranchiae*** and ***elutus*** were anthropophilic, although all species would under experimental conditions readily feed on either host. Mer et al. (1947) confirmed the attraction of ***An. elutus*** to human expired air. In the other study Brouwer (1960) demonstrated that the mosquitoes can differentiate between individual humans. Also he showed that ***An.stephensi*** responded in different degrees of attractiveness to arm emanations from four different persons.

Mosquitoes of the ***An. gambiae*** complex show different host preferences, ***An. gambiae s.s.*** being highly anthropophilic and ***An. quadriannulatus*** being zoophilic (Gillies and Coetzee 1987). Moreover, many ***Culex spp.*** are ornithophilic, but some species show a seasonal shift in feeding preference , changing from bird feeding to mammal feeding. Edman (1979) studied the effect of wind-direction and distance of chickens, rabbits and their CO_2 equivalent on mosquito attraction in Florida and found that ***Culex nigripalpus*** showed no preference for chickens or rabbits, while ***Cx. pilosus*** hardly responded to either bait, probably because this mosquito feeds mainly on poikilothermic vertebrates. In other research (Snow 1983) compared the attractive effect of traps baited with chickens, pigeons, ducks, goats, man or calf. ***An. melas*** and ***Cx. Thalassus*** were common at all baits, but ***An. melas*** predominated in the catches from mammals. ***Aedes spp***. and ***Cx. tritaeniorhynchus*** were significantly represented in mammal-baited traps and poorly at the avian baits. In contrast, ***Cx. invidiosus* , *Cx. neavei* , *Cx. tigripes*** and ***Cx. weschei*** made up a major part of the catches only in the bird-baited traps. ***An. gambiae s.s.*** was taken in significantly larger numbers with man compared with the other baits. ***Aedes spp.*** usually feed on mammals. Downe (1960) reported that, birds appeared to be relatively uncommon hosts in nine species of ***Aedes*** in Canada. In another study Mclver (1968), ***Ae. aegypti*** preferred mice to chicks, while ***Cx. tarsalis*** preferred chicks to mice when their weight was two times that of the mice . ***Cx. tarsalis*** also was attracted to frogs, toads and lizards, but preferred chicks and mice. ***Ae. aegypti*** was not attracted to cold-blooded animals.

Finally, it can be said that blood-seeking mosquitoes use air-borne olfactory cues produced by the host to orientate themselves to the host. Carbon dioxide and organic-chemical emanations from the skin, expired air and urine are attractive to mosquitoes, although little is known about host-specific kairomones other than lactic acid and CO_2. more studies on host-seeking behavior, in particular on flight behavior after odor recognition and on the role of odor mixtures during the host-oriented flight, are required to understand the role of each olfactory cue engaged in the host-seeking process (Takken 1991).

1. 3. 5 The role of olfactory in the oviposition process

Oviposition process is the final result of series complex behavioral processes, beginning from feeding behavior, meeting behavior, host-seeking behavior and finally oviposition behavior. The result of these series processes is reproductive to survival of the species. Therefore, reproductive success in mosquitoes is dependent in part on the ability of the gravid female to locate and select an appropriate oviposition site. Its choice is determined largely by volatile chemical cues that emanate from potential sites. These oviposition-site related substances are detected by chemosensory neurons located on the antennae of the female (Kuthiala et al. 1992).

Perry and Fay (1967) reported that, gravid ***Ae. aegypti*** were attracted to water that contained methyl and ethyl esters of short-chain fatty acids such as ethyl propionate and methyl butyrate. Klowden and Blackmer (1987) demonstrated that ethyl propionate would stimulate preoviposition behavior (attraction to an oviposition site) by gravid female in an olfactometer. Davis (1976) described the electrophysiological responses to these fatty acid esters by chemosensory neurons associated with the antennal sensilla trichodea type 2 (A2-II). While Mclver (1978) subsequently redescribed the sensilla as comprising both a short sharp-tipped and a short blunt-tipped sensilla trichodea in ***Ae. aegypti*** , it is unclear which of these two types of A2-II sensilla contains the receptors that respond to the substances. Although several studies have tried to identify substances of larval, egg, bacterial, fungal, and plant origin from the oviposition sites of various mosquito species that attract gravid females to ovitraps, and a few studies have examined the repellent-deterrent activity of natural products and insecticides (Bentley and Day 1989), it is surprising that there are ont much studies on the effects of insect repellents such as deet (N,N-diethyl-m-toluamide) and Bayrepel (1-piperidinecarboxylic acid,2-(2-hydroxyethyl)-1-methylpropylester, CAS No.119515-38-7) on oviposition behavior have been reported. These substances affect host-seeking and biting behavior of avid female mosquitoes and some exert their effects on behavior by rendering the female anosmic to a host and thus unable to detect volatile host attractants (Davis 1985; Davis et al. 1987). In another study on effect of the repellent deet on the antennal chemoreceptors for oviposition in ***A. aegypti*** Kuthiala et al. (1992) demonstrated that, the mosquito repellent Deet greatly reduced the behavior oviposition response when presented together with ethyl propionate. Also, the electrophysiological recordings from the short, sharp-tipped sensilla trichodea on antennae of ***A. aegypti*** showed that the response of the receptors to ethyl propionate was reduced during the simultaneous presence of Deet and ethyl propionate. The depression of ethyl propionate by Deet was similar to the depression of sensitivity of the lactic acid-excited cell to the host attractant lactic acid in the presence of deet. This similarity of effect on two different types of olfactory receptors suggests a mode of action for deet that is not related to oder recognition specificity of the receptors, but rather to some more general aspect of neuronal excitability. The result of Kuthiala et al. (1992) provided further support for the hypothesized mechanism that repellents may interact with and inhibit the response of a sensory neuron to a normally attractive chemical signal. In addition, for the female to respond behaviorally to these oviposition site stimuli, it must be gravid (Klowden 1989). During the time it is gravid, host-seeking behavior is absent (Klowden and Lea 1979) and lactic acid sensitivity is suppressed.

1. 4 Odorant-binding proteins (OBPs) and olfactory function

OBPs were first discovered in moths (Vogt and Riddiford 1981), but they now appear to be a consistent feature of insect chemosensory sensilla and are found in other orders, such as ***Diptera*** (Mckenna et al. 1994; Pikielny et al. 1994), ***Phasmidoptera*** (Tuccini et al. 1996) and ***Heteroptera*** (Dickens et al. 1995). Similar proteins are also present in taste sensilla (Ozaki et al. 1995). OBPs are soluble proteins of low molecular weight (about 17kDa), they have an acidic isoelectric point (pH = 4.7) and they can be divided into three subclasses according to their amino acid sequence : the pheromone-binding proteins (PBPs), which are particularly abundant in male moths ; and two classes of general odorant-binding proteins (GOBP1 and GOBP2), which are found in equal amounts in both sexes (Pelosi and Maida 1995). Immunolabelling studies have shown that OBPs are biosynthesized in the sensillar auxiliary cells and secreted into the sensillum lymph (Steinbrecht et al. 1992). Biosynthesis continues through adult life and is balanced by simultaneous endocytosis and breakdown (Vogt et al. 1989; Steinbrecht et al. 1992). The functional significance of OBPs was unclear for many years, but it now appears that they may serve as transporters and deactivators of the stimulus molecules (Ziegelberger 1995). In addition, they may play a role in stimulus recognition.

The number of fully sequenced OBPs is increasing rapidly. In particular, PBPs are very diverse and in moths some have as little as 29% amino acid identity (Pelosi and Maida 1995). It has already been noted by Vogt et al. (1991) that those species which use the most different pheromones also have the most different PBPs. comparative immunolabelling studies of Steinbrecht (1996) confirmed this notion. On the other side, PBP and GOBP2 have not been found to be co-localized in the same sensillum. However, the distribution of PBP and GOBPs in moths is not correlated strictly with the morphological types of sensilla trichodea and sensilla basiconica, respectively (Steinbrecht 1996).

It is needed to mention that the presence of OBPs in the sensillum lymph has solved at least one problem, such as, the question of how can a highly polar molecule, such as pheromone compound, pass through an aqueous fluid to reach the dendritic membrane (Ziegelberger 1996), summarized some proposals for functions of PBPs :

Pheromone-binding protein as a carrier : the best studied OBP is the pheromone-binding protein (PBP) of ***Antheraea polyphemus*** because of its high concentration in male antennae (Vogt and Riddiford 1981; Klein 1987) and thus, its availability for functional studies. In perfusion experiments of the aqueous sensillum lymph space, the PBP strikingly increased the electrophysiological response of the receptor cell when introduced together with the lipophilic pheromone (Van den Berg and Ziegelberger 1991). Thus, the PBP is able to act as solubilizer and carrier. The diffusion velocity of the pheromone is decreased when bound by the PBP, but it is compatible with diffusion coefficient found for longitudinal pheromone transport in the olfactory hair (Kanaujia and Kaissling 1985).

PBP made complex with pheromone to activation the receptor cell : Almost all incoming pheromone molecules will be bound by the PBP because of its millimolar concentration 10mM (Vogt and Riddiford,1981) and its nanomolar dissociation constant 60nM (Kaissling et al. 1985), 640nM (Du and Prestwich 1995), so it seems likely that the pheromone-PBP complex activates the presumed receptor molecules. Therefore, the PBP might possess two binding sites: one for the odorant and the other for the receptor or another component of the receptor cell membrane.

PBP as a deactivator : a rapid pheromone deactivation has been postulated because the excitatory action of the pheromone occurs within a short, limited time period. The degrading enzyme found in the sensillum lymph could, in principle, be responsible for a rapid stimulus termination. (Vogt et al. 1985) calculated a half-life of about 15ms for the isolated esterase and the free pheromone. In vivo, however, most of the pheromone is bound by the PBP (Kaissling 1986) and is probably protected from the enzyme (Vogt and Riddiford,1986). (Kasang et al. 1988)estimated the half-live of 3H-labelled pheromones in intact antennae of ***A. polyphemus*** as being about 3 min. this observation and the large variability of the esterase activity in individual males (including those with apparently no enzyme activity but with a normal electrophysiological response) suggest that the esterase is not responsible for the rapid deactivation of the pheromone. However, the esterase may be responsible for the final sequestration of the pheromone (Maida et al. 1995). The above results, together with the finding that the pheromone is still present in the sensillum lymph after the electrophysiological response has terminated (Kanaujia and Kaissling 1985), also (Kaissling 1986) led to suggest the idea that the PBP deactivates the pheromone. Indeed, on the presence of two redox states of the PBP support the hypothesis that PBP in addition to its carrier function, also plays a role in stimulus termination (Ziegelberger 1995) .

OBPs and odour discrimination : Binding proteins from different subclasses show a complex and distinct distribution pattern among different sensillum types and are not co-localized within the same sensillum. Immunocytochemical studies on ***A. polyphemus*** show that PBP is found exclusively in the sensilla trichodea innervated by pheromone

receptor cells, whereas GOBP is present in the sensilla basiconica tuned to general odours (Steinbrecht et al. 1992; laue et al. 1994; Steinbrecht 1996). Therefore, it is possible that the soluble OBPs are already involved in the first step of stimulus recognition before the highly specific receptor cell is activated. This notion is supported by the observation that the number of OBPs found in a given is species is increasing steadily. Five different OBPs have been sequenced in ***Drosophila*** (Pikielny et al. 1994; Mckenna et al. 1994) and similar results are obtained in vertebrates, where
up to eight OBPs have been identified in the old world porcupine ***Hystrix criststa*** (Felicioli, et al. 1993).

However, at least in the case of ***A. polyphemus,*** OBPs are not associated with specific ligands. In this species, the GOBP of males and females binds, in a concentration-dependent manner, more labeled pheromone than does PBP. Homogenates of male antennal branches and female antennae were incubated with the pheromone component 3H-(E,Z)-6,11-hexadecadienyl acetate and analyzed by native polyacrylamide gel electrophoresis. The labeled pheromone is bound in male homogenates by two PBPs and one GOBP, and in female by only a single GOBP. The binding sites of PBP and GOBP for the same pheromone are probably different because the two proteins share an amino acid sequence identity of only about 30%. Therefore, the classification of OBPs into PBPs and GOBPs is not substantiated by specific odorant binding, although the localization of a given OBP, is correlated with the receptor cell specificity. It is worth mentioning that PBP is present in female homogenates because female silkmoths do not respond to their own pheromone in electrophysiological or behavioral studies. The natural stimuli of the PBP-containing sensilla of females have not been identified (Ziegelberger 1996). In the other side, the OBPs play some additional roles when it in high concentrations :

1- OBPs as buffers of toxic compounds : in addition to sex pheromones and general plant odours, toxic compounds also enter the sensilla through the pore tubules. If toxic compounds reach the dendrites,they might interfere with the pheromone response. It is conceivable that OBPs may also serve as scavengers, buffering unexpected compounds and maintaining a functioning sensillum.

2- OBPs as polyanions : the ionic composition of the sensillum lymph has been analysed by flame photometry and X-ray microanalysis, and has revealed a high potassium, low sodium and low calcium content (Kaissling and Thorson 1980; Steinbrecht and Zierold 1987) the total positive charges of the cation electrolytes are not balanced by the total negative charges of chlorine. Histochemical studies on olfactory sensilla of the plow fly, (Gnatzy and Weber 1978) and estimations of the isoelectric points of OBPs (pI for PBP = 4.5) Klein (1987) suggest that the balance is most probably achieved by organic polyanions such as acid mucopolysaccharides and acidic OBPs.

3- OBPs keep pheromones in solution : the lipophilic nature of most odorants suggests that free odorant molecules are inserted in the receptor cell membrane. The binding of the incoming pheromone molecule by the OBP (at millimolar concentrations) might keep the pheromone in solution. It is not known whether a pheromone that is inserted in the lipid membrane of the receptor cell will activate the cell and how it could be deactivated.

4- OBPs protect pheromone from degrading enzymes : the binding of the pheromone by the PBP might prevent the pheromone from enzymatic degradation. Pheromone perception is very sensitive; one pheromone molecule is able to elicit a nerve impulse. Therefore, molecules should not be degraded before they activate the receptor cell. Whether the addition of PBP slows down the degradation rate as suggested by (Vogt and Riddiford 1986)

1. 5 The use of plant materials for pest control

Plants, insects, and other organisms co-exist since more than three hundred million years. During this time, plants have been under a continuous selection pressure from predators and numerous environmental factors. Due to their lack of mobility, plants must rely on both physical and chemical defense mechanisms such as producing various toxic metabolites to survive the predatory attacks of other organisms such as insects, bacteria, and fungi, and to adequately compete with other plant species for light and nutritional resources. The defense chemicals or secondary metabolites of plants can serve several types of functions. They can be insecticidal (Schoonhoven,1993 ; Tsao and Coats,1995), or antimicrobial to bacteria, fungi and viruses (Oh et al. 1967 ; Hubbell et al.,1983 ; Kemp and Burden,1986 ; Harbone,1988). Some are also herbicidal (Whittaker and Feeny,1971 ; Tsao and Eto,1996), and some possess other types of biological activities (Leopold et al.,1976 ; Sutherst et al.,1982). These beneficial, bioactive chemical substances are found in abundance in plant species. Of the 5–10% of the higher plants which have been phytochemically analyzed, more than 30,000 secondary metabolites have been reported (Wink,1993). Due to the public concern over the toxicity and environmental impact of conventional synthetic pesticides, exploitation and utilization of naturally occurring products in order to combat harmful agricultural and public health pests have been increasingly the focus of researchers, environmentalists and industry. Because natural pesticides, or pesticides derived from natural products, support both crop production and the environment by being effective in pest control, less toxic to non-target organisms and biodegradable at the same time, they may be safer than synthetic pesticides. Repeated use of a single synthetic pesticidal ingredient can result in resistance amongst the target populations, whereas, natural products in plant defense mechanisms often consist of a variety of toxins which make adaptation of the predator unfavorable (Wink,1993).

The use of plant material to control domestic and agricultural pests was widespread in ancient cultures (Secoy and Smith,1983). In China as early as 25 – 220 A.D. Shengnong Ben Cao Jing [Classical Pharmacopoeia of the Heavenly Husbandman] mentioned the anthelmintic effect of ***Melia azedarach*** a tree closely related to the neem tree ***Azadirachta indica*** . In the late Wei Dynasty (533 – 544 A.D.) Chi Ming Yao Shu [Important Arts for the People's Welfare] reported that, a boiling water extract of the roots of ***Veratrum spp.*** cured sheep scab. This and other scattered information in ancient literature suggest that the use of folk herbal pesticides including washing hair with a boiling-water extract of the roots of ***Stemona*** spp. to control lice, and repelling fleas and mosquitoes with smokes by burning the dried leaves of ***Artemisia*** spp. or peach branches was common knowledge (Yang and Tang,1988).

Jacobson (1982) has classified the kinds of effect of plant materials on insects in 6 groups, including:

1- those attractive to insects

2-those repellent to insects

3- those that kill insects

4- those inhibiting or abnormally accelerating insect growth or development

5- those that sterilize insects

6- those that deter feeding by insects (antifeedants)

Phytochemicals can be extracted from either whole plants or specific parts of the plant, depending on the activity of the derivatives. Some plants accumulate bioactive chemicals differentially in various parts of the plant, such as leaves, fruits, flowers, roots and bark. Investigators have found that the effectiveness of chemicals derived from specific plant parts often varies with the mosquito species. Certain phytochemicals have photo-activated toxins that are reported effective against mosquitoes. Some phytochemicals act as general toxicants to all life stages of the mosquito, whereas others interfere with growth and reproduction, or act on the olfactory or repellency (Sukumar et al. , 1991). Many studies were conducted to create new mosquito-control products from plant sources. Wilcoxon et al. (1940) reported that extracts derived from the male fern, ***Aspidium filixmas*** contain a toxic constituent, filicin, a phloroglucinol propyl ketone, which proved toxic to ***Culex quinquefasciatus***. In a following study that was conducted by (Hartzell and Wilcoxon,1941) they evaluated extracts from 150 species of plants for their toxicity to mosquitoes and found several to be very effective. Jacobson (1958) reviewed the insecticides derived from plants and reported that several phytochemicals were used against mosquitoes. Jilani and Su (1983) evaluated three plant materials that are common in Pakistan : rhizomes of ***Curcuma longa***, L. (turmeric) , leaves of ***Azadirachta indica***, A. Juss. (neem), and leaves of ***Trigonella foenum-graecum***, L. (fenugreek) for their repellency against the adults of the three species of stored-product insects. All the three plants showed repellency effects on the target insects with differences between extracts on the base of the kind of the solvent. In the comprehensive study that was conducted by Pascual-Villalobos and Robledo (1999) in Spain on plant extracts of 57 species from 21 different families, being harvested from the wild in Southeastern Spain, the grain pest ***Tribolium castaneum***, Herbst was found The results further showed that, ten plants of them were active in most tests performed. One plant produced growth inhibition in larvae. Three plants showed contact toxicity in pupae (100% mortality). Compositae species had a tendency to induce either growth inhibition (with or without mortality) or repellency effect. Overall, 70% of the extracts tested showed some activity and 21% of them were more active.

1. 5. 1 The limited factors of toxicity effects : Many of the plant chemicals are toxic for many species of insects, some of them have selectivity to species or specific stage. The differential responses induced by phytochemicals on various species of mosquitoes were influenced by extrinsic and intrinsic factors such as the species of plant, the parts of the plant, the solvents used for extractions, the geographical location where the plants were grown and the methods employed for evaluation. The extracted portion is very important and leads to different degrees of toxicity. Marcard et al. (1986) reported that among the different plant portions from ***Ajuga remota*** and ***A. reptans*** , the effectiveness of the derived extracts against ***Aedes aegypti*** , ***Ae. togoi*** and ***Culex quinquefasciatus*** larvae decreased starting with roots, leaves, shoots, being least in flowers respectively.

The solvents also have strong effects on the degree of the toxicity of the extracts. It is possible that the active constituent responsible for activity is extracted in larges amounts only with certain solvents. Sherif and Hall (1985) observed that, when ***Macrocystis pyrifera*** and ***Artemesia cana*** were extracted with water and with organic solvents, the organic extract produced higher mortality in ***Culex quinquefasciatus*** . This probably depends on the polarity range of the solvents (Sukumar et al. , 1991). In early studies acetone extracts and water extracts of certain plant products were tested against ***Culex quinquefasciatus*** larvae . Acetone was the better solvent (Hartzell,1944).

Toxicity of plants against insects may possibly influenced by their geographical distribution. Novak (1985) did not observe any toxicity with acetone and alcohol extracts of garlic ***Allium sativum*** in ***Aedes*** larvae when tested in Czechoslovakia, but in the USA Amonkar and Reeves (1970) reported that the extracted oil and crude methanolic extract of garlic at very low concentrations could control larval mosquitoes of five species. In another study on acetone extract of ***Vetiveria zizanoides*** roots from the USA failed to induce larval toxicity (Jacobson,1958), but in a later study Murthy and Jamil (1987) found the oil of ***Vetiveria*** roots from India is very effective against ***Culex quinquefasciatus*** larvae .

1. 5. 2 Growth regulated inhibition and sterilization effect of some plants : Within a large number of toxic plants we can find a few plants that show selective interference with growth and reproduction. The unique action of precocene from ***Ageratum*** interfering with growth by transgressing certain stages of development was noted. In mosquitoes it prevented pupal formation and adult emergence when newly hatched young larvae were exposed (Cupp et al. ,1977) . Also in adult females it inhibited trypsin synthesis and retarded ovarian maturation, when females were treated with precocene after blood feeding, resulting in abnormal oviposition (Kelly and Fuchs,1978). Some other plant chemicals such as aristolochic acid from ***Aristolochia bracteata*** inhibited reproduction, inducing sterility in mosquitoes (Saxena et al. ,1979). Biotin from plants, aflatoxin from ***Aspergillus flavus*** , pactamycin and porfiromycin from lower plants have also sterilized mosquitoes (Borkovec,1987). Although numerous plants have shown tendencies to interfere with growth and reproduction, neem (***Azadirachta indica***) occupies an important place because of its strong action in inducing toxicity through inhibition of growth and reproduction. Although the mode of action of azadirachtin and other components present in neem seed kernels is not clearly understood, it seems likely that there is an interference in hormonal balance (Sukumar et al. , 1991). Also azadirachtin acts as an anti-ecdysteroid or affects the neuroendocrine control of the ecdysteroids (Zebitz,1984). The unique mode of action of azadirachtin by its controlling effect on hormones and its favorable toxicological and selective properties from the ecological perspectives, provides a basis for emergence of promising phytochemical in mosquito control. Patterson et al. (1975) found that extracts from several plants of North Dakota exhibit mimics of insect ecdysones and juvenile hormone activity at various levels based on the parts of the plant used for extraction. As with toxicity, growth inhibition from phytochemicals can also be species specific. Sujatha et al. (1988) observed that ***Acorus calamus*** extracts induced malformations to a greater extent in ***Anopheles stephensi*** and to a lesser extent in ***Culex quinquefasciatus*** and ***Aedes aegypti*** , while ***Madhuca longifolia*** induced greater growth inhibition in ***Culex quinquefasciatus*** .

Phytochemicals growth inhibitors are also affected by the solvents used in the extraction process. Dhillon et al. (1982) reported that. Only the methanol-eluted fraction of petroleum ether extracts from the filamentous algae ***Rhizoctonum heiroglyphicum*** exhibited insect growth inhibitory activity and introduced various abnormalities in ***Aedes aegypti*** , ***Culex quinquefasciatus*** and ***Culiseta incidens*** .

1. 5. 3 Repellency and deterrents effect of some plants : the phytochemicals that have repellency or deterrents properties were evaluated previously to be used in the integrated pest management programs against both agricultural pests and medically important insects. Thorsell et al. (1970) reported that extracts from three plant species (***Ledum palustre*** , ***Lycopersicon lycopersicon*** , and ***Myrica gale***) exhibited repellency to ***Aedes aegypti*** adults. Likewise many essential oils of certain plants often exposed repellent effects against mosquitoes and other blood feeders, like leaf oil of ***Ocimum suave*** (Chogo and Crank,1981). Repellency effects mostly are associated with oviposition behavior of mosquitoes. Ethanolic, hexanic and lyophilized extracts of eight plants deterred oviposition by ***Aedes*** mosquitoes (Consoli et al.,1989). Acetone extracts of four species of the ***Labiatae*** family are reported to have ovipositional deterrents to ***Aedes aegypti*** (Sharma et al.,1981b). ***Lavendula gibsonii*** has also an ovicidal and general repellent effect on ***Aedes aegypti*** (Sharma et al.,1981a). The solvent used for extraction and the species specificity are the limiting factors that affect ovipositional deterrence of phytochemicals. Aqueous extracts of ***Lemna minor*** significantly deterred oviposition of ***Aedes aegypti*** but had no effects against ***Culex pipiens*** . Also the methanolic extract of the same plant disturbed oviposition in ***Aedes aegypti*** , but the pentane extract showed no ovipositional deterrent activity (Judd and Borden,1980).

Concluding, future mosquito management programs will use plant derived chemicals (phytochemicals) that offer not only effective mosquito control agents, but also are biorational alternatives to organic synthetic pesticides. The fact that these chemicals are originate from natural sources with a high degree of biodegradation, makes them environmentally sound control agents. With an ever increasing public interest and awareness on the environment, in both developed and developing countries, positive public perception of natural pesticides is an added incentive for their development and use.

Many plants offer great promise as sources of phytochemicals for the control of mosquitoes. Six plant families with several representative species (***Asteraceae*** , ***Cladophoraceae*** , ***Labiatae*** , ***Meliaceae*** , ***Oocystaceae*** , and ***Rutaceae***) appear to have the greatest potential for providing future mosquito control agents (Sukumar et al. , 1991).

1. 6 The essential oils of plants

Essential oils were defined as natural volatile substances found in a variety of odoriferous plants (Zhu et al., 2001). Likewise an essential oil is a fragrant, volatile liquid extracted by distillation from a single botanical source. In the case of citrus fruits the essential oil may also be obtained by means of expression (Tisserand,1990). It is not fully understood why some plants contain essential oils and others not. It is clear that the aromatic quality of oils plays a role in the attraction or repulsion of certain insects or animals. It has also been suggested that they play an important part in the transpiration and life processes of the plant itself. Aromatic plants and oils have been used for thousands of years as incense, perfumes and cosmetics and for their medical and culinary applications. There is literature of India dating from around 2000 BC, listing over 700 substances including many aromatic plants like cinnamon, spikenard, ginger, myrrh, coriander and sandalwood. But aromatics were considered to be more than just perfumes. In the Indo-Aryan tongue "atar" means smoke, wind, odour and essence, and some Indian old books refer their use for both liturgical and therapeutic purposes.

The Chinese also have an ancient herbal tradition which accompanies the practice of acupuncture, The earliest records are found in the Yellow Emperor's Book of internal medicine dating from more than 2000 years BC.

Egyptian civilization has various contributions in this field. Papyrus manuscripts dating back to the reign of Khufu around 2800 BC, record the use of many medical herbs, while another papyrus written about 2000 BC speaks of fine oils and choice perfumes.

Between the seventh and thirteenth centuries the Arabs produced many great men of science, among them Avicenna (AD 980-1037). This highly gifted physician and scholar wrote over a hundred books in his lifetime, one of which was devoted entirely to the rose flower. Among his discoveries he has been credited with invention of the refrigerated coil, a breakthrough in the art of distillation, which he used to produce pure essential oils and aromatic water (Lawless,1992).

1. 6. 1 Original habitat : Main oil producing plants are represented in over thirty families of plants, comprising some ninety species. The majority of spices (allspice , cardomon , clove , nutmeg , ginger , etc.) originate from tropical countries. Conversely the majority of herbs grow in temperate climates (bay , cumin , dill , marjoram , fennel , lavender , rosemary , thyme , etc.). The same plant grown in a different region and under different conditions can produce essential oils of widely diverse characteristics, which are known as "chemotypes" . Common thyme (***Thymus vulgaris***) for example produces several chemotypes depending on the conditions of its growth and dominant constituent, notably the citral or linalool types, and the thymol or carvacrol type. It is therefore important not only to know the botanical name of the plant from which an oil has been produced, but also its place of origin and main constituents which concerned the main ways of defining the qualities (Lawless,1992).

Essential oils are extracted from almost every conceivable plant part, such as : flower like rose and chamomile , leaves as peppermint and rosemary, fruits of orange and lemon , seeds as in coriander and fennel , grasses like lemongrass and gingergrass , roots and rhizomes as ginger and vetiver , wood of cedarwood and sandalwood , bark like in cinnamon and sassafras , gum as in frankincense and myrrh and blossom like neroli and ylang-ylang (Tisserand,1990). There are also essential oil from bulbs like garlic, dried flower buds like clove, and from stems or twig like clove stem and savin.

1. 6. 2 Properties of essential oils: Usually they are liquid but can also be solid or semisolid, according to temperature such as guaiacwood and rose. The majority of essential oils are clear or pale yellow in color, although a few are deeply colored like German chamomille (blue) or valerian (green). Essential oils are by definition, volatile, and they evaporate at varying rates. They are damaged by the effects of light, heat, air and moisture , and should always be kept in a cool environment, in well stoppered dark glass bottles. They dissolve in pure alcohol and also in fats and oils and are not soluble in water (Tisserand,1990).

1. 6. 3 Extraction of essential oils : The method of extraction which used depends on the quality of the material which is used and the type of aromatic products that is required.

Lawless (1992) reported different ways, by help of which aromatic material can be prepared (Figure 1).

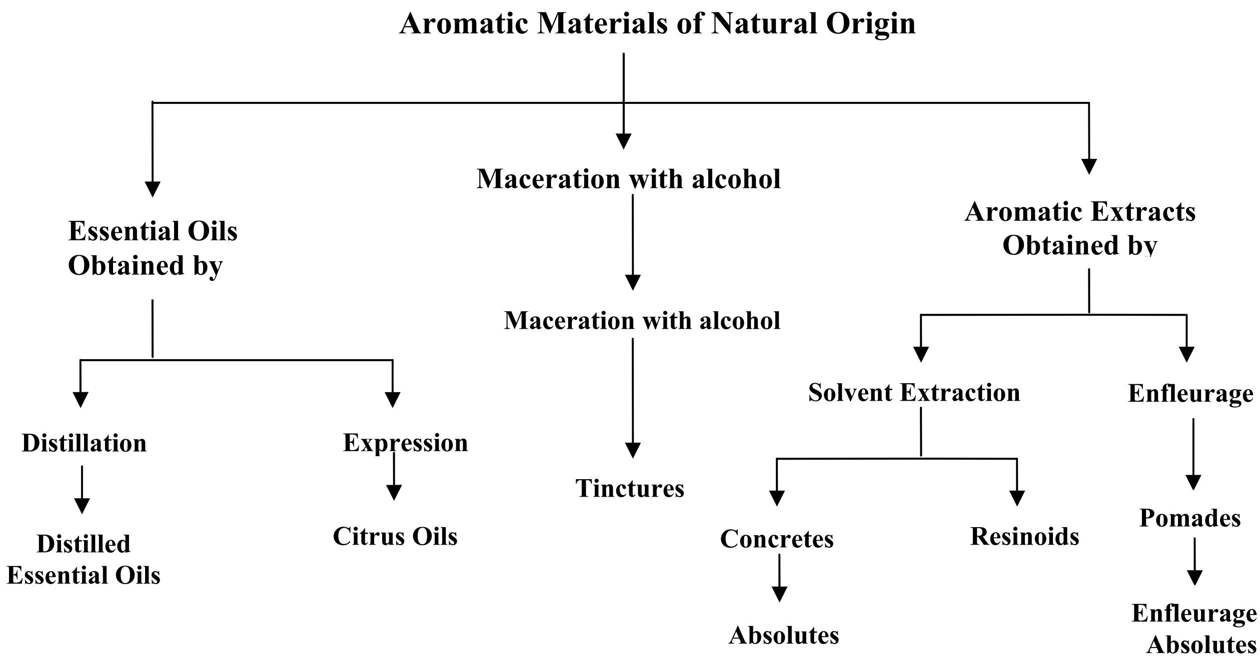

Figure (1) Chart shows different ways to obtain aromatic material.

Some plant materials (especially flowers) are subject to deterioration and should be processed as soon as possible after harvesting. Others (including seeds and roots) are either stored or transported for extraction.

All essential oils are extracted from the plant materials by two main methods: (1) by simple expression as is the case in most of the citrus oils including lemon and bergamot. Or (2) by steam water or dry distillation . The majority of oils such as lavender, myrrh, sandalwood and cinnamon are produced by steam distillation. This process only isolates the volatile and water-insoluble parts of a plant. Many other constituents such as tannins, mucilage and bitters are consequently excluded from the essential oil (Lawless,1992).

1. 6. 4 Yield: Essential oils yield from plant materials is different and limited by many factors. The average yield of essential oil is between 1% and 2% although it can be as little as 0.015% in rose and as much as 10% or 15% in the case of most gums. Gurjun balsam can yield up to 80% of essential oil (Tisserand,1990). Studies on the differences in the yield of plant essential oils and their main components during the life cycle of ***Origanum vulgare***,L. showed that the percentages of the essential oil ranged from 2.2% to 4.9% according to the plants growth stage. As well the percentages of components of essential oil were more different, the phenols alternated between 20.4% and 76.7% based on the groups and growth stages. The monoterpene hydrocarbons ranged between 11.3% and 34.9% (Putievsky et al.,1985). Other studies approached the influence of the district of cultivation and type of soil in ***Salvia sclarea***,L. containing essential oil and the percentages of their components. Many diversities were observed in color of oil, density, and solubility. Moreover the percentage of total esters ranged from 59.5% to 93.4% and the percentage of linalyl acetate alternated between 41.7% and 65.35% (Verzar-Petri et al.,1985)

1. 6. 5 Chemical analysis of essential oils : Many chemical compounds can be found in essential oils, but in general essential oils consist of chemical compounds which have hydrogen, carbon, and oxygen. Tisserand (1990) divided these compounds into two groups:

1- Hydrocarbons which almost exclusively comprise terpenes such as (aromadendrene, bisabolene, cadinene, camphene, carene, caryophyllene, cedrene, copaene, cymene, dipentene, elemene, farnesene, heerabolene, humulene, limonene, myrcene, ocimene, phellandrene, pinene, sabinene, selinene, terpinene, terpinolene, and ahujene).

2- Oxygenated compounds that will be divided into five groups:

A- Alcohols as (benzyl alcohol, borneol, carotol, cedrol, citronellol, farnesol, geraniol, linalool, menthol, nerol, nerolidol, olibanol, phenyl ethyl alcohol, sabinol, santalol, terpineol, terpinenol, and vetiverol) .

B-Aldehydes such as (acetaldehyde, benzaldehyde, cinnamic aldehyde, citral, citronellal, cuminic aldehyde, neral, and perillaldehyde).

C- Esters such as (benzyl acetate, bornyl acetate, eugenyl acetate, geranyl acetate, lavandulyl acetate, linalyl acetate, menthyl acetate, methyl anthranilate, methyl salicylate, neryl acetate, sabinyl acetate, terpinyl acetate, and vetiveryl acetate)

D- Ketones as (acetophenone, camphor, carvone, fenchone, irone, jasmone, menthone, methyl nonyl ketone, pinocamphone, pulegone, and thujone)

E- Phenols as (anethole, apiol, asarone, carvacrol, chavicol, eugenol, methyl chavicol, methyl eugenol, myristicin, safrole, and thymol).

There are some other groups of compounds that may be occur in certain essential oils:

- Acids as (alantic acid, benzoic acid, phenyl acetic acid, and anisic acid).
- Oxides as (ascaridol, bisabolol oxide, bisabolone oxide, and cineol).
- Lactones such as (alantolactone, ambrettolide, bergaptene, coumarin, and costuslactone).
- Nitrogen compounds such as (indol, prussic acid).
- Sulphur compounds as (allyl isothiocyanate, diallyl disulphide, and phenylethyl isothiocyanate).

Most essential oils contain between 10 and 200 components, Many of the trace components and some of the minor ones of certain essential oils have not yet been identified. For long time only large constituents in essential oils have been known. Now by using the gas liquid chromatography (GLC) and gas chromatography (GC) very small and even trace constituents had been detected and were often identified. The same constituents such as cineol may be present in quite a number of different essential oils, in some of these oils it may be a major constituents, in others it may be a minor or only a trace constituent.

Trace constituents are present in very small amounts (sometimes too small to be measured). For example cineol is the principal constituent (PC) of eucalyptus oil and usually reaches about 80%. However, in the case of mandarin oil this component only reaches 0.002% (Thus being 40,000 times less than in eucalyptus oil). Many essential oils contain two or three major components, e. g. bergamot oil contains limonene 28.5%, linalool 28.5%, linalyl acetate 27.7% and also contains many other minor and trace components. Moreover some essential oils contain only one major component which can constitute as much as 95% of the total e. g. (mustard oil contains allyl isothiocyanate 99% , wintergreen oil contains methyl salicylate 98%, sandalwood contains santalol 95% and mandarin oil consists of limonene with two other terpenes (95%) while the remaining 5%consists of at least 74 identified minor and trace components.)

1. 6. 6 Use of essential oils in insect control programs: Essential oils are used in four primary ways : 1- as odorants in fragrances. 2- as flavor enhancers in many food products. 3- as pharmaceuticals. 4- against insects (Zhu et al.,2001). The use of plant extracts including essential oils with known effects on insects could be a useful complementary or alternative method to the heavy use of classical insecticides. This could improve the biodegradability of insecticide treatments and therefore decrease the quantity of toxic insecticide residues, increase insecticide selectivity and develop a better respect for the environment. This alternative strategy based on the identification of plant insecticidal molecules, is not now Humans have used aromatic plants and essential oils in order to protect themselves or their domestic animals from blood feeders arthropods (Secoy and Smith,1983 ; Yang and Tang,1988). Recently essential oils have received much attention as useful bioactive compounds against insects, since insecticide activity of essential oils has been shown against many insect groups such as cockroaches (Ahmad et al.,1995), stored product insects (Shaaya et al.,1991), house flies (Singh and Singh,1991), or termites. Bultman et al. (1979) in their study tested 42 tropical African woods suggested that volatile allelochemicals might be one of the major factors for their natural resistance to termites. Some other studies confirmed that some essential oils such as cedar wood (Adams,1991), ***Litsea cubeba*** (Lin and Yin,1995a), and ***Cinnamomum spp.*** (Lin and Yin,1995b)were natural repellent to termites. Zhu et al. (2001) evaluated 8 essential oils (vetiver grass, cassia leaf, clove bud, cedarwood, ***Eucalyptus globules***, ***Eu. citriodora***, lemongrass, and geranium) against the Formosan subterranean termite, most of them showed good effects against termite some as repellents and others as insecticides. Tripathi et al. (2003)

evaluated the effects of *d*-limonene (main constituent of many citrus essential oils) on three stored-product beetles and they found that, *d*-limonene reduced oviposition up to 92.3% at a concentration of 2.14mg/cm^2. Hatching of *d*-limonene treated eggs was reduced by 94.5% with no subsequent adult survival at 2.14mg/cm^2 concentration. Furthermore 87.7 – 96.8% feeding deterrency by d-limonene was observed at a high concentration. In previous studies the repellency and feeding deterrent effects of turmeric oil, sweetflag oil, neem oil, and neem-based insecticide (margosan-O) were tested against the lesser grain borer ***Rhyzopertha dominica***, F. For eight weeks, turmeric oil and sweetflag oil were significantly more repellent during the first 2 weeks than neem oil and margosan-O , however, thereafter their repellency decreased more rapid than that of neem oil or margosan-O (Jilani and Saxena,1990). Neem oil strongly repelled ***Anopheles darlingi*** and other anopheline mosquitoes. The protection rate provided was 98.2% during an 8 hours period (Caraballo,2000). A study of Sharma et al. (1993) showed the neem oil can an excellent repellent of ***An. culicifacies*** (the major vector of rural malaria in India) and other anophelines, even at concentrations as low as 0.5 and 1% . At a concentration of 2% the anophelines did not bite and the protection provided was 100% during a 12 hours period.

Today essential oils represent a market estimated at 700 million Dollar and a total world production of 45000 tons. About 90% of this amount is concentrated on fifteen products, particularly mints (***Mentha piperita***, ***M. arvensis***, and ***M. spicata***) and citrus (orange, lemon, lime) and some other important species such as ***Eucalyptus globulus*** , ***Litsea cubeba*** (***Lauraceae***), clove, cedar and patchouli. In the course of last few years, the utilization of essential oils has been modified. There has been a steady increase in the production for the food aroma industry (citrus, rose, mints). In the contrary, the use of essential oils in hemisynthetic reactions (citronella, clove, eucalyptus, camphor, lemongrass) or in alcoholic perfumery (patchouli) has decreased. Thus the diversified use of essential oils by the development of their use on the pest management sector could be both on economic and ecological advantage.

2. Materials and methods

2 . 1 The mosquito strains

Three mosquito strains were used in most tests that were conducted in this study :

Aedes aegypti (Linnaeus) , (***Diptera : Culicidae***), called Yellow Fever Mosquito.

Anopheles stephensi (Liston) , (***Diptera : Culicidae***), one of the most important malaria vectors.

Culex quinquefasciatus (Say) , (***Diptera : Culicidae***), vector of filariasis and encephalitis.

2. 1. 1 Sources of the strains : the two mosquito strains of ***Aedes aegypti and Culex quinquefasciatus*** were obtained from the insectaria of the scientific section of Bayer company (Leverkusen : Germany). Their source was the Tropical Institute in Hamburg (Germany) . Both species were cultivated at Bayer insectaria for forty years .

Anopheles stephensi strain was obtained from Professor Dr. Walter A. Maier (Parasitology Department of the University of Bonn). The source of this strain is a mixture of three colonies from the London Tropical Institute, Basel Tropical Institute and Parasitology Institute at Zürich University.

2. 1. 2 Laboratory rearing of mosquito strains : the three mosquito strains were brought into a special insect room in the Institute of Zoology, Cell biology and Parasitology at Heinrich Heine University (Düsseldorf : Germany) and reared at a temperature 25± 2 C^0, relative humidity 70%± 10 , and a photoperiod of 12h light:12h dark since January , 2002 .

2. 1. 2. 1 Rearing of *Aedes aegypti*, L. **mosquitoes :** this species was brought from the source as dry eggs on white filter papers. For hatching the eggs were placed in the normal water with some of yeast extract to reduce the oxygen content of the water to stimulate the hatching process (Service,1993). In the our case the hatching process began after four hours from eggs stimulation. The new larvae were translated to plastic pans (size 9.8 or 4 liter ; Varesal : Italy) with tap water about 1 liter / 500 first and second instars larvae and/or 1 liter/ 100 third and fourth instars larvae. The water was replaced every three days to remove excess food and larval faeces. The larvae were allowed pass all four larval instars in the ten days period under our conditions to reach the pupa stage. In this phase, they were collected by use of a plastic pipette (Alpha Laboratories Ltd, UK.) into 250 ml glass piker with some clean water and transported to adult rearing cages (aluminum frame, they were sized 48.5 × 40 × 30 cm with glass cover and characterized by a spherule 20 cm diameter service opening being provided with fabric sleeve to serve the adult mosquitoes and prevent the flight. 6 small openings were covered by netted weir to aeration) . The adult mosquitoes stayed in the rearing cages all along their lifetime (i. e. for feeding, mating, and egg production). To collect the produced eggs some petri dishes were placed into the rearing cage with some filter paper and clean water, so that filter paper ended in the water and the other side stayed out of water in order to supply a suitable place for female to deposit eggs two days after the blood meal.

2. 1. 2. 2 Rearing of *Anopheles stephensi* (Liston) **mosquitoes :** this species was received as a mixture of first/second instar larvae. They were placed in plastic pans (size 9.8 or 4 liter ; Varesal : Italy) in tap water about 1 liter / 500 first and second instars larvae and/or 1 liter/ 100 third and fourth instars larvae. To remove excess food and larval waste the water was replaced every three days. The total period of larval development until fourth instar was about ten days under our conditions to reach pupa stage. They were collected using plastic pipettes (Alpha laboratories Ltd, UK.) and deposed into 250 ml clean water. Inside adult rearing cages (aluminum frame 48.5 × 40 × 30 cm . see above). In these cages the adults mosquito were kept until they mate, feed and produced eggs. The eggs were laid on the water surface of small Petri dishes they were placed into adult cages. these dishes were provided by pieces of filter paper to a supply suitable layer inside the water allowing the females to lay eggs without drowning. The females laid their eggs mostly three days after blood meal. The new eggs were placed directly in water to stimulate the hatching process

2. 1. 2. 3 Rearing of *Culex quinquefasciatus* (Say) **mosquitoes :** a mixture of larval instars of ***Culex quinquefasciatus*** (Say) were obtained to continue these their life stages in our insectaria. There they were distributed into plastic pans (size 9.8 or 4 Liter ; Varesal : Italy) with tap water about 1 liter / 500 first and second instars larvae and/or 1 liter/ 100 third and fourth instars larvae, the water was replaced every three days to remove excess food and larval remains (For further proceeding see above). Through two to three days the adults stage were emerged inside the rearing cages to mate, feed, and lay their eggs as rafts on water surface in 200 ml glass wares were laid into rearing cages after blood meal. Thereafter the new eggs were transformed to larval plastic pans with water to hatching .

2. 1. 3 Feeding of mosquito stages

2. 1. 3. 1 Feeding of larvae : larval instars of all three species were fed by fish food (Tetra Rubin : Tetrawerke, Ulrich Baensch GmbH : Germany) 20g / 2000 first or second instar larvae daily and/or 20g / 500 third or fourth instar larvae daily, being sprinkled on the surface of water in larval pans.

2. 1. 3. 2 Feeding of adults : the adults of the three species were fed on two sources to prolong as long as possible the time for eggs production.

- feeding of the adult mosquitoes on sugar solution: adult female and male of all mosquitoes needed a source of sugar solution to get their requirements of energy for flight and other activities (Service,1993). Thus the three mosquito colonies were supplied by a sugar solution inside 20 ml glass bottles filled with a 10% sugar solution as reported by Villarreal et.al., (1998). There pieces of toilet paper were placed to allow mosquitoes landing and feeding without drowning. Such bottles were placed inside the adult cages and replaced every three days.

- feeding the adult mosquitoes on blood : female of most mosquito species need a blood meal to produce their eggs. Thus without blood meal they will be unable for eggs production. Therefore the stock colonies of all three species were supplied by blood meals via anesthetized rats being placed five to ten minutes into the colonies after shaving their abdominal hairs. Day feeders (***Aedes aegypti***. L.) were fed at day and the nocturnal feeders (***Anopheles stephensi***.(Liston), and ***Culex quinquefasciatus*** . (Say)) were fed at night.

- anesthetic solution : to anesthetize the rats a solution compacting of Ketamin 10% (Ketaminhydrochlorid ; WDT ; Germany), Rompun 2% (Xylazinhydrochlorid ; Bayer ; Germany) and 0.9% NaCl solution. Was used. The solution was prepared as (0.25 ml Rompun 2% + 2 ml Ketamin 10% + 9ml 0.9% NaCl solution). This solution was injected into the abdominal cavity (0.1 ml per 10 g of body weight). Thereafter, to protect the rat eyes from dry, LACRYBIOTIC-C by crame was placed onto the eyes of the rats eyes after anesthetizing them.

2. 2 The group of essential oils

A large group of essential oils was used in this study. These oils were produced by different companies and being obtained from several geographical locations as stated in (Table 2) .

Table (2) . Oils that were used in this study, their producers and habitats.

NO	Name of material	The producer	The habitats
1	Citronella (*Cymbopogon winterianus*)	Aromara ; Germany	China
2	Rosewood (*Aniba rosaeodora*)	Aromara ; Germany	Brazil
3	Lavender (*Lavandula angustifolia*)	Primavera Life ; Germany	France
4	Camphor (*Cinnamomum camphora*)	Spinnrad ; Germany	China
5	Catnip (*Nepeta cataria*)	Cavallier Freres ; France	France
6	Geranium (*Pelargonium graveolens*)	Spinnrad ; Germany	Reumon
7	Thyme (*Thymus serpyllum*)	Primavera Life ; Germany	Torky
8	Eucalyptus (*Eucalyptus globulus*)	Spinnrad ; Germany	France
9	Jasmine (*Jasminum grandiflorum*)	Aromara ; Germany	France
10	Broad-Leaved (*Eucalyptus dives*)	Spinnrad ; Germany	Australia
11	Lemongrass (*Cymbopogon citratus.*)	Aromara ; Germany	China
12	Lemonscented Eucalyptus(*Eucalyptus citriodora*)	Aromara ; Germany	China
13	Fichtennadel (*Picea excelsa*)	Aromara ; Germany	Korea
14	Amyris (*Amyris balsamifera*)	Aromara ; Germany	Haiti
15	Lemon (*Citrus limon*)	Spinnrad ; Germany	Italy
16	Narrow-Leaved Eucalyptus (*Eucalyptus radiata*)	Spinnrad ; Germany	Australia
17	Carotin oil (*Glycina soja*)	Spinnrad ; Germany	

18	Cedarwood (*Juniperus virginiana*)	Aromara ; Germany	Florida
19	Frankincense (*Boswellia carteri*)	Spinnrad ; Germany	Somalia
20	Dill (*Anethum graveolens*)	Caelo ; Germany	
21	Myrtle (*Myrtus communis*)	Spinnrad ; Germany	Corsica
22	Chamomile (*Anthemis nobilis*)	Spinnrad ; Germany	France
23	Cinnamon (*Cinnamomum zeylanicum*)	Spinnrad ; Germany	Srilanka
24	Juniper (*Juniperus communis*)	Spinnrad ; Germany	Osterreich
25	Sage (*Salvia sclarea*)	Spinnrad ; Germany	France
26	Peppermint (*Mentha piperita*)	Spinnrad ; Germany	Corsica
27	Basil (*Ocimum basilicum*)	Spinnrad ; Germany	Cameron
28	Cajeput (*Melaleuca leucadendron*)	Spinnrad ; Germany	
29	Soya bean(*Glycina max*)	Vitaquell ; Germany	
30	Rosemary (*Rosmarinus officinalis*)	Spinnrad ; Germany	Spain
31	Niaouli (*Melaleuca quinquenervia*)	Spinnrad ; Germany	Madagascar
32	Olive (*Olea europaea*)	Libyan oils Company	Libya
33	Black pepper (*Piper nigrum*)	Primavera Life ; Germany	Srilanka
34	Verbena (*Lippia citriodora*)	Primavera Life ; Germany	France
35	Tagetes (*Tagetes minuta*)	Primavera Life ; Germany	Egypt
36	Violet (*Viola odorata*)	Primavera Life ; Germany	Egypt
37	Sandalwood (*Santalum album*)	Primavera Life ; Germany	India
38	Litsea (*Litsea cubeba*)	Primavera Life ; Germany	China
39	Helichrysum (*Helichrysum italicum*)	Primavera Life ; Germany	France
40	Galbanum (*Ferula galbaniflua*)	Primavera Life ; Germany	Iran
41	Chamomile (*Chamaemelum nobile*)	Primavera Life ; Germany	Italy

2. 3 Scanning electron microscope (SEM)

To study the morphology of the mosquito stages the Scanning Electron Microscope was used to get clear photos for each stage and/or instar of the three target mosquito species (***Aedes aegypti*** (Linnaeus). ***Anopheles stephensi*** (Liston) and ***Culex quinquefasciatus*** (Say).

2. 3. 1 Fixation the mosquito specimens

The mosquito stages were picked up from cages and/or water into 50ml glass bottles contained 25ml tap water for water stages (larvae and pupa) when the eggs and adults samples transferred into empty glasses. All samples were killed by use the fridge under (4 C^0) for 4 hours . then the samples ran over fixation procedures:

1- the samples were immersed in 5% gluteraldehyd in 0.1 M Na-Cacodylat puffer and reserved in fridge at 4 C^0 for 24 hours.

2- wash the samples (6x / 15min) using the 0.1 M Na-Cacodylat puffer.

3- passing the washed samples without dryness through series of concentrations of acetone solutions. (20% / 10 min ; 40% / 10min ; 50% / 10min ; 60% / 10min ; 70% / 20min ; 80% / 10min ; 90% / 10min ; 96% / 10min ; 100% 2x / 20min ; 100% Molecular sib 3x / 20min).

4- the fixed samples were reserved in the fridge at 4 C^0 for 24 hours.

2. 3. 2 Drying of the specimens by critical point

After fixation the fixed samples reserved into small screen capsules, then the capsules contained samples inserted into drying room of critical point gadget (Balzers Union II 120) with suitable amount of 100% acetone. By use the CO_2 gas the samples in the drying room were cooled till 5 C^0 next washed by passing the CO_2 gas through the acetone in the drying room, after 7 times the acetone in the drying room was missed, thereafter the gas was closed and begin the drying 10 minutes under (37 C^0 ; 85 bar). The dried samples were carefully getting out and loaded on SEM plates readiness for golden overlaying .

2. 3. 3 Gold sputtering

Before scanning under electron microscope the fixed dried patterns must be overlaid by a layer of gold (Balzers SL 9496), thereafter the vacuum pump was turned on until the chamber reached 0.1 Torr. Then the Argon gas was opened into the chamber. This step was repeated three times till the specimens were under negative pressure of 0.15 torr . Here the stream switched on at force 25 Volt in Argon atmosphere 3 minutes for larval samples and/or 5 minutes for adults samples. Thereafter the specimens obtained were exposed under scanning electron microscope (Leitz-AMR 1000) and the photos were taken on Agfa APX 25 films.

2. 4 Mass spectrometer / Gas chromatograph analysis

The oils that were used in this study were analyzed using MS/GC (HEWLETT PACKARD – 5890 – SERIES II) system . The analysis was performed by resolving small amount of target oil in Ethyl acetate ($CH_3COOC_2H_5$) to produce 0.1% solution. The gadget was adjusted on suitable work method for essential oils analysis. When the system is ready one micro liter of oil solution was injected in the MS/GC unit, thereafter the procedure begin automatically to need 39 minutes to whole sample evaporated. Thus we obtained the pikes of all oil constituents, retention times, and their molecular weights. The Dictionary of natural products was used to compare the molecular weight of main components for each oil with our MS/GC results.

3. Biology and morphology of the three mosquito species

3. 1 Introduction

Biology and morphology of mosquitoes are not often considered in recent studies especially not the common species, that had been studied and described many years ago. In the last two centuries most mosquito species were described with their biological, morphological, and ecological aspects.

Life cycle of mosquitoes

Mosquitoes as all typical ***Diptera***, exhibit a complete metamorphosis. The juvenile form passes through both larval and pupal stages (Figure 2). The larvae are anatomically different from the adults, live in different habitats and feed on a different type of food.
Transformation to the adult takes place during the non-feeding pupal stage.
The eggs : depending on the species about 50 to 500 eggs are laid by the female at one time, depositing them on water or on sites that will be flooded.
Mosquito eggs can be classified into three groups:
1- eggs are laid singly on still or very slow-moving water surface (***Anopheles***), each egg having a series of floats on its surface.
2- eggs are laid in groups forming rafts that float on water surfaces (***Culex*** and ***Culiseta***).
3- eggs are laid singly outside of the water in the mud (***Aedes*** and ***Psorophora***). Each egg is protected by an egg shell, which in many species is elaborately sculpted. Spermatozoa stored by the inseminated female fertilize the oocytes as soon as they are ovulated. The further embryonic development starts almost immediately after the eggs have been laid. Depending on climatic conditions like temperature and humidity it takes one to two days up to a week or more, until the embryo develops into a fully formed larva. In most species the larva hatches once it is formed, and it may survive for a few days in the absence of water. Mosquitoes of the tribe Aedini have water-proof egg shells being capable to resist desiccation and remain fully-formed but unhatched. Aedine larvae can survive for months or even years in the absence of free water. Aedine species often lay their eggs in places that may not be flooded for days or even weeks. E.g. high tide flooding of a salt marsh stimulates hatching and can lead to an apparent population explosion.

The larvae : These shapes are characterized by a large head and thorax, they are legless with a slender abdomen and look hairy. The microhabitats of mosquito larvae are small or shallow water with little or no movement. Typically shallow pools, are sheltered stream edges, marshes and water filled tree holes, leaf axils or man-made containers. The habitats range in size from animal footprints to marshes or saline water in salt marshes. All aquatic animals have problems with their salt balance, living in fresh or salt water. The mosquito larvae solved these problems so that they can live in the different water salinities. The young larva is fully adapted for living in water after it hatches from the egg. The larvae use atmospheric oxygen for respiration and water borne particles as food. The characteristic food resource of mosquito larvae includes aquatic microorganisms such as bacteria, diatoms and algae. Further important component food particles as detritus is largely derived from decayed plant tissues. Such as particles provide food for diverse aquatic invertebrates, which filter them from the water by a variety of mechanisms. Mosquito larvae which live mainly in still water, are exceptional in not relying on natural water currents to bring the particles to them. By means of the regular beating of their mouth brushes mosquito larvae generate water currents which flow towards the head, and in a manner, that is not well understood, they separate particle of a certain size from the water. ***Anopheline*** larvae typically feed at the water surface in a particle-rich layer just below the surface membrane. ***Culicine*** larvae feed on particles suspended in the water column and many species supplement this feeding mode by abrading with their mouthparts the layers of organic matter that cover submerged surfaces thus generating new particles. ***Toxorhynchitine*** larvae are predatory on small invertebrates as are a very few species in the other two subfamilies, too except for ***Anopheles*** larvae, which rest horizontally on the surface of the water and breath directly through holes in their abdomen. The other genera use a siphon tube at their abdomen for oxygen uptake. Larval stages usually take 4 -10 days or more under unsuitable circumstances. The organs that compose the larval body serve larval functions and are mostly very different in structure from the adult organs. Within these larval organs there remain groups of undifferentiated cells that will eventually become adult organs. The growing mosquito larva moults four times i. e. there are larval shapes. After the first three moults it appears very much as before. During the period of the fourth moult the imaginal disks develop rapidly, changing the form of the insect crudely to that the organism that leaves the fourth larval skin is a pupa. The rapid growth rates of many tropical species permit the exploitation of transient water bodies (Clements, 1992).

The pupae : Mosquito pupae are the most active of any insect pupae. They are mobile and swim actively in the water. They have a large combined head and thorax and a slender abdomen giving it a comma shape (Tredten,2002). The larval integument splits along the middorsal line allowing the pupal thorax to emerge and very soon the trumpets spring up and come into contact with the water surface, settling pupal respiration into operation while the still-encased abdomen wriggles free of its larval integument. About three to five minutes are required to finish the whole process. Pupae are non-feeder stage, being less dense than water, they normally spend most of their time at the water surface breathing through the paired respiratory trumpets. Exceptions are pupae of the genera ***Mansonia*** , ***Coquillettidia*** and a few species of ***Mimomyia*** which like their larvae pierce submerged plants to breath. Buoyancy is due to air trapped in a ventral air-space formed by the developing mouthparts, legs and wings. When pupae are disturbed by shadows or vibrations they descend in a quick zigzagging fashion by alternately flexing and stretching the abdomen. When swimming stops, pupae slowly float to the water surface, where their position is held by the hydrophilic outer coating of the trumpets and by the large paired dendritic float hairs on the first abdominal segment. Temperature is a very effective factor for the pupal duration. In hot tropical countries the duration is usually two to three days but can be as short as 26 hours at temperatures of about 30 C . In temperate regions pupal life usually lasts about one week but can be stretched to two to three weeks in cold weather. Duration also varies according to species : e. g. in ***Mansonia*** and ***Coquillettidia*** the pupae stage lasts six to nine days. No species overwinters as pupae. In dry conditions pupae tolerate partial desiccation better than larvae, they survive well on damp substrates. A few hours before emergence the pupa noticeably darkens some five to ten minutes before the adult emergence the abdomen is straightened and the pupa lies almost parallel to the water surface. Air appears beneath the integument which then splits mid-dorsally. The thorax and head of the adult is followed by the antennae and mouthparts, and finally the legs and abdomen begin to emerge. Emergence usually takes 12 to 15 minutes and within minutes afterwards the newly emerged adult can start for very short flights. Male larvae generally pupate before females and in most species male pupal life is shorter than female pupal life. Male usually emerge a day or so before females. Most species emerge in the early evening or late at night. The presence of pupae in a habitat reflects its productivity but not all pupae will give rise to sexually mature adults, since many will be eaten previously by predators. Emerging and newly emerged adults are also very susceptible to predation, and adults have to live sufficiently long to reproduce before there is any new input into the habitat (Service,1993).

The sex ratio of the emerging population calculated over the entire emergence period is usually about 1:1 , although in high arctic ***Aedes impiger*** and ***A. nigripes*** females predominate. There can be seasonal variations, e. g. ***A. triseriatus*** a tree-hole species produces predominantly males at the beginning of the season but females are most common in the later part of the season (Scholl and DeFoliart, 1978).

The adult : Adults are small and fragile, two winged with long, slender legs, capable of flying one to several miles. A mosquito can fly up to 300 miles in its lifetime by its own and lives by wind. Some mosquitoes won't go more than 500 feet from the larval habitat – others can cover 6 – 8 miles a day with the help of the wind. The female flies into a swarm of males and mating takes place almost immediately in midair. Mating takes from 4 to 40 seconds but other specimens stay together for over an hour. Mosquitoes can only fly at 25 mph, but they can fly up, down, sideways and backwards. Wings, legs and other body parts are more or less covered by tiny scales. Males have bushy feather-like antennae; females do not. Mosquitoes are generally 3-6 mm long, some of the largest belonging to the mainly tropical genus ***Toxorhynchites*** (19 mm long, 12-24 mm wing) (Service,1993). They smell with their antennae and live from 10 – 60 days while females are capable to reach up to 5 months or more depending on predator pressures. Throughout the mosquito's lifetime it can bite up to 6 times – even more if a blood meal is interrupted. However, only female mosquitoes bite. They can sip up to 1-1/2 times their own weight in blood and still fly away. They can sense a person 20 feet away. They are species depending attracted by carbon dioxide, odor, heat, moisture and wind their activity peaks are most prominent at dawn or dusk. Male mosquitoes locate females by the sound of their wings in flight- the sounds range from 500-800 vibrations a second. Males will come to any source (e.g. a tuning fork) that produces these sounds.

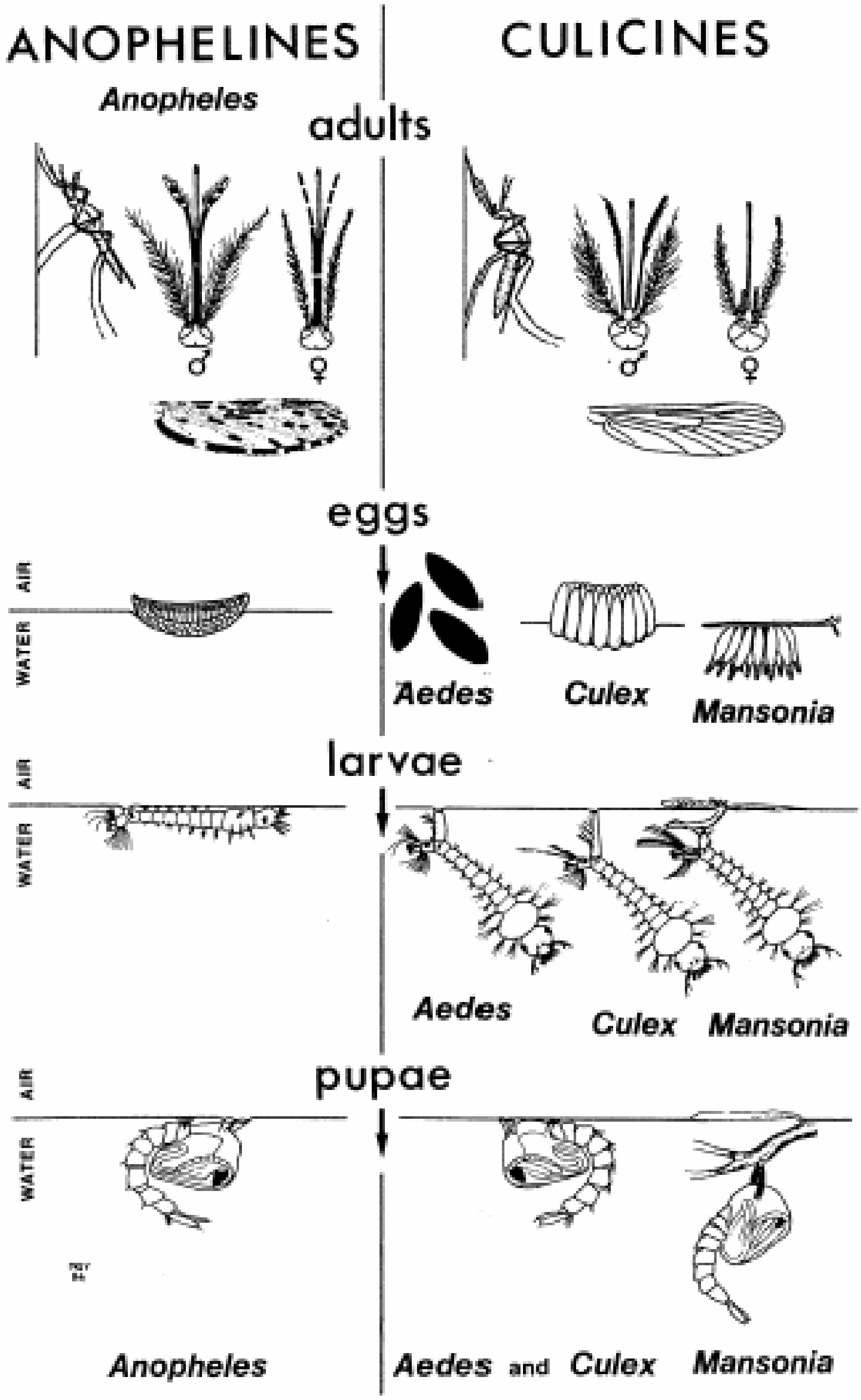

Figure (2) Sequence of mosquito life cycle stages in Anophelines and Culicines (Service,1993).

3 . 2 Materials and Methods

3. 2. 1 Biological studies

The study targeted to watch the life cycle of the three mosquito species: Yellow Fever Mosquito ***Aedes aegypti*** (Linnaeus); ***Anopheles stephensi*** (Liston), one of the more important malaria vectors; ***Culex quinquefasciatus*** (Say) , vector of filariasis and encephalitis. 100 individuals from each species were used in these studies. 100 larvae from each species were picked up directly after hatching from the eggs, each larva was transferred alone with 40 ml tap water into 50 ml glass bottle. Fifty larvae were reserved on the laboratory table under room temperature (ranging between 19^0 and 22^0C) , while the other fifty larvae were reserved in the incubator under 28^0 C. The studied samples were exposed three times daily to recording every changes especially the dates of molting between each followed two instars.

3. 2. 2 Morphological studies

This study was performed under laboratory conditions (20.5^0 C , 70 RH) to measure the stages sizes of the three mosquito species. 100 eggs from each species were measured. Then immediately after hatching 100 larvae from each species were isolated in 40 ml tap water within 50 ml glass bottle. The bottles contained larvae were dept during the study period on the laboratory table. The individuals were measured daily under binocular microscope till the adult emergence. During the study period photos were taken of each stage and/or instar of the target species.

3. 3 Results

3. 3. 1 Life cycle of *Aedes aegypti*

The averages of the time periods of the life cycle stages of ***Aedes aegypti*** mosquitoes that were reared in laboratory at room temperature and/or in incubator at 28^0 C were shown in Table 3. The time between egg lying and hatching ranged from 160 to 200 hours at room temperature and between 150 and 168 hours inside the incubator. The age of first instar larvae ranged between 70 and 96 hours in the room and between 40 and 70 hours at incubator temperature. Also the time of second instar larvae varied from 48 to 120 hours at room temperature and from 42 to 48 at incubator temperature. The third instar larva needed 42 to 90 hours in the room and only 48 hours at 28^0 C at incubator, while the fourth instars time ranged from 94 to 144 hours at room temperature and 72 and 96 hours in the incubator. While the pupa in the room temperature remained from 90 to 120 hours, those in incubator fluctuated between 48 and 96 hours.

Table (3) The averages of time periods of life cycle stages of ***Aedes aegypti*** mosquitoes at room temperature and/or inside incubator at 28^0 C .

No.	The stages	Time periods / Hours ± Standard Deviations		T value
		At room temperature	At 28^0 C	
1	The time between egg lying and hatching	173.2 ± 15.46	162.8 ± 7.95	1.34*
2	Larva 1	81.4 ± 13.37	50 ± 11.66	3.96*
3	Larva 2	72.4 ± 29.41	46 ± 2.83	1.97*
4	Larva 3	64 ± 20.54	48 ± 0	1.74*
5	Larva 4	114.8 ± 20.57	76.8 ± 10.73	3.66*
6	Pupa	101.2 ± 13.89	71.6 ± 16.99	3.01*

$P > 0.05$ * Significant difference

3. 3. 2 Life cycle of *Anopheles stephensi*

Life cycle stages of ***Anopheles stephensi*** mosquitoes that were reared at room temperature and/or inside an incubator at 28^0 C were recorded. The averages of time periods are presented in Table 4. The time between egg lying and hatching ranged from 72 to 80 hours at room temperature and from 72 to 75.5 hours at 28^0 C . First larval instar time fluctuated between 144 to 150 hours at room temperature and only 72 hours inside the incubator. Second larval instar needed in incubator 96 hours longer than at room temperature varying between 48 to 72 hours. The period of the third instar larvae ranged from 72 to 100 hours at room temperature and between 48 and 72 hours at 28^0 C. Fourth instar larvae stayed between 120 and 200 hours at room temperature, but inside the incubator it took only 48 to 72 hours. The time of pupal period in this species varied between 80 and 100 hours at room temperature and from 48 to 100 hours at 28^0 C .

Table (4) The averages of time periods of life cycle stages of ***Anopheles stephensi*** mosquitoes at room temperature and/or inside incubator at 28^0 C .

No.	The stages	Time periods / Hours ± Standard Deviations		T value
		At room temperature	At 28^0 C	
1	The time between egg lying and hatching	75.2 ± 4.38	72 ± 0	1.63*
2	Larva 1	147.6 ± 3.28	72 ± 0	51.44*
3	Larva 2	67.2 ± 10.73	96 ± 0	6*
4	Larva 3	80.8 ± 11.45	62.4 ± 11.75	2.36*
5	Larva 4	158 ± 40.25	57.6 ± 11.75	5.3*
6	Pupa	92 ± 10.95	72.8 ± 16.47	2*

P > 0.05 * Significant difference

3. 3. 3 Life cycle of *Culex quinquefasciatus*

In the case of ***Culex quinquefasciatus*** mosquitoes the averages of time periods of life cycle stages are shown in Table 5. The time between the egg lying and hatching was about 80 hours at room temperature and 70 hours in the incubator at 28^0C. The time of first larval instar continued from 120 to 200 hours in room temperature, while it was decreased at 28^0C ranging from 90 to 96 hours. Second larval instar stayed from 80 to 102 hours at room conditions, and this period decreased to 48 and 50 hours in incubated individuals. The period of the third instar larvae ranged between 50 and 80 hours at room temperature and between 48 and 72 hours at 28^0 C. Life period of larvae four took time fluctuated from 90 to 125 hours at room conditions, while it increased to 96 and 216 hours at incubator conditions. Pupa periods ranged from 100 to 150 hours at room temperature, while it lasted between 48 to 72 hours inside an incubator at 28^0 C.

Table (5) The averages of time periods of life cycle stages of ***Culex quinquefasciatus*** mosquitoes at room temperature and/or inside incubator at 28^0 C .

No.	The stages	Time periods / Hours ± Standard Deviations		T value
		At room temperature	At 28^0 C	
1	The time between egg lying and hatching	82.2 ± 2.28	72.4 ± 2.07	7.1*
2	Larva 1	142 ± 34.92	93.2 ± 3.03	3.11*
3	Larva 2	93 ± 10.19	48 ± 1.41	9.77*
4	Larva 3	60 ± 14.14	53 ± 10.9	0.88*
5	Larva 4	106 ± 15.57	139.2 ± 59.75	0.47*
6	Pupa	116.4 ± 20.61	52.8 ± 10.73	6.12*

P > 0.05 *Significant difference

3. 3. 4 Morphological measurements

Specimens of each larval stage and/or instars of the three mosquito species were measured, in the Table 6. Maximal, minimal and averages of length were presented.

Table (6) The maximal, minimal and averages of lengths of all stages and/or instars of the three mosquitoes species.

		The length of mosquito stages / mm							
		Egg	Larva 1	Larva 2	Larva 3	Larva 4	Pupa	Adult	
								M	F
Aedes	Max	0.6	1.2	2.5	4	6	5	4.2	5.5
	Min	0.5	1	1.9	3	5.4	4	3.7	5
	Av	0.58	1.1	2.23	3.35	5.6.9	4.24	3.99	5.15
Anopheles	Max	0.6	1	1.6	3	5	4	4.5	5
	Min	0.4	0.7	1.3	2	4	3	4	4.5
	Av	0.5	0.91	1.51	2.62	4.51	3.77	4.4	4.85
Culex	Max	6	1.2	1.9	4.5	6.3	4	5.2	6.4
	Min	3.5	0.8	1.4	3.2	5.5	3	4	5
	Av	4.6	1.01	1.63	3.8	5.92	3.55	4.6	5.58

(Max = Maximal ; Min = Minimal ; Av = Average)

To show the morphological structure of each mosquito stages whole stages of each mosquito species were photographed in its habitat. See (Appendages 1, 2, and 3).

3 . 4 Discussion

3 . 4 . 1 The differences in the life cycles

The differences between the two environments become very clear. Especially the effects of temperature was the limiting factor for the periods of time that was taken by the various life cycle stages. In ***Aedes aegypti*** mosquitoes the time between the egg lying and hatching was the longest period in their life cycle. The averages were 173.2 and 162.8 hours at room and incubator temperatures, respectively. Larva three was the shortest period in the life cycle at room temperature, while the time of larva two was the shortest time in the incubator at 28^0C. The whole life cycle from egg lying to reach the adult stage needed 538 to 721 hours (averaging 607 hours) at room temperature, while it decreased to 424 and 518 (average 455.2 hours) in the incubator. In the case of ***Anopheles stephensi*** the longest period in their life cycle was that of larva one at room temperature (averaging 147.6 h), and larva two in 28^0C. (averaging 96 h). The whole life cycle in this species took 557 to 682 (by average 621.4 hours) in the room, while in the incubator it ranged between 432 and 436 (at average 432.8 hours). In ***Culex quinquefasciatus*** mosquitoes the second larval instar period was the longest time in their life cycle at room temperature (by average 142 h), while the fourth larval instar took the longest time in their life cycle at 28^0C (at average 139.2 h). The whole life cycle from the egg lying till reaching the adult stage needed in average 602 hours at room temperature, while the average decreased to 460.8 inside the incubator at 28^0C.

Thus it can be concluded, that if the temperature in the room was not stable, variations between individual life cycle were larger than between individuals that were reared in the incubator at stable temperatures. Furthermore the life cycle time decreased inside the incubator compared to those that were reared at room temperature.

4 . Essential Oils As Repellents of Mosquitoes

4. 1 Introduction

The use of repellents to protect humans and his animals from bites of mosquitoes already has been accepted as part of an overall integrated mosquito-borne disease control program (Chavasse and Yap 1997; Yap et al. 2000). Insect repellents are an alternative to the use of insecticides . Dethier et al. (1960) defined a repellent in terms of the specific behavior pattern evoked : a repellent is a chemical which causes an insect to make oriented movements away from its source. The repellent products may be applied to the skin to protect an individual from the bites of mosquitoes of mites of ticks or of lice . Less commonly they may be used to exclude insects from an area, such as in packaging to prevent infestation of stored products (Peterson and Coats 2001).

4. 1. 1 Historical review of insect repellents : with respect to common repellents there are many materials that derived from plant sources, such as various plant oils, smokes or tars were used during the ancient and medieval periods. This was documented in many references from several cultures. But here we will talk only about the insect repellents during last 100 years. In 1919 , Bacot and Talbot (1919) published what is probably the first well-planned laboratory evaluation of mosquito repellents. They used human volunteers treating the forearm from wrist to elbow with 1 g of the experimental material and than exposed it in a cage of ***Aedes aegypti*** mosquitoes. The protection period was determined by testing at 2, 3, and 5 hrs intervals after treatment. Except for some minor changes, this method is even today the most commonly practiced test procedure worldwide. Furthermore ***A. aegypti*** is still the most commonly used species. The progress in this field has been relatively slow except for the war years of the 1940s and 1950s. During these periods high priorities were set to develop better, more broadly effective insect repellents (Schreck 1977). Before the Second World War there were only four principal repellents, oil of Citronella was used sometimes as hair treating for head lice, dimethylphthalate being discovered in 1929, indalone in 1937, and Rutgers 612 became available in 1939. Many formulations were prepared by the combination of some or all last three compounds to increase their properties as broad insect repellent to use it in many parts of world and at different times. During the Second World War six parts of dimethylphthalate, two parts of indalone, and two parts of Rutgers 612 were mixed and used by military personnel deployed around the world. Moreover they all failed to provide a satisfactory protection (Peterson and Coats 2001). Likewise, there is another mixture called M-250 which was used by U. S. armed forces. This product containing 60% dimethylphthalate, 20% ethylhexandiol, and 20% indalone , later was replaced by an improved mixture M2020 containing 4 parts dimethylphthalate, 3 parts ethylhexandiol, and 3 parts dimethylcarbate (Travis and Morton 1946; Smith et al. 1952). The mixture M-1960 containing 3 parts 2 butyl-2-ethyl-1,3-propanediol, 3 parts N-butylacetanilide, 3 parts benzyl benzoate and 1 part emulsifier was developed for treatment of military uniforms for protection against mosquitoes, ticks, chigger mites, and biting flies (Smith and Cole 1951). In fact all military repellent mixtures failed to protect the individuals from insect attacks. Thus the United States government screened over 20,000 potential mosquito repellent compounds. In 1953 the insect repellent properties of N,N-diethyl-m-toluamide (DEET) were discovered and the first DEET product was introduced in 1956. DEET is still the most widely used mosquito repellent it has generally been regarded as safe, but toxic effects have been recorded, including encephalopathy in children, urticaria syndrome, anaphylaxis, hypotension and decreased heart rate (Robbins and Cherniack 1986; Peterson and Coats 2001). Another discouraging effects of DEET is its capability to act as a solvent of paints, varnishes, some polyethylene materials or synthetic fabrics (Trigg 1996). Because of these undesired effects of DEET research was actively carried out to find an alternative compound that is safer in use and is equally or more effective than DEET (Robert et al. 1991; Schreck and Leonhardt 1991; Sukumar et al. 1991; Dua et al. 1996; Walker et al. 1996). Recently Bayer AG (Leverkusen , Germany) developed and registered a new active compound named Bayrepel (1-piperidinecarboxylic acid, CAS No. 119515-38-7), which is a broad range insect repellent. This compound was previously known as KBR 3023 (Yap et al. 1998). This new repellent compound was investigated according to toxicological standards for skin repellents under U. S. Environmental Protection Agency requirements. The median lethal doses for oral and dermal acute toxicity of this compound on rats were 4,743 and 2,000 mg/kg , respectively. This product also was found to be nonneurotoxic and it did not accumulate in tested rats (Yap et al. 1998; Yap et al. 2000).

4. 1. 2 Natural insect repellents : The use of natural products such as plant extracts or oils is not common until now due to many reasons, Thus it is rather easy to find out the chemical compounds that have repellency properties and to use them as active ingredient in the final repellent product. Furthermore the development of natural active ingredients need a long time for research and large financial effort. Leading to fact that the final product will be more expensive than chemical products. The US Department of Agriculture (USDA) has tested between (1953) and (1974) 901 substances (872 synthetics and 29 botanical oils) for repellency to four species of domiciliary cockroaches, 127 compounds repelled 94% or more of the German cockroach, 61 compounds repelled 100% and 13 substances repelled 100% of all four species tested. None of those 13 was a pure plant product (Peterson and Coats 2001). In the last few years with the increase of public concern on the safety of many chemical products that were used previously as insecticides or insect repellents, several institutes and researchers were directed to the development of natural active ingredients especially from plant sources. Jilani et al. (1988) tried the repellent and growth-inhibiting effects of turmeric oil (***Curcuma longa***, L.) , sweetflag oil (***Acorus calamus***, L.) , neem oil (***Azadirachta indica***, A. Juss) , and Margosan-O (a commercial neem-based insecticide) on red flour beetle (***Coleoptera : Tenebrionidae***). They found that, the repellency increased with increasing concentration of the oils and Margosan-O , as well as the turmeric oil or sweetflag oil repelled insects during the first 2 hours. Thereafter repellency decreased more rapidly than with Neem oil or Margosan-O. Some essential oils have been employed as insect repellents since ancient ages such as citronella and pennyroyal. Freeborn (1928) and Dover (1930) cited some insect repellent formulations consisting of a number of essential oils such as citronella, camphor, tar, pennyroyal and castor oils that provided a long-lasting protection from insect bites. Forty essential oils extracted from Australian plants were evaluated against mosquitoes, march flies, and sand flies, the most effective of these were ***Dacrydium franklini , Backhousia myrtifolia , Melaleuca bracteata ,*** and ***Zieria smithii*** (Penfold and Morrison 1952). Repellency properties of nepetalactone (cyclopentanoid monoterpene) isolated from the catnip plant ***Nepeta cataria*** against seventeen species of insects were reported by Eisner (1964). Also many monoterpenes were reported for their insect repellents properties such as ά-pinenen, limonene, terpinolene, citronellol, citronellal, camphor, rotundial, dolichodial, teucrein, and isoborneol (Perttunen 1957; Moore 1974; Takikawa et al. 1998; Eisner et al. 2000; Blaske et al. 2003). Among 29 tested alkaloids obtained from ***Delphinium***, ***Consolida***, and ***Aconitum*** species 21 compounds showed a promising insect repellent activity, while eight of them were not found to be active. Hetisine had the highest activity and the lowest activity had venulol (Ulubelen et al. 2001). The use of essential oils alone or as mixtures of two oils or more in insect repellent formulations is now beginning.

Jantan and Zaki (1998) evaluated four essential oils of ***Litsea elliptica*** , ***Cinnamomum mollissimum*** , ***Cymbopogon nardus*** , and ***Pogostemom cablin*** respectively for their repellency effect against ***Aedes aegypti***,L. . They established an aqueous cream containing 15% of the leaf oils (***L. elliptica , C. mollissimum , Cy. nardus*** in the ratio of 1:1:1) that provided 96.6% protection against mosquito bites for the duration of the test. Barnard (1999) tested the repellency of five essential oils (Bourbon geranium, cedarwood, clove, peppermint and thyme) alone at different concentrations (5, 10, 25, 50, 75, and 100%) or combinations against two mosquito species ***Aedes aegypti***, L. and ***Anopheles albimanus***, Wiedemann . Thyme and clove oils were the most effective mosquito repellents. Palsson and Jaenson (1999) conducted research in several villages in Guinea Bissau (West Africa) on eight plant species being used traditionally as mosquitoes repellents by native people (***Hyptis suaveolens***, Poit. (***Lamiaceae***) ; ***Daniellia oliveri***, Rolfe (***Caesalpiniaceae***) ; ***Elaeis guineensis***, Jacq. (***Arecaceae***) ; ***Parkia biglobosa***, Jacq.&Benth. (***Mimosaceae***) ; ***Azadirachta indica***, A.Juss. (***Meliaceae***) ; ***Eucalyptus sp***. (***Myrtaceae***) ; ***Ocimum canum***, Sims (***Lamiaceae***) ; and ***Senna occidentalis***, L. (***Caesalpiniaceae***)) . They found that all the products tested except for ***S. occidentalis*** were significantly more effective than the control. Also they had done questionnaire in these villages on the kind of mosquito control that already had been used by these people. A total of 54.6% households had used plants to repel mosquitoes, and of these 54.4% were using plants in combination with bed nets. Mosquito coils were used by 14.4% households and 38.8% of these used coils together with bed nets. A total of 31.2% households were using bed nets only. In another study (Ansari et al. 2000) suggested that, the peppermint oil (***Mentha piperita***, L.) showed strong repellent action against adult mosquitoes when applied on human skin. The protection obtained against ***Anopheles annularis*** , ***An. culicifacies*** , and ***Culex quinquefasciatus*** was 100%, 92.3% and 84.5%, respectively. Tawatsin et al. (2001) studied the repellency of some volatile oils (turmeric ***Curcuma longa*** ; kaffir lime ***Citrus hystrix*** ; citronella grass ***Cymbopogon winterianus*** ; and hairy basil ***Ocimum americanum***) against three mosquito vectors (***Aedes aegypti*** ; ***Anopheles dirus*** ; and ***Culex quinquefasciatus***), and demonstrated the potential of volatile oils extracted from turmeric, citronella grass and hairy basil as topical repellents against both day and night-biting mosquitoes. Effect of catnip essential oil (***Nepeta cataria***, L.) as a barrier to subterranean termites was studied by Peterson and Ems-Wilson (2003) finding that the termites avoided treated sand.

4. 1. 3 Chemical and physical properties of potential insect repellents : many people asked for the chemical structural and physical properties needed for a repellent? Skinner and Johnson (1980) commented that the vapor pressure or boiling point are the only parameters that to correlate with repellent activity. This is a rather clear that a chemical that is going to repel mosquitoes, will most likely act in the vapor phase. In addition, it should not evaporate too fast, otherwise it will have its ability to protect. Thus, it has been possible to define a range of boiling-point temperatures into which most repellents will fall. Other properties, such as partition coefficients, melting points (except that liquids work better than solids), molecular weights (except as they relate to boiling points), infrared absorption, viscosity, surface tension, and molecular polarizability, have been shown to have no clear correlation with repellency.

Regarding functional groups and structural correlations (Garson and Winnike 1968) stated of 4308 compounds examined for repellent activity, that the most effectives were amides, imides, alcohols, and phenols. Furthermore, an oxygen function seemed to be necessary for repellency. Skinner and Johnson (1980) used a computer-assisted search for help to describe some common chemical structures associated with known repellent and non repellent compounds. Otherwise, the structures shown are rather common and can be found in many compounds having no repellent properties.

4. 1. 4 Mode of action of insect repellents : the mechanisms of insect repellent substances were and are a difficult point in the insect repellent studies. Davis (1985) suggested at least five potential mechanisms underlying the activity of chemical substances commonly referred to as repellents.

1- Repellents may interact with and inhibit the response of a sensory neuron to a normally attractive chemical signal. For example DEET and Rutgers 612 inhibit the response of the lactic acid (Davis and Sokolove 1976). The degree of interference of the repellent with the cell response to lactic acid is dependent on the intensity of the repellent.

2- A chemical that is an attractant at low stimulus may at higher intensities alter the response of the insect from attraction to repulsion. For example it has been reported that at low levels of DEET will attract female ***Aedes aegypti*** (Kost et al. 1971). Repellency is observed only when the amount of DEET in the vapor phase is increased. Similar paradoxical response patterns have been observed in the oviposition attraction response of

Aedes triseriatus . At normal low levels (3ppm), p-cresol attracts these mosquitoes to an oviposition site (Bentley et al. 1979), but at higher levels it repels them. Moreover lactic acid which is normally a host attractant, at high intensity levels will repel female ***Aedes aegypti*** (Müller 1968).

3- Repellents may activate a receptor system that mediates a competing or inappropriate behavior pattern. Normally the chemosensory neurons of the short, pointed and possibly the short, blunt sensory hairs respond to oviposition-site attractants with an increase in spike frequency (Davis 1976). When presented with a repellent such as 612 or SRI-C6 (n-hexyl-triethylene glycol-monoether), these neurons may also increase their spike frequency. Thus, certain chemical repellents mimic substances that are chemical signals for the location of an oviposition site.

4- Repellents may activate a noxious odor receptor. DEET and other repellents excite some of the blunt sensory hairs (Lacher 1971; Davis and Rebert 1972). If activity in these neurons uniquely signaled the presence of an odor that mediated avoidance, a repellent behavior is introduced. These are called noxious odor receptors.

5- A repellent simultaneously activating several different receptor types that mediate various behavior patterns, may cause such a barrage of sensory input that any signal specific to host finding is lost in the noise. This repellent could be thought jamming the sensory information system. Davis (1985) introduce of data showing activation of five identified chemoreceptor types.

4. 1. 5 Some very important factors for repellent tests : Schreck (1977) suggested : it is not only the amount of material important in the test, but also the species to be tested, the strain of species, the number of test insects, their sex, whether they are mated, their age, the host, attractant, or standard used, time of day, temperature, humidity, etc. The species and its peculiarities are extremely important, final one species may become repelled, another may not or may even be attracted. Kost et al. (1971) reported that DEET is an attractant at lower concentrations. Smith (1970) refers to published data showing lactic acid to be a repellent for ***Aedes aegypti*** under some conditions and an attractant under others.

4. 2 Materials and methods

4. 2. 1 Preparation of the oils for the test : forty-one oils mentioned in Table 2 were used in this trial. In the first stage the oils were tested to detect their repellent properties against the three mosquito species (***Aedes aegypti*** , (Linnaeus) ; ***Anopheles stephensi*** , (Liston) ; ***Culex quinquefasciatus*** , (Say) (***Diptera : Culicidae***)), using 20% oil solutions in the complex solvent composed by 20% genapol , 10% PEG , 20% Ethanol and 50% water to mimic the final formulation in some cosmetic products and fix the essential oil on the skin for time as long as possible. The oils that showed good repellent properties in the first stage were transferred to the second stage to test them in other solvents such as ethanol and aceton and/or with vanillin as fixation materials as suggested by Tawatsin et al. (2001). Thereafter, the oil concentrations were decreased to test groups of oil mixtures (Table 7) with the result that some oils had very good properties as mosquito repellents. To compare our products with some common chemical repellents DEET (N,N-diethyl-m-toluamide) and Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester) (Appendix 9) they were tested as 20 % solutions using equal solvents and formulations against the same mosquito strains.

Table (7). Combination of oil mixtures with different solvents.

NO	The Mixture	The Solvent	The oil concentrations combined in the mixtures				
			Litsea cubeba	***Melaleuca leucadendron***	***Melaleuca quinquenervia***	***Viola odorata***	***Nepeta cataria***
1	**M1**	Complex	1%	1%	1%	1%	1%
2	**M2**	Complex	1%	1%	1%	1%	0
3	**M3**	Complex	1%	1%	1%	0	1%
4	**M4**	Complex	1%	1%	0	1%	1%
5	**M5**	Complex	1%	0	1%	1%	1%
6	**M6**	Complex	0	1%	1%	1%	1%
7	**M7**	Ethanol	1%	1%	1%	1%	1%
8	**M8**	Acetone	1%	1%	1%	1%	1%
9	**M9**	Complex	2%	0	0	0	2%
10	**M10**	Ethanol + 1%Vanillin	1%	1%	1%	1%	1%
11	**M11**	Ethanol + 1%Vanillin	1%	1%	1%	1%	0

4. 2. 2 Repellent test procedure : The tests were conducted in the Institute of Zoology, Cell biology and Parasitology (Heinrich Heine University Dusseldorf , Germany) within laboratory rooms maintained at 27 ± 2 ^{o}C and relative humidity 70 ± 10% as suggested by Thavara et al. (2001). The repellent effect of essential oils was evaluated using the human-bait technique (Schreck and McGovern 1989; WHO 1996). The testing period lasted up to eight hours depending on the efficacy. Since the target mosquitoes were day or night biters, ***Aedes aegypti*** was tested from 0800 h to 1600 h, while ***Anopheles stephensi*** and ***Culex quinquefasciatus*** were tested between 1600 h and 2400 h. The evaluation method used was similar to that described by Tawatsin et al. (2001). For testing, 0.1 ml of test material [oil solution, or oils mixture solution, or DEET or Bayrepel solution] was applied onto a 30 cm^2 marked area of a forearm of a human volunteer . Each arm was covered by a paper sleeve with a 30 cm^2 exposed area corresponding to the marked and treated site. After treatment, the volunteer introduced his arm every 30 minutes into a mosquito cage (48.5 ×

40 × 30 cm with glass caver) containing 250 nulliparous female mosquitoes being between 5 and 15 days old as recommended by Debboun et al. (1999) and left the arm there for 2 minutes. Before the start of each exposure period, the mosquitoes were tested for their readiness to bite by placing an untreated bare hand of a volunteer into a test mosquito cage for up to 15 sec. for ***Aedes aegypti*** , and for up to 30 sec. for ***Anopheles stephensi*** and ***Culex quinquefasciatus*** . The mosquitoes were blown from the hand before any blood was taken. If at least 2 mosquitoes bit the hand of the control person the repellency test was carried out, otherwise the test was not conducted. For the actual test, the number of landing mosquitoes without biting and the number of biting mosquitoes on marked area was recorded at each interval until either 2 bites occurred in a single 2 minutes exposure period, or 1 bite occurred in each of 2 consecutive exposure periods. At this point the test was terminated. On each day only one repellent preparation was tested, in order to leave time for residues to be lost from the skin before the next test (Curtis and Hill 1988).

- The duration period between the application of a repellent and the first 2 bites or 2 bites in successive observations was recorded as the **protection time**.

- The **percentage of repellency** was calculated at the end of every test by using the formula that was mentioned by Tawatsin et al. (2001) and Thavara et al. (2001) :

$$\textbf{\% Repellency} = \frac{\mathbf{C - T}}{\mathbf{C}} \mathbf{\times 100}$$

In this formule **C** is the total number of mosquitoes landing and/or biting at the control area (30 cm^2 on a human volunteer forearm without repellent material) and **T** is the total number of mosquitoes landing and/or biting at a treated area.

- The **percentage of landing mosquitoes** was calculated at the end of every test by using this formula :

$$\textbf{\% Landing} = \frac{\mathbf{L}}{\mathbf{250}} \mathbf{\times 100}$$

L represents the total number of landing mosquitoes at the end of test.

-The **percentage of biting mosquitoes** was calculated at the end of every test by using this formula:

$$\textbf{\% Biting} = \frac{\textbf{B}}{\textbf{250}} \times 100$$

B is the total number of biting mosquitoes at the test end.

4. 3 Results

4. 3. 1 Screening of oils according to their repellency properties : in the first stage oils were tested against the three mosquitoes species. The oils were tested in a 20% solution in a complex formulation (20% Genapol ; 10% PEG ; 20% Ethanol ; 50% water) to detect their repellency ability by estimating the protection period and calculating the percentage of repellency, percentage of landing mosquitoes and percentage of biting mosquitoes. Table 8 shows the protection period and percentage of repellency of the tested oils, DEET and Bayrepel against the three mosquitoes species. The protection period against ***Aedes aegypti*** ranged between 480 minutes for catnip (*Nepeta cataria*) , niaouli (*Melaleuca quinquenervia*), and litsea (*Litsea cubeba*) and only 60 minutes for eucalyptus (*Eucalyptus globulus*), tagetes (*Tagetes minuta*), and Chamomile (*Chamaemelum nobile*). The protection period of DEET 20% in the same formulation was 360 minutes and Bayrepel 20% in the same formulation reached only 240 minutes. The protection periods against ***Anopheles stephensi*** were 480 minutes for 26 oils from 41 tested ones, while in the remaining they were rather short: only 180 minutes for fichtennadel (*Picea excelsa*), and pepper,black (*Piper nigrum*) . However, DEET 20% and Bayrepel 20% in the complex formulation reached 480 minutes for each species. The protection periods for all tested oils, DEET and Bayrepel were 480 minutes against ***Culex quinquefasciatus*** except for dill (*Anethum graveolens*) which provided only 180 minutes protection against this species.

The percentage of repellency for ***Aedes aegypti*** ranged between 89.2 % for rosewood (*Aniba rosaeodora*) and 13.5 % for jasmine (*Jasminum grandiflorum*), but only 45.9 for DEET and 29.7 % for Bayrepel in the same formulation. In the case of ***Anopheles***

stephensi the repellency was 4.8 % for rosewood (*Aniba rosaeodora*) and 100 % for 13 other oils from 41 oils tested as well as for DEET and Bayrepel. In ***Culex quinquefasciatus*** the repellency was 57.1 % for camphor (*Cinnamonum camphora*) and dill (*Anethum graveolens*) and was 100 % for 33 oils from 41 tested oils, DEET and Bayrepel in the same solvent, as shown in (Table 8)

Table (8) The protection period and percentage of repellency of tested oils, DEET and Bayrepel against the three mosquito species.

NO	Name of material	*Aedes*		*Anopheles*		*Culex*	
		PP	%R	PP	%R	PP	%R
1	Citronella (*Cymbopogon winterianus*)	120	75.7	480	52.4	480	100
2	Rosewood (*Aniba rosaeodora*)	90	89.2	390	4.8	480	85.7
3	Lavender (*Lavandula angustifolia*)	180	24.3	480	80.9	480	85.7
4	Camphor (*Cinnamonum camphora*)	150	32.4	480	42.8	480	57.1
5	Catnip (*Nepeta cataria*)	480	83.8	480	100	480	100
6	Geranium (*Pelargonium graveolens*)	150	78.4	480	61.9	480	100
7	Thyme (*Thymus serpyllum*)	150	56.7	450	33.3	480	100
8	Eucalyptus (*Eucalyptus globulus*)	60	56.7	330	28.6	480	100
9	Jasmine (*Jasminum grandiflorum*)	270	13.5	480	100	480	100
10	Broad-Leaved (*Eucalyptus dives*)	210	18.9	480	38.1	480	100
11	Lemongrass (*Cymbopogon citratus*)	180	70.3	480	100	480	100
12	Lemonscented *Eucalyptus*(*Eucalyptus citriodora*)	150	59.4	480	52.4	480	100
13	Fichtennadel (*Picea excelsa*)	120	21.6	180	19	480	85.7
14	Amyris (*Amyris balsamifera*)	240	29.7	480	100	480	100
15	Lemon (*Citrus limon*)	90	67.6	420	9.5	480	100
16	Narrow-Leaved Eucalyptus (*Eucalyptus radiata*)	150	64.9	480	42.8	480	100
17	Carotin oil (*Glycina soja*)	180	16.2	480	9.5	480	100
18	Cedarwood (*Juniperus virginiana*)	180	37.8	480	38.1	480	100
19	Frankincense (*Boswellia carteri*)	120	75.7	300	19	480	100
20	Dill (*Anethum graveolens*)	90	78.4	210	71.4	180	57.1

21	Myrtle (*Myrtus communis*)	150	56.7	390	42.8	480	85.7
22	Chamomile (*Anthemis nobilis*)	240	64.9	480	76.2	480	100
23	Cinnamon (*Cinnamomum zeylanicum*)	330	70.3	480	100	480	100
24	Juniper (*Juniperus communis*)	210	43.2	480	76.2	480	100
25	Sage (*Salvia sclarea*)	120	45.9	300	19	480	100
26	Peppermint (*Mentha piperita*)	120	59.4	390	57.1	480	100
27	Basil (*Ocimum basilicum*)	120	81.1	210	66.7	480	100
28	Cajeput (*Melaleuca leucadendron*)	360	43.2	480	100	480	100
29	Soya bean(*Glycina max*)	180	54	480	76.2	480	100
30	Rosemary (*Rosmarinus officinalis*)	330	43.2	480	100	480	100
31	Niaouli (*Melaleuca quinquenervia*)	480	75.7	480	100	480	100
32	Olive (*Olea europaea*)	210	67.6	480	71.4	480	71.4
33	Black pepper (*Piper nigrum*)	90	64.9	180	61.9	480	100
34	Verbena (*Lippia citriodora*)	150	70.3	330	38.1	480	100
35	Tagetes (*Tagetes minuta*)	60	83.8	480	100	480	100
36	Violet (*Viola odorata*)	360	67.6	480	100	480	85.7
37	Sandalwood (*Santalum album*)	150	59.4	480	100	480	100
38	Litsea (*Litsea cubeba*)	480	73	480	100	480	100
39	Helichrysum (*Helichrysum italicum*)	120	43.2	360	47.6	480	100
40	Galbanum (*Ferula galbaniflua*)	150	70.3	480	100	480	100
41	Chamomile (*Chamaemelum nobile*)	60	70.3	330	47.6	480	100
42	Bayrepel 20% in complex solvent	240	29.7	480	100	480	100
43	DEET 20% in complex solvent	360	45.9	480	100	480	100

On the other side, the differentiation between repellent effect and feed deterrent effect of tested oils should be clear, Thus in Table 9 the percentage of landing and biting mosquitoes in every tested oil were shown. In ***Aedes aegypti*** the highest landing percentage was 12% for jasmine (*Jasminum grandiflorum*) and the smallest was 0.8% for rosewood (*Aniba rosaeodora*), while the percentage of biting mosquitoes ranged between 0% for niaouli (*Melaleuca quinquenervia*) and litsea (*Litsea cubeba*), and 1.6% for five tested oils such as thyme (*Thymus serpyllum*) , carotin oil (*Glycina soja*) , dill (*Anethum graveolens*), myrtle (*Myrtus communis*), juniper (*Juniperus communis*), and helichrysum (*Helichrysum italicum*). In ***Anopheles stephensi*** the percentage of landing mosquitoes was 0% for 13 oils from 41 oils tested as mentioned in Table 9 and 7.2% for carotin oil (*Glycina soja*). However, the rate of biting mosquitoes was 1.6% for rosewood (*Aniba rosaeodora*) and 0% for 18 oils as shown in (Table 9). In ***Culex quinquefasciatus*** the landing rate was 1.2% for camphor (*Cinnamomum comphora*) and 0% for 33 other tested oils as in (Table 9), but the percentage of biting was 0% for all tested oils except for dill (*Anethum graveolens*) 0.8%.

Table (9) The percentage of landing and the percentage of biting mosquitoes after application of the oils, DEET and Bayrepel tested against the three mosquito species.

NO	Name of material	*Aedes*		*Anopheles*		*Culex*	
		%L	%B	%L	%B	%L	%B
1	Citronella (*Cymbopogon winterianus*)	2.4	1.2	3.6	0.4	0	0
2	Rosewood (*Aniba rosaeodora*)	0.8	0.8	6.4	1.6	0.4	0
3	Lavender (*Lavandula angustifolia*)	10.4	0.8	1.6	0	0.4	0
4	Camphor (*Cinnamonum camphora*)	8.8	1.2	4.4	0.4	1.2	0
5	Catnip (*Nepeta cataria*)	2	0.4	0	0	0	0
6	Geranium (*Pelargonium graveolens*)	2.4	0.8	2.8	0.4	0	0
7	Thyme (*Thymus serpyllum*)	4.8	1.6	4.4	1.2	0	0
8	Eucalyptus (*Eucalyptus globulus*)	5.2	1.2	4.8	1.2	0	0
9	Jasmine (*Jasminum grandiflorum*)	12	0.8	0	0	0	0
10	Broad-Leaved (*Eucalyptus dives*)	11.2	0.8	4.8	0.4	0	0
11	Lemongrass (*Cymbopogon citratus.*)	3.2	1.2	0	0	0	0
12	Lemonscented Eucalyptus(*Eucalyptus citriodora*)	5.2	0.8	4	0	0	0
13	Fichtennadel (*Picea excelsa*)	10.4	1.2	6	0.8	0.4	0
14	Amyris (*Amyris balsamifera*)	9.6	0.8	0	0	0	0
15	Lemon (*Citrus limon*)	3.6	1.2	6.8	0.8	0	0
16	Narrow-Leaved *Eucalyptus* (*Eucalyptus radiata*)	4.4	0.8	4.4	0.4	0	0
17	Carotin oil (*Glycina soja*)	10.8	1.6	7.2	0.4	0	0

18	Cedarwood (*Juniperus virginiana*)	8	1.2	5.2	0	0	0
19	Frankincense (*Boswellia carteri*)	2.8	0.8	6	0.8	0	0
20	Dill (*Anethum graveolens*)	1.6	1.6	1.6	0.8	0.4	0.8
21	Myrtle (*Myrtus communis*)	4.8	1.6	4	0.8	0.4	0
22	Chamomile (*Anthemis nobilis*)	4	1.2	2	0	0	0
23	Cinnamon (*Cinnamomum zeylanicum*)	3.2	1.2	0	0	0	0
24	Juniper (*Juniperus communis*)	6.8	1.6	2	0	0	0
25	Sage (*Salvia sclarea*)	7.2	0.8	6	0.8	0	0
26	Peppermint (*Mentha piperita*)	4.8	1.2	2.8	0.8	0	0
27	Basil (*Ocimum basilicum*)	2	0.8	2	0.8	0	0
28	Cajeput (*Melaleuca leucadendron*)	7.6	0.8	0	0	0	0
29	Soya bean(*Glycina max*)	5.6	1.2	1.6	0.8	0	0
30	Rosemary (*Rosmarinus officinalis*)	7.2	1.2	0	0	0	0
31	Niaouli (*Melaleuca quinquenervia*)	3.6	0	0	0	0	0
32	Olive (*Olea europaea*)	4	0.8	2	0.4	0.8	0
33	Black pepper (*Piper nigrum*)	4	1.2	2.4	0.8	0	0
34	Verbena (*Lippia citriodora*)	3.2	1.2	4.4	0.8	0	0
35	Tagetes (*Tagetes minuta*)	1.6	0.8	0	0	0	0
36	Violet (*Viola odorata*)	4	0.8	0	0	0.4	0
37	Sandalwood (*Santalum album*)	4.8	1.2	0	0	0	0
38	Litsea (*Litsea cubeba*)	4	0	0	0	0	0
39	Helichrysum (*Helichrysum italicum*)	6.8	1.6	3.6	0.8	0	0
40	Galbanum (*Ferula galbaniflua*)	3.6	0.8	0	0	0	0
41	Chamomile (*Chamaemelum nobile*)	3.6	0.8	3.6	0.8	0	0
42	Bayrepel 20% in complex solvent	9.6	0.8	0	0	0	0
43	DEET 20% in complex solvent	6.8	1.2	0	0	0	0

From these experiments the best five oils were selected according to their properties in the Tables 8 and 9. **These five oils derived from :-**

1- Litsea (*Litsea cubeba*).
2- Cajeput (*Melaleuca leucadendron*)
3- Niaouli (*Melaleuca quinquenervia*).
4- Violet (*Viola odorata*) .
5- Catnip (*Nepeta cataria*) .

4. 3. 2 The test of the five best oils in comparison with DEET and Bayrepel at different formulations

To exhibit the effect of the formulation on the repellency properties of oils, the five best oils, DEET, and Bayrepel were tested using 20% solutions in three formulations against the three mosquito species :

The three formulations were:

1- Ethanol alone .
2- A complex formulation containing 20% Genapol, 10% PEG, 20% Ethanol, 50% water .
3- Ethanol containing 5% vanillin.

- The protection periods of the five best oils, DEET and Bayrepel at 20% solutions against the three mosquito species were shown in Figure 3. The complex formulation was the best one in all trials, the maximum period of 480 minutes was recorded using this formulation against ***Aedes aegypti*** for Litsea (*Litsea cubeba*), niaouli (*Melaleuca quinquenervia*) and catnip (*Nepeta cataria*) . Thus it was the best formulation against this mosquito species with respect to all tested oils except violet (*Viola odorata*), while the vanillin formulation was the best one with viola. Statistically there were no significant differences between these seven substances (five best oils, DEET and Bayrepel) against ***Aedes aegypti*** mosquitoes ($F=1.426$, $df=6$, $P>0.05$), but the differences were significant between the complex formulation and Ethanol and Vanillin formulations ($F=15.658$, $df=2$, $P>0.05$, $LSD=139.626$).

Against ***Anopheles stephensi*** the protection periods of these seven substances were 480 minutes using the complex formulation. The protection period with the vanillin formulation was 480 minutes for Violet (*Viola odorata*), Catnip (*Nepeta cataria*) and DEET but it decreased for others substances to unacceptable levels. With ethanol the protection periods were 480 minutes for Bayrepel and DEET, and 390 minutes for violet (*Viola odorata*), while decreased for others substances. Statistical analysis showed significant differences between the substances. DEET differed significantly from cajeput (*Melaleuca leucadendron*), litsea (*Litsea cubeba*), and niaouli (*Melaleuca quinquenervia*). Cajeput differed from Bayrepel, violet (*Viola odorata*), and catnip (*Nepeta cataria*), while other comparisons were not significant ($F=3.33$, $df=6$, $P>0.05$, $LSD=197.25$).

In the case of ***Culex quinquefasciatus*** mosquitoes all substances in the three formulations induced a 480 minutes protection period. Therefore no significant differences appeared against this mosquito species.

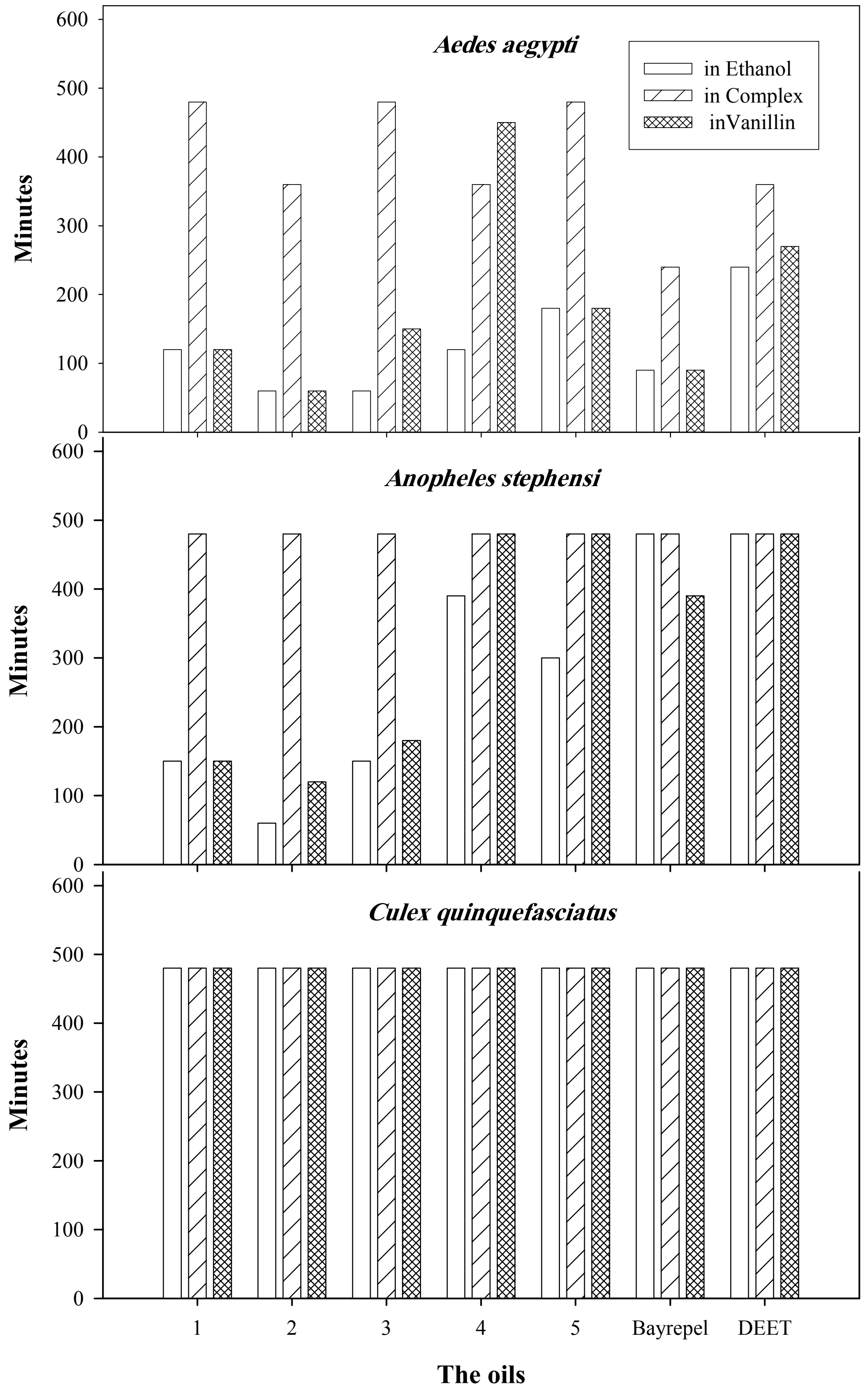

Figure (3). Protection period of the best five oils, Bayrepel and DEET with the three formulations against the three mosquito species.

-The percentages of repellency for seven substances (five best oils, DEET and Bayrepel) were presented in Figure 4. The repellency was very extremely different with all three formulations. With the complex formulation repellency against ***Aedes aegypti*** ranged between 29.7% for Bayrepel and 83.8% for catnip (*Nepeta cataria*), while from 5.4% for violet (*Viola odorata*) to 75.7% for DEET and cajeput (*Melaleuca leucadendron*) with the vanillin formulation, and between 48.6% for catnip (*Nepeta cataria*) and 86.5% for DEET with the ethanol formulation. The statistical analysis gave insignificant differences between the seven substances ($F=0.92$, $df=6$, $P>0.05$), as well as among the three formulations ($F=0.2$, $df=2$, $P>0.05$).

Against ***Anopheles stephensi*** the complex formulation was the best one, 100% repellency for all the seven substances was recorded. While the repellency of substances using the vanillin formulation ranged between 23.8% for violet (*Viola odorata*) and 80.9% for DEET, and from 28.6% for niaouli (*Melaleuca quinquenervia*) and catnip (*Nepeta cataria*) to 85.7% for DEET within ethanol. Statistically the analysis exhibited no significant differences between the seven substances ($F=2.207$, $df=6$, $P>0.05$). However, among the formulations the complex formulation was significantly better compared to the other two formulations ($F=29.19$, $df=2$, $P>0.05$, $LSD=14.43$).

In the case of ***Culex quinquefasciatus*** mosquitoes the complex formulation recorded 100% repellency for all substances except for violet (*Viola odorata*) reaching 85.7%, while the vanillin formulation recorded 100% repellency for all substances except for cajeput (*Melaleuca leucadendron*) 71.4%. All substances mixed in ethanol showed a 100% repellency except for cajeput (*Melaleuca leucadendron*) 28.6%. Statistical analysis appeared not significant differences between oils ($F=2.25$, $df=6$, $P>0.05$) and also not differences were seen among formulations against this species ($F=0.61$, $df=2$, $P>0.05$).

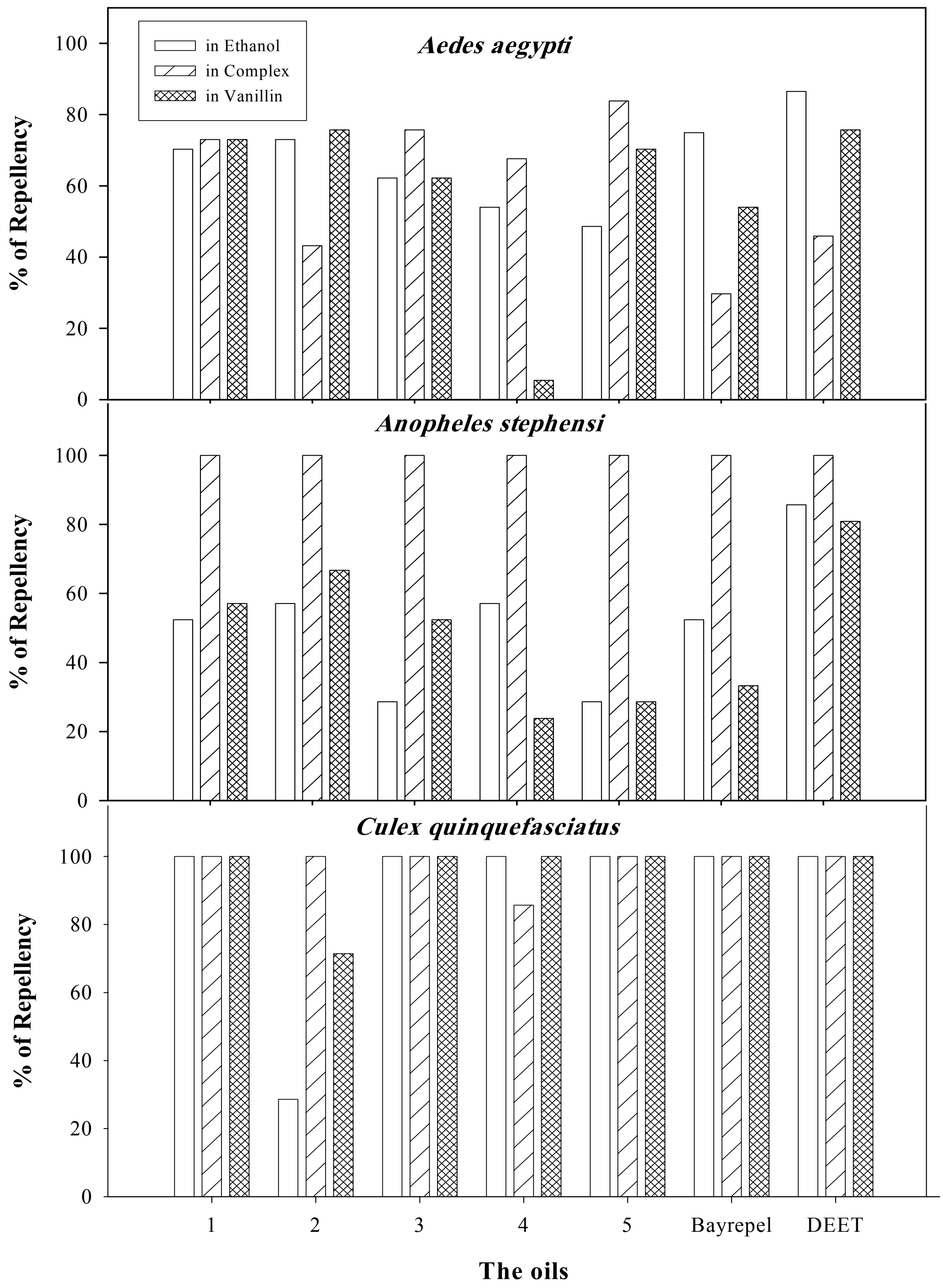

Figure (4). Percentage of repellency of the best five oils, Bayrepel and DEET with the three formulations against the three mosquito species.

- The percentages of landing mosquitoes on the areas treated by the seven substances (five best oils, DEET and Bayrepel) were shown in Figure 5. In ***Aedes aegypti*** the highest percentage of landing was 13.2% obtained on an area treated by violet (*Viola odorata*) with in a vanillin formulation, and the smallest percentage was 1.2% recorded in an area treated by DEET with ethanol. However, in ***Anopheles stephensi*** 6% was the highest percentage of landings obtained in violet areas with vanillin formulation and 0% was recorded from all the seven substances with the complex formulation. ***Culex quinquefasciatus*** landed in a rate of 2% on cajeput (*Melaleuca leucadendron*) areas with ethanol, 0.8% on cajeput areas with a vanillin formulation, and 0.4% in cases of violet with complex formulation, while 0% for others treatments. In the ***Aedes aegypti*** trials it was not clear which formulation was better, since each formulation was the best one with some substances and the worst with others, therefore no significant differences were noted between the different formulations ($F=0.339$, $df=2$, $P>0.05$), and between the seven substances ($F=0.15$, $df=6$, $P>0.05$) against this mosquito species.

Concerning the ***Anopheles stephensi*** experiments the complex formulation was the best one with all substances, statistically the differences were significant between the complex formulation and the other two formulations ($F=22$, $df=2$, $P>0.05$, $LSD=1.177$), while there were no significant differences between the seven substances ($F=1.82$, $df=6$, $P>0.05$).

All formulations with either of substances were good against ***Culex quinquefasciatus***. Thus these existed no statistical differences between the substances ($F=2.26$, $df=6$, $P>0.05$) or between the formulations ($F=0.614$, $df=2$, $P>0.05$) in this test system.

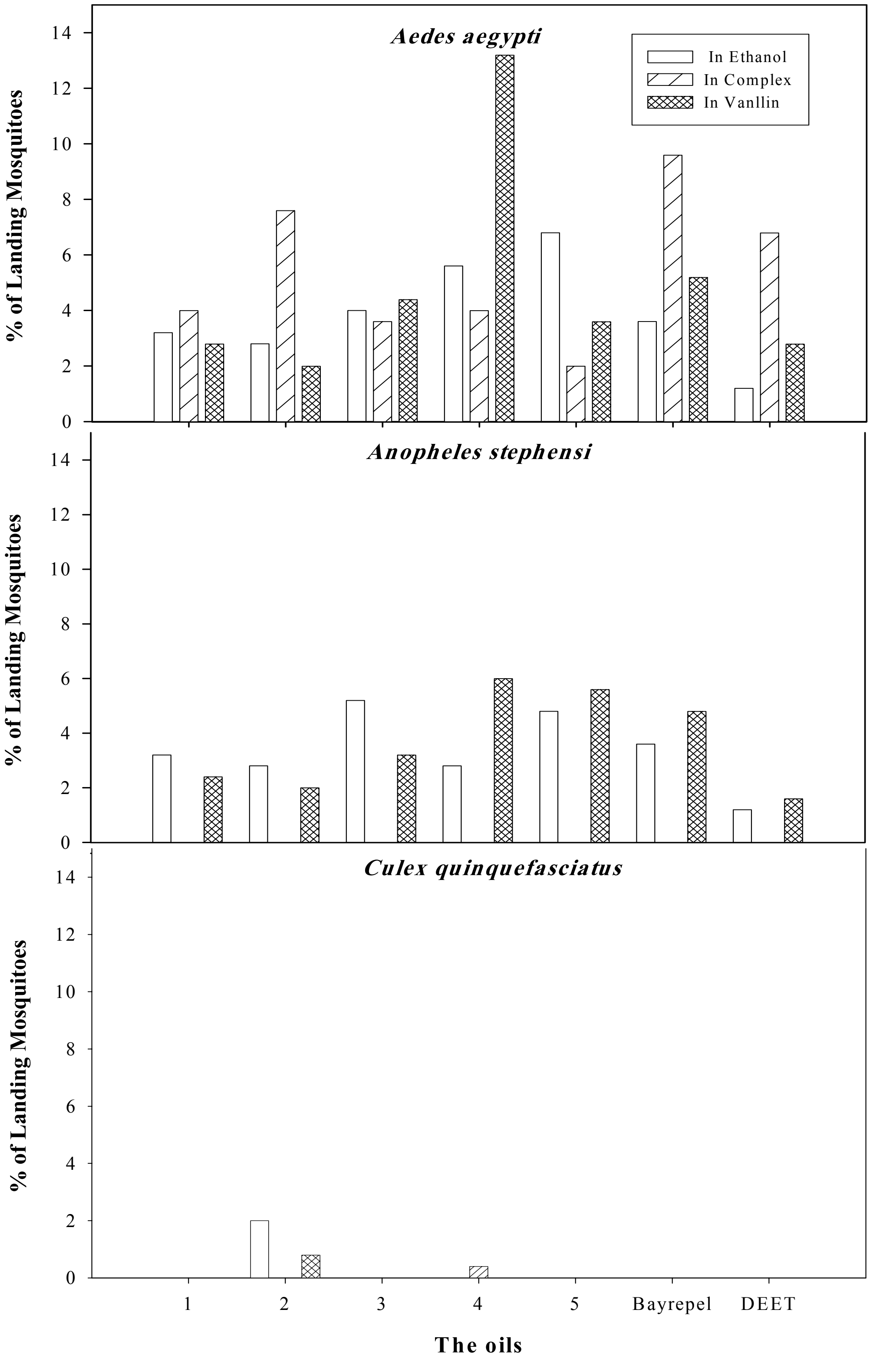

Figure (5) . Percentage of landing mosquitoes on skin treated with the best five oils, Bayrepel and DEET using the three formulations against the three mosquito species.

-The percentage of biting mosquitoes after treatment with the seven substances (five best oils, DEET and Bayrepel) and the three formulations were shown in Figure 6 . Against ***Aedes aegypti*** the complex formulation was the best one in combination with all substances except DEET, while the percentage of biting mosquitoes ranged from 0% for litsea ***(Litsea cubeba)*** and niaouli (*Melaleuca quinquenervia*) to 1.2% for DEET. The vanillin formulation allowed 1.6% of biting mosquitoes with cajeput (*Melaleuca leucadendron*) and Bayrepel , 1.2% with litsea and niaouli and 0.8% with the remaining substances. Statistical analysis showed insignificant differences among the seven substances ($F=0.155$, $df=6$, $P>0.05$), but the complex formulation was significantly different from the other two formulations ($F=5.9$, $df=2$, $P>0.05$, $LSD=0.42$).

With respect to ***Anopheles stephensi*** tests, the complex formulation was also the best one, because the biting rate was 0% for all substances with this formulation. The percentage of biting mosquitoes ranged between 0% for DEET and 1.2% for litsea with vanillin formulation, and from 0% for DEET to 1.2% for catnip (*Nepeta cataria*) with ethanol. Statistically the difference in the percentages of bitings was significant only between litsea and DEET, ($F=14.21$, $df=6$, $P>0.05$, $LSD=0.639$). Thus the complex formulation differed significantly from the other two formulation ($F=12.67$, $df=2$, $P>0.05$, $LSD=0.418$).

In the case of ***Culex quinquefasciatus***, mosquitoes the percentages of bitings were 0% for all seven substances using all three formulations.

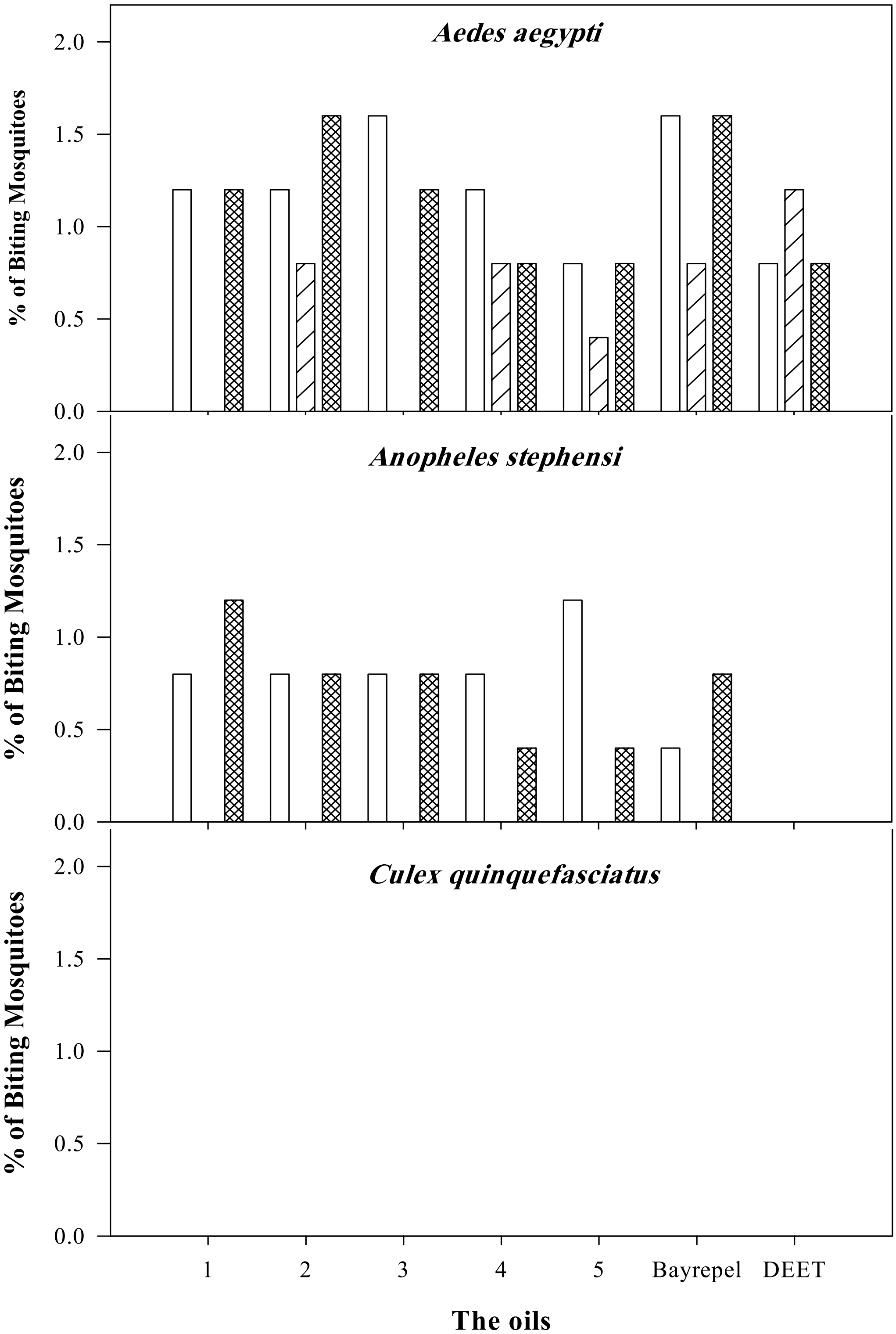

Figure (6) . Percentages of biting mosquitoes after treatment with the best five oils, Bayrepel and DEET using the three formulations against the three mosquito species.

4. 3. 3 Tests using oil mixtures

The five best oils that had been obtained from the basic screening in the first stage were used to prepare some mixtures at small concentrations (not more than 5%) in different solvents and formulations. Eleven mixtures mentioned in Table 7 were tested against the three mosquito species. Protection time and percentage of repellency of those mixtures were presented in Table 10. According to protection time and repellency, five mixtures were excellent repellent products against ***Aedes aegypti*** mosquitoes, offering 480 minutes of protection. However, their percentages of repellency ranged from 70.3% for M9 to 100% for M5. The protection time for other mixtures decreased to less than 270 minutes.

Concerning ***Anopheles stephensi*** mosquitoes nice results were obtained using seven mixtures, producing a 480 minutes protection period . 100% repellency were obtained with M1, M2, M3, M4, M5, M6, or M9, while the periods of protection and repellency decreased for other mixtures.

The protection periods were 480 minutes for all mixtures in the case of ***Culex quinquefasciatus*** mosquitoes. Furthermore the percentages of repellency were 100% for M1, M2, M3, M4, M5, M6, or M9, and decreased to 28.6% for M8 and 0% for M7, M10, and M11.

The statistical analysis of the protection times for all mixtures showed that, the differences were significant between ***Culex quinquefasciatus*** mosquitoes and other two species, while the difference was not significant between ***Aedes aegypti*** and ***Anopheles stephensi*** ($F=9.346$, $df=2$, $P>0.05$, $LSD=109.47$). Thus the differences were significant between M1, M4, M5, M6, and M9 on one side and M7, M8, M10, and M11 on the other, while the other comparisons between the mixtures were not significant ($F=3.307$, $df=10$, $P>0.05$, $LSD=209.63$). In percentage of repellency there is no significant differences between the mosquito species ($F=1.386$, $df=2$, $P>0.05$) but between the mixtures there are many significant differences, while, the differences were significant between M1, M2, M3, M4, M5, M6, and M9 on one side and M7, M8, M10 and M11 on the other. However the differences among M11 and M2 were not significant ($F=7.478$, $df=10$, $P>0.05$, $LSD=43.68$).

Table (10) . Protection periods and repellency of eleven oil mixtures against the three mosquito species.

NO	The Mixture*	*Aedes*		*Anopheles*		*Culex*	
		PP	% R	PP	%R	PP	%R
1	M1	480	86.5	480	100	480	100
2	M2	120	67.6	480	100	480	100
3	M3	270	73	480	100	480	100
4	M4	480	97.3	480	100	480	100
5	M5	480	100	480	100	480	100
6	M6	480	80.5	480	100	480	100
7	M7	120	40.5	150	38.1	480	0
8	M8	120	64.9	180	28.6	480	28.6
9	M9	480	70.3	480	100	480	100
10	M10	150	32.4	180	52.4	480	0
11	M11	120	81.1	210	57.1	480	0

* The oil mixtures formulas are explained in table 7.

The percentages of landing and biting mosquitoes for the eleven mixtures against the three mosquito species were shown in Table 11. In ***Aedes aegypti*** trials the percentages of landing ranged from 0% for M5 to 7.6% for M7 or M10, while the biting percentages ranged between 0% for M4, M5, M6, or M9 and 2.4% for M10.

In ***Anopheles stephensi*** mosquitoes the percentages of landing and/or biting mosquitoes were 0% for M1, M2, M3, M4, M5, M6 and M9, while the landing rate was 4.8% , 4% , 2.8%, and 2.4% for M8, M7, M10 or M11 respectively. The biting rate was only 1.2% for other four mixtures as seen in (Table 11).

Culex quinquefasciatus mosquitoes were weaker than previous the two species. The landing percentages were 0% for M1, M2, M3, M4, M5, M6 and M9, 2.8% for M7, M10 and M11, and 2% for M8, while the biting rate was 0% for all mixtures (Table 11).

The statistical analysis showed significant differences in percentages of landing between ***Aedes aegypti*** and the other two species (F=8.65, df=2, P>0.05, LSD=1.07). Among the mixtures the differences were significant between M7, M8, M10, and M11 on one side and M4, M5, or M6 in the other side. There were also significant differences between M1, M2, and M3 on one side and M7, M8, and M10 on the other, as well as among M9 and M7 and M10, and between M7 and M11 (F=5.5, df=10, P>0.05, LSD=2.149). Concerning the percentages of biting the differences were significant between ***Culex quinquefasciatus*** and the other two species ($F=8.03$, $df=2$, $P>0.05$, $LSD=0.368$). Among the mixtures occurred significant differences between M2, and M10 on one side and M4, M5, M6, and M9 on the other side, There were also significant differences among M2 and M3 ($F=2.76$, $df=10$, $P>0.05$, $LSD=0.887$).

Table (11). Percentages of the landing and the biting mosquitoes for eleven oil mixtures against the three mosquito species.

NO	The Mixture*	*Aedes*		*Anopheles*		*Culex*	
		%L	%B	%L	%B	%L	%B
1	M1	1.6	0.4	0	0	0	0
2	M2	4	0.8	0	0	0	0
3	M3	2.8	1.2	0	0	0	0
4	M4	0.4	0	0	0	0	0
5	M5	0	0	0	0	0	0
6	M6	2.2	0	0	0	0	0
7	M7	7.6	1.2	4	1.2	2.8	0
8	M8	4	1.2	4.8	1.2	2	0
9	M9	4.4	0	0	0	0	0
10	M10	7.6	2.4	2.8	1.2	2.8	0
11	M11	1.6	1.2	2.4	1.2	2.8	0

* The oil mixtures formulas are explained in table 7.

4 . 4 Discussion

4 . 4 . 1 The differences between repellent effect and feed deterrent effect

Many workers in field of the effects of plant materials against insects ignored the sensitive difference between these two effects. Therefore the present thesis tried to show this difference by recording the biting and landing mosquitoes separately in each trial. Therefore the protection times were determined as the time from material application until the first two bites. The percentage of repellency depended on the total number of landing and biting mosquitoes in treated and control areas. If the protection time was long and the material had feed-deterrent properties, the mosquitoes should more often land than try to bite. If the protection period of the material is long, the percentage of repellency should decrease because of the large number of landing mosquitoes. For example the protection period for jasmine (*Jasminum grandiflorum*) against ***Aedes aegypti*** was 270 minutes, while the repellency decreased to 13.5%. The protection time of rosemary (*Rosmarinus officinalis*) was 330 minutes, but the repellency was only 43.2% . This shows that these two oils are feed deterrents rather than repellents for ***Aedes aegypti*** mosquitoes. Against ***Anopheles stephensi*** mosquitoes carotin oil (*Glycina soja*) gave 480 minutes protection and only 9.5% repellency. This strongly shows that this oil is only a feed deterrent and is not repellent. There are many examples of feed deterrent effects against this mosquito species in Table 8. Only one oil in the tested group was a feed-deterrent against ***Culex quinquefasciatus*** : it was olive (*Olea europaea*) giving 480 minutes protection and 71.4% repellency.

By calculation the percentage of landing and biting mosquitoes using the number of landing mosquitoes and the number of biting mosquitoes dividend on total number of mosquitoes in test cage (250 female) and multiplied by 100. Thus the difference between the two effects becomes very clear in Table 9, although the number of biting mosquitoes was the limiting factor for the test duration. Therefore the percentages of biting were not high. But in any way the percentage of biting was always smaller than the percentage of landing except in the case of dill (*Anethum graveolens*) where the ***Culex quinquefasciatus*** the landing rate of mosquitoes was 0.4%, while the percentage of biting was 0.8%. In the case of jasmine the difference between landing and biting percentages was more extreme: ***Aedes aegypti*** mosquitoes reached 12% and 0.8% respectively. This means that this oil was a feed deterrent rather than a repellent. Also the percentage of landing was 7.2% for carotin oil, while the biting was only 0.4% in the case of ***Anopheles stephensi***. Therefore it was more a feed deterrent than a repellent. There are shown many more examples in Table 9.

4. 4. 2 Effects of formulation on repellency properties

The introduced method is very important to get any cosmetic or repellent product. The formulation must be the better way to make appear the repellency properties of a product with consideration to other factors like healthy and economical aspects. To use the essential oils as a commercial repellent product, their formulation must contain some fixation materials to fix the aromatic materials on the skin for as long as possible. Some workers mentioned, that the repellency properties of some essential oils against many arthropods are based on their aromatic constituents (Zhu et al 2001). The present study compares between vanillin as fixation material, a complex formulation used in some cosmetic products and only a mixture of essential oil in ethanol. The Figures (3) (4) (5) and (6) except for few a cases indicated that the complex formulation was the best one against all the three mosquito species, apparently because this formulation contained 20% Genapol and 10% Polyethylene glycol as fixation materials. In many previous studies the effects of base material of plant products as insect repellents were discussed. Das et al. (1999) evaluated the repellent properties of ***Zanthoxylum armatum*** D C. Syn. , ***Z. alatum*** Roxb. (Timur), ***Curcuma aromatica*** (Jungli haldi) and ***Azadirachta indica*** (Neem) against mosquitoes in two bases of mustard oil (***Brassica*** sp.) and coconut oil (***Cocos*** sp.). They found that all the herbal oils and DMP exhibited a better protection against the bites of mosquitoes in mustard oil base than in coconut. The use of vanillin as fixation material in the formulation of essential oils as insect repellents was evaluated by Tawatsin et al. (2001) using four plant oils. The authors mentioned that three volatile oils of four tested one can be formulated with vanillin in various forms to replace DEET. But our study did not confirm these results since the vanillin in our study was not strong enough to formulate the oils to induce an acceptable mosquito repellency.

Also the chemical insect repellents induced various repellent properties with different formulations. Gupta and Rutledge (1989) evaluated a six arthropod repellent formulations of DEET against two species of ***Aedes*** mosquitoes under three climates. The effects of formulations were more different, some formulations induced more bitings than the concurrent untreated control. Thus they mentioned that. repellency was not directly related to the DEET concentration in the various controlled-release repellent formulations.

4. 4. 3 Synergistic effects of essential oils in oil mixtures

This property occurs in oil mixtures using low concentrations . For example a mixture of the five best oils (e. g. 1% or 2% for each oil) the total concentration was not higher than 5% in the complex formulation gaving 480 minutes protection against all the three mosquito species as did M1, M4, M5, M6, and M9 with a repellency rates of 86.5%, 97.3%, 100%, 80.5% and 70.3% respectively against ***Aedes aegypti*** mosquitoes . In the oil mixtures usually some oils were more important than others. In the study against ***Aedes aegypti*** mosquitoes the following effects were seen. When the catnip and violet were removed from mixtures M2 and M3 the protection periods decreased to 120 and 270 minutes, and the percentage of repellency also decreased to 67.6% and 73.3% respectively, as well as the percentage of landing and biting of mosquitoes. Likewise, the formulation effect existed with respect to other mixtures (e. g. in M7 and M8), when the complex formulation was replaced by ethanol. When acetone was the solvent used without fixation material, the protection time decreased to 120 minutes for two mixtures against ***Aedes aegypti*** and the percentages of repellency were only 40.5% and 64.9% respectively. Against ***Anopheles stephensi*** mosquitoes only a 150 and 180 minutes protection time was reached, and only 38.1% and 28.6% repellency for M7 and M8 respectively. The vanillin was not good enough to induce the same effect that was induced by the complex formulation (e. g. in M10 contained 5% of all the five best oils at 1% for each one in ethanol and vanillin), their protection against ***Aedes aegypti*** was 150 minutes and only 32.4% repellency, moreover it had recorded the higher percentages of landing 7.6% and biting 2.4%. Thus against ***Anopheles stephensi*** it had resulted 180 minutes protection time, 52.4% repellency, 2.8% landing mosquitoes and 1.2% biting mosquitoes.

This means that a synergistic property occurred between these best five oils through the ability of small concentration mixtures to induce repellency properties better than large amount of any single oil. Furthermore the formulation containing fixation constituents was very important for the repellency properties.

4. 4. 4 The differences in response of mosquito species to essential oils

The responses of the three mosquito species studied in this thesis were extremely different. ***Culex quinquefasciatus*** was more sensitive for all oils and mixtures, while ***Aedes aegypti*** was tolerant to many oils and oils mixtures compared to ***Anopheles stephensi*** as is shown in Tables 8 , 9 , 10 and 11. Barnard (1999) explained the differences in responses of mosquito species for their preference of food sources. ***Aedes aegypti*** is an anthropophilic species with high biting pressures in laboratory bioassays, ***Culex quinquefasciatus*** is an ornithophilic biter. Thus it has only small appetite in laboratory trials, while ***Anopheles stephensi*** is a general mammalophilic. Thus it ranged in the middle between the previous two species.

5 . The sensilla of mosquitoes and their importance in repellency

5. 1 Introduction

The sensilla are sensory receivers with peculiar locations at the insect body being placed at antennas, maxillary bulbs, proboscis, tarsi, back side etc. The sensilla exist in several forms. Every one is specialized to receive a well defined stimulus such as mechanical effects, temperature or humidity changes, and any kind of odors.

The complex diversity of sensillar structures on insect antennae has puzzled scientists since the 19th century. The advent of electrophysiological recording methods enabled this diversity to be explained in terms of function. The grouping of receptor cells in separate entities enables a reproducible recording to be made from defined receptor cells. These recordings are more difficult with sensory epithelia, such as the vertebrate olfactory mucosa (Steinbrecht 1996).

Extensive literature on the types of sensilla exist on the antennae of nearly every insect taxon. The scanning electron microscope is useful for obtaining the first survey of external structure and distribution, but important aspects of innervation and modality specific structures require transmission electron microscopy for clarification. Since the heroic days of Slifer et al. (1959), considerable progress in specimen preparation and structural resolution has been made as in the introduction of cryofixation techniques (Steinbrecht 1980).

Zacharuk (1985) reviewed insect sensillar structure and classified olfactory sensilla on the basis of pore distribution and wall thickness. He distinguished between smooth-sided "multiporous pitted" or (**MPP**) sensilla, which my be thin-walled or thick-walled, and sculpted "multiporous grooved" or (**MPG**) sensilla.

There is also an aporous category, into which fall sensilla generally considered to be hygro/thermoreceptive. In such sensilla, convection or conduction heat and water vapor affect the dendrites either by passing through the overlying cuticle or by altering the micro conformation of the cuticle thus affecting the dendrites indirectly (Altner and Loftus 1985).

5. 1. 1 The important types of sensilla

In the past, various types of insect sensilla were identified at the basis of the shape of their cuticular parts and their position on, within or below the cuticle. The classification of nine basic types was brought together by (Snodgrass 1926 ; 1935).

Schneider et al. (1964) added another type of antennal sensilla, and two or three others types have appeared in the literature since then.

Zacharuk (1985) said that, except for the minor addition of types and some division of types into subtypes, the basic scheme of Snodgrass is very much in use today. It has a purely morphological basis, primarily being interpreted within the limits of the resolution of the light microscope. Through the years workers continually felt the need to describe functions to the various types of sensilla. Initially this was by inference from structure, position on specific parts or appendages of the body, or behavior of the insect after some form of incapacitation of specific sensory fields. Recently function of specific type of sensilla were identified electrophysiologically in some insects and were inferred for other sensilla in these and other insects by structural or distributional association with the known functional types. Inference of function from structure and position of sensilla was later corroborated electrophysiologically for some types of sensilla, but not for others. Inference by ultrastructural association with known function types can be similarly erroneous especially when the morphological knowledge is superficial.

Mclver (1982) was the last one that has published new information on the morphology of mosquito olfactory sensilla. He reviewed literature dating from the early 1950s and provided detailed summaries of distribution, numbers of different species and each sex, and of the ultrastructure of all Mclver's review (1982) provides information on aspects of the sensillar complement of mosquitoes from 11 genera. The most thoroughly studied species are ***Anopheles stephensi* , *Culex pipiens*** and ***Aedes aegypti***.

In ***Aedes aegypti*** five types of olfactory sensilla occur on the antennae (large and small sensilla coeloconica, sensilla ampullaceae, grooved pegs, sensilla trichodea). Another type occurs on the palps (capitate pegs) (Mclver 1982 ; Sutcliffe 1994).

The same sensillar types appear to occur with some variations in ***Anopheles stephensi*** and in other species. In addition, large sensilla coelconica occur only in anopheline mosquitoes. The morphology of these sensillar types is summarized in the following sections. In addition, the possible sensitivities and biological roles of these sensilla in mosquitoes, and in other insects, where equivalent types occur, is discussed:

Capitate pegs : this type of sensillum occurs on palpal segments 2-4 in female anophelines, on palpal segment 4 in male anophelines (Maclver and Siemicki, 1975), and on segment 4 only in male and female culicines (Mclver and Charlton 1970 ; Mclver 1971). Numbers present range from less than 20 per palp in female ***Uranotaenia* sp.** (Omer and Gillies 1971) to more than 200 in various ***Culex*** species (Mclver 1970) Males generally have smaller numbers than females.

Capitate pegs have been studied ultrastructurally in a number of mosquitoes including ***Aedes aegypti*** (Mclver 1972) and ***Anopheles stephensi*** (Mclver and Siemicki 1975). They are thin walled MPP sensilla possessing. In these species 3 neurons 2 of these have branching, digitiform dendrite and the 3rd produces a highly lamellate dendrite. Palpal ablation studies suggest a CO_2 detection role for these sensilla in ***Ae. aegypti*** and ***Culex quinquefasciatus*** (Bassler, 1958 ; Omer and Gillies, 1971). Carbon dioxide responsiveness within a behaviorally significant range (additions of 0.01% to ambient CO_2 concentration) for capitate pegs of ***Ae. Aegypti*** was confirmed electrophysiologically by Kellogg (1970). He also found that capitate pegs in this species respond to odors of n-heptane, acetone, and amylacetate (the latter induces inhibition).

To determine which dendrites in the capitate pegs responds to specific stimuli is not yet possible. However, morphological evidence can be used to build a strong circumstantial case for which dendrite is the probable CO_2 detector. Capitate peg equivalents occur in other members of the ***Diptera*** such as biting flies of the **Cerasimuliids** (Mercer and Mclver 1973) and **Ceratopogonids** (Rowley and Cornford 1972), where they are found in deep pits on the 3rd segment of the maxillary palp. In the black fly, ***Simulium arcticum*** , these pegs are innervated by a single neuron producing a lamellar dendrite (Sutcliffe et.al.,1987), whereas in the **Ceratopogonid *Culicoides furens***, they are innervated by 2 neurons, one producing a digitiform dendrite, the other a lamellate dendrite (Chuwang et. al. 1975).

Sutcliffe et. al. (1987) argued, that because all 3 biting groups (**Simuliidae, Ceratopogonidae,** and **Culicidae**) respond to CO_2 and because the lamellate dendrite is the only dendrite common to capitate pegs of the all three groups, the lamellate is the probable CO_2 detector.

In fact, many insects, not just bloodfeeders, are known to respond to CO_2. For example Bogner et.al. (1986) and Lee et. al. (1985) described palpal sensilla possessing only a lamellate dendrite in the moth ***Rhodogastria sp.***(**Arctiidae**) and in the butterfly ***Pieris rapae,*** **(Pieridae)**, respectively. Furthermore, Bogner et. al. (1986) demonstrated electrophysiologically that these palpal sensilla and therefore the lamellate dendrite within are CO_2 sensitive. This provides further circumstantial support for the lamellate dendrite of mosquitoes and other biting flies being the CO_2 sensitive unit. It would be very interesting to know what it is about CO_2 detection that necessitates such an elaborate dendritic structure (Sutcliffe 1994).

Morphological evidence also points out a seeming incongruity with respect to the sex specific occurrence of the capitate pegs, that is although females always have more such pegs, ***nematoceran*** males, which have no apparent need to locate the host, generally have some. What use could males make of this information?

Sutcliffe et. al. (1987) pointed out that some male black flies **(*Simulium arcticum*, *Boophthora erythrocephala*(De Geer), *Odagmia ornata* (Meigen))** intercept females at or near the hostand may, therefore orient to host odors including CO_2. Although males of a few mosquito species (including ***Ae. aegypti***) are also known to seek mates at or near hosts, this is not thought to occur widely (Sutcliffe et. al. 1987).

In studies involving animal baited trapping , male of the mosquito ***Aedes diantaeus*** made up more than 50% of trap catches and apparently are attracted by odors, because the bait animals were hidden behind a screen (Jaenson 1985). Perhaps male mosquitoes, black flies etc., orient to hosts to locate mates more than generally realized. Their absence from many trap collections may be due to differences in near-host response that normally keep them at a distance. Alternatively perhaps males of some species only seek mates at hosts under certain special conditions.

Although still not definitive, further evidence in support of mate seeking as a role for CO_2 detection by male ***nematoceran*** biting flies come from the finding that male ***Tx. brevipalpis*** lack capitate pegs altogether. This mosquito species does not feed blood. If the females do not mass around hosts, perhaps there is no point in the males possessing sensory equipment to detect CO_2 (Sutcliffe 1994).

Grooved pegs : males and females of all mosquito species examined to date possess the short deeply grooved sensilla called A3 sensilla in ***Ae. aegypti*** by Steward and Atwood (1963). Grooved pegs occur on all flagellar segments in females varying form 10 per antenna in female ***Uranotaenia lateralis***(Ludlow) to 350 per antenna in female ***Culex restuans*** (Theobald) (Mclver 1970). Male ***Ae. aegypti*** and ***An. stephensi*** have less grooved pegs than conspecific females (36 in male ***Ae. aegypti*** versus more than 100 in females) and they are restricted to antennal segments 12 and 13 (Mclver 1970).

Mclver (1974) described the grooved pegs in ***Ae. aegypti*** as thick-walled and having 3-5 but usually 3 unbranched dendrites that make contact with the outside by a single apical pore. In ***An. stephensi*** the grooved pegs are categorized into A1 and A2 subtypes but have the same number of dendrites and similar ultrastructure as in ***Ae. aegypti*** (Boo and Mclver 1976; Boo 1980). Grooved peg sensilla in ***Culex pipiens*** also resemble those of ***Ae. aegypti*** but possess only 2 dendrites (Elizarov and Chaika 1972).

Despite what appears to be a contact chemosensillar morphology, single apical pore, Mclver (1974)concluded that the grooved pegs must operate as olfactory sensilla because their shortness and the fact that they are located among much longer setae, would make contact with a substrate virtually impossible for them. Kellogg (1970)confirmed an olfactory function for the grooved pegs with physiological evidence that the grooved pegs of ***Ae. aegypti*** respond to vapors of ammonia, acetone, and water (by excitation) and of acetic acid and anisole by inhibition. Davis and Sokolove (1976) could only partially confirm this response spectrum, but were able to show that the grooved pegs of ***Ae. aegypti*** respond to lactic acid, a known mosquito attractant (Acree et. al. 1968). The lactic acid response comes from 2 cells, one is inhibited by increasing lactic acid concentration, whereas the other is excited by it.

The grooved pegs of ***Ae. aegypti*** also respond to organic vapors including those of certain fatty acids possibly skin associated and essential oils possibly flower and nectar source associated (Davis 1977). In addition, both lactic acid sensitive cells in the grooved pegs of ***Ae. aegypti*** are inhibited by DEET (Davis and Sokolove 1976). Moreover, the electrophysiological data were obtained a total of 120 grooved peg sensilla on the antenna of 30 virgin female of ***Ae. aegypti***. Of the 120 sensilla whose responses were examined, he obtained clear lactic acid-excited responses from 104 and clear lactic acid-inhibited responses in 16 . (Davis 1984).

The grooved pegs as described by (Mclver 1974) are very unusual as insect olfactory sensilla in that they have but a single apical pore instead of usual for olfactory sensilla numerous pores in the side walls. According to Zacharuk (1985) MPG sensilla which resemble strongly the grooved pegs have very small pores that open in bottoms of grooves in the peg shaft. These connect by means of spoke canals the lumen of the peg. Electron dense material from the dendritic chamber often fills these spoke canals and flows out to coat the bottoms of the grooves. Odorant molecules are thought to dissolve in this material and eventually diffuse to pores and into the peg lumen through the canals. Although Mclver (1974) observed electron dense strands, that look like spoke canals, this possibility was rejected because so few were seen. Spoke canals are easily missed, however, because they are not numerous (as few as 200 per sensillum) and may be as small as 5 nm in diameter (Zacharuk 1985). The grooved pegs also have electron dense material in their grooves. It is possible that the multiporous nature of these sensilla has been misinterpreted and that the single apical pore is actually a molting pore which occurs at the tips of many sensilla (Zacharuk 1985).

Finally there is enough morphological evidence to justify leaving the possibility that the antennal grooved pegs of mosquitoes are conventional MPG sensilla (Sutcliffe 1994).

Large sensilla coeloconica : Called sunken pegs by Boo and Mclver (1976) and simply sensilla coeloconica by Ismail (1962), the large sensilla coeloconica occur only in ***Anopheline*** mosquitoes**.**

Female of ***Anophelines*** which usually have a few such sensilla on each of the seven basal flagellomeres, have more than conspecific male which have between 8 and 14 mainly on the subterminal flagellomere (Mclver 1982).

Large sensilla coeloconica consist of short pegs (5µm in length) in ***An. stephensi*** (Boo and Mclver 1976). Four or five neurons extend branched dendrites into the peg lumen. The dendrites appear to have contact with the exterior by means of up to 16 grooves that run from just above the peg base to just below its tip. These grooves contain electron dense material along their bottoms that appears to come from the peg lumen. Although no spoke canals are observed in the micrgraphs, these sensilla fit the description of Zacharuk (1985) MPG sensilla may have closer affiliations with the grooved pegs. The large sensilla coeloconica are probably olfactory in function although no electrophysiological evidence exists to support this. However, if the large sensilla coeloconica are another form of grooved pegs, they may also have grooved peg type sensitivities to skin associated volatiles, DEET and/or lactic acid.

Small sensilla coeloconica : Two or three so called small sensilla coeloconica occur at the tip of both antennae of both sexes of all Culicine, Anopheline, and Toxorhynchitine species that have been examined (Mclver 1973; Boo and Mclver 1975; Mclver 1982). Each small sensillum coeloconicum consists of an aporous peg approximately 2-3 µm long set into the bottom of a shallow pit. Each peg is innervated by 2 neurons, the dendrites of which are closely packed into the peg lumen. The dendrite of a 3rd cell extends toward the peg base but stops well short of it and takes on a lamellate form. Typically, these sensilla occur in very small numbers usually at the antennal tips and at the ends of the antennal segments and typically, they are innervated by a triad of cells as in mosquitoes.

These sensilla have been studied physiologically in many insects, for example in stick insects (Tichy 1987), locusts (Ameismeier and Loftus 1988), and in certain lepidopteran caterpillars (Schoonhoven 1967; Dethier and Schoonhoven 1968). Although much variation in response has been found, such sensilla often have 2 cells that respond to changes in humidity (one cell being inhibited, the other excited by moisture increase) and a 3rd cell that responds positively to decreasing temperature that is a cold cell. The fact that air temperature and relative humidity may vary often poses problems in properly interpreting the responses of these sensilla.

Altner and Loftus (1985) point out that the microtubule filled dendrites of the aporous peg respond mechanically to moisture induced distortions of the peg cuticle. Although morphologically similar to mechanosensitive tubular bodies (Mclver 1975; 1985), these units may be functional moisture detectors. The lamellate dendrite may be the temperature sensor, the extent of development of the lamellae has been suggested to relate to the temperature range the dendrite is designed operate in the more lamellae, the lower the operate temperature range may be (Altner and Loftus 1985).

In the early ablation experiments that were conducted by Roth and Willis (1952) suggested that sensilla at the antennal tip of ***Ae. aegypti*** are thermosensitive. Davis and Sokolove (1975) made physiological recordings from the small sensilla coeloconica in the tip of the antennae of ***Ae. aegypti*** and showed that these sensilla possess 2 thermosensitive cells one being excited by increasing temperature, one being inhibited by it. No evidence was found for the detection of water vapor, CO_2 , or infrared radiation by these sensilla. Considering the extent of evidence showing such sensilla in other insects to be humidity sensitive, it may be premature, based on this one report, dismiss the possibility of hygroreception for such sensilla in all mosquitoes (Sutcliffe 1994).

Tempting to ascribe a role in host seeking behavior to the small sensilla coeloconica, those of ***Ae. aegypti*** are capable of responding to the small temperature changes that might occur within a meter or so from the foreface of the warm blooded host (Davis and Sokolove 1975). A role for the lamellate dendrite in host seeking is also suggested by morphological evidence that this dendrite is much reduced in size in the antennae of the nonhost seeker ***Tx. brevipalpis*** (Mclver and Siemicki 1978).

The lamellate dendrite is most elaborate in male ***Deinocerites cancer*** than in others insects, which spend much of their lives in dark crab holes where they attend female pupae and mate with the female adults as they emerge. Mclver and Siemicki (1976) suggested that the elaborate lamellate dendrite of male ***De. cancer*** is an infrared radiation receptor used to identify the older female pupae. The highly developed lamellate dendrites of cave beetles which also spend their lives in darkness were also suggested as infrared detectors . No subsequent support for detection by these sensilla has been forthcoming. On the other hand Altner and Loftus (1985) suggested that the more highly developed the lamellate dendrite the more sensitive it may be to small temperature changes through conduction or convection. This may explain the elaborate lamellate dendrite in the crab hole mosquito, because if about to emerge pupae are warmer than younger ones .

Although presumed thermo/hygrosensilla on biting insects may be involved in host seeking or in specialized cases mate seeking, such sensilla occur in virtually all insects, where they have been looked for. The function of these sensilla in insects including mosquitoes may be a general one, because for animal as small as insects sunlit biotopes may be quite unmanageable if not quickly lethal in the absence of instant clues about their temperature and humidity (Sutcliffe 1994).

Sensilla ampullaceae : (pegs in deep pits)also occur in small numbers along the antennae of male and female ***An. stephensi*** and ***Ae. aegypti*** . Their morphological similarity to small sensilla coeloconica suggests that the sensilla ampullaceae are not of the pore inflexible socket type sensilla (Boo and Mclver 1975; Mclver and Siemicki 1979), which use sensitive to thermal and moisture stimuli, however, no physiological investigations have been done to confirm their sensitivities.

Sensilla trichodea : these are the most numerous and varying sensilla on the mosquito antenna. Antenna of ***Tx. brevipalpis*** female bear more than 1200 of such sensilla (Omar and Gillies 1971), whereas those of female of smaller species bear less, e. g. 650 in ***Ae. aegypti*** (Steward and Atwood 1963), 550 in ***Anopheles*** **spp.**(Omer and Gillies 1971).numbers of sensilla trichodea on males are significantly lower than on conspecific females. Sensilla trichodea occur generally distributed over the antennal segments, are much longer than the grooved pegs, though shorter than the mechanoreceptive sensilla chaetica at the bases of most antennal segments, and occur in a number of variants based on length and with sharp or blunt at the tip. Mclver (1982) noted that all sensilla trichodea of the mosquito are innervated by 2 sensory cells and that each sensory cell produces an unbranched dendrite that extends the length of the inside of the seta. These conform to the thick walled MPP type of olfactory sensillum described by Zacharuk (1985) occurring widely in insects.

It is generally true that identifiable differences in morphology correspond to differences in sensory function, although it is not necessarily correct to conclude that sensilla of a given morphological type share the same sensory function.

In mosquitoes then, it is probable that the sensilla trichodea serve many specific sensory functions. A number of physiological studies have been done on the response spectra of sensilla trichodea of ***Ae. aegypti*** and other mosquito species. Many of them are summarized by Mclver (1982). Briefly sensilla trichodea types and subtypes have been found that respond to oviposition site related compounds (Davis1976; Bentley et al. 1982). Essential oils are often associated with nectar sources (Lacher 1967; Davis 1977), fatty acids and oils are associated with skin and to certain repellents (Lacher 1971; Davis and Rebert 1972). Interestingly none of the sensilla trichodea have been found to be sensitive to lactic acid. Zacharuk (1985) introduce nine basic type of sensilla that may be occur in different species in the Class ***Insecta*** , they served many kinds of sensory functions. As is shown in (Figure 7) .

[1- sensilla trichodea as chemosensitive, olfactory or thermosensitive ; 2-sensilla chaetica as tactile and perhaps chemosensitive ; 3-sensilla basiconica as hygrosensitivity ; 4-sensilla coeloconica as chemo, thermo or hygrosensitive. ; 5-sensilla ampullacea in function similar to those of coeloconica ; 6-sensilla squamiformia as mechano or chemosensitive ; 7-sensilla campaniformia as mechanosensors ; 8-sensilla placodea as chemosensory ; 9-sensilla scolopophora as chordotonal organs ; 10-sensilla styloconica as mechano or chemosensitive].

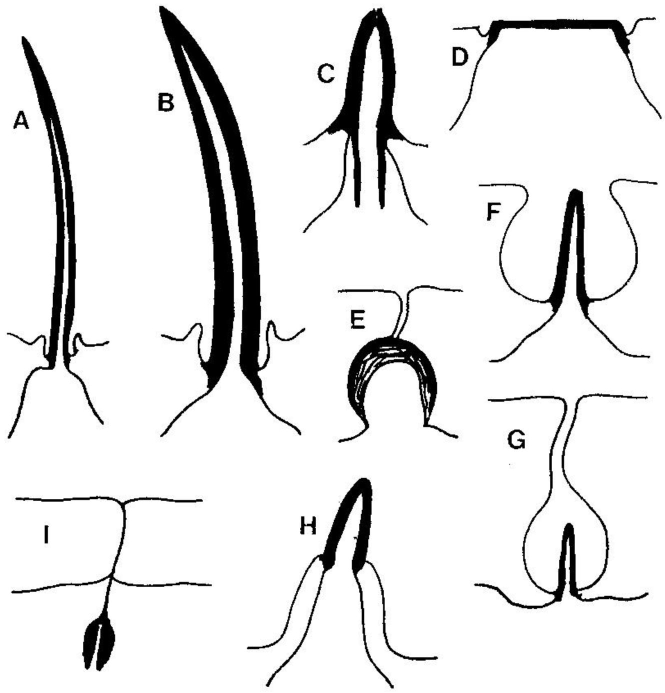

Figure (7) Primary cuticular features of the types of sensilla. A: trichodea, B: chaetica, **C**: basiconica, **D**: placodea, **E**: campaniformia, **F**: coeloconica, **G**: ampullacea, **H**: styloconica, **I**: scolopophora. (Zacharuk, 1985)

In general it is difficult to identify the sensilla or to include them to one of above types, since those types of sensilla are not stable yet and they need a lot of research to discover their types and their functions.

5. 1. 2 The nervous cells in sensilla

Two types of sensory cells were identified by Zawarzin (1912), their fine structure and relationships were clarified is classification still seems to be the most suitable today in the light of these (Figure 8) His type I neurons are bipolar and are provided with a dendrite that has a ciliary structure and is for the most part unbranched. The extreme tip of its cilium is simple, branched or lamellate in different types of sense organs, almost all of which are associated in one way or another with the cuticle. His type II neurons are bipolar or multipolar, with dendrites that arborize into many fine branches along their lengths. Typically they do not have a ciliary structure. Both types are ensheathed by glial cells to varying extents. But the type I neurons typically have at least two other cells associated with them peripherally. They and their associated cells are of epidermal origin. The specific organs that we normally term sensilla in insects typically contain type I neurons.

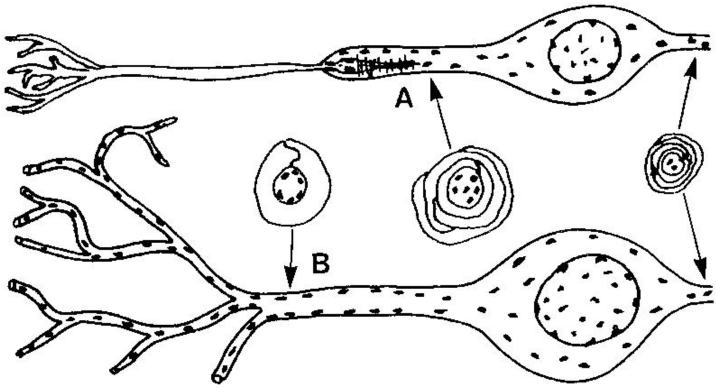

Figure (8) A: Type I neuron with a terminally branched dendritic ciliary segment, **B**: bipolar type II neuron with a small part of its terminal dendritic branches (Zacharuk, 1985).

An insect sensillum is suitably defined as a sense organ that has one or more bipolar type I neurons associated with cuticular parts or vestiges of these that extend above the surface of the cuticle or are within or beneath it, and the dendrites of which are enveloped by at least tow associated cells that form the cuticular parts or have other functions. Most workers agree that all insect sensilla evolved from an integumental hair or seta.

Thus , the basic components of all sensilla should be homologous. Most of the types of insect sensilla reported have either direct or indirect association with cuticle, thus and are considered here as cuticlar (Zacharuk 1985).

Mosquito cuticular sensory receptors (sensilla) are typical of those of insects in general. They consist an external cuticular process in the form of a seta or modified seta (depending on specific function). The seta is underlain by a small group of modified epithelial cells including a bipolar sensory cell and various sheath cells. Each sensory cell(s) extends a dendrite that associates with seta in a specific sensory modality manner. Each sensory cell also sends an axon to the central nervous system without aynapsing or fusing with others (Sutcliffe 1994).

Insect odor sensitive sensilla have from one (Schmidt and Gnatzy 1972) to more than fifty (Barlin and Vinson 1981) sensory cells, although the number 2-6 is more common (Zacharuk 1985). Dendrites from sensory cells usually extend, either branched or unbranched, into the hollow seta process, where they come into contact or close proximity with pores or pore-tubules extending from the pores. Odorant molecules apparently diffuse into the pores eventually coming into contact with, or affecting in some indirect manner the dendrites (Sutcliffe 1994).

5. 1. 3 Sensilla structure in relation to olfactory function

Sensilla structure is made to serve their function. This becomes clear in the morphological structure, numbers of several cell types, and their connections. The uniform structure of insect sensilla is one or several primary receptor cells and usually three auxiliary cells in combination with a special cuticular apparatus (seta) is well known and can be explained by a similar development from epidermal mother cells (Keil 1992). Modality specific structures in the sensory dendrites and in the cuticular apparatus are remarkably conserved throughout the insect orders. Consequently, predictions of sensillar function can be made from morphological data in (Figure 9) (Steinbrecht 1996). Mechanosensitive, olfactory sensilla, thermo/hygrosensitive sensilla and pheromone-sensitive olfactory receptors are reviewed by many workers. Olfactory sensilla, for example, are characterized by numerous pores in the sensillar wall, which are the supposed site of entry of odor molecules. The wall pores may be of the pore-tubule type, such as in single-walled sensilla, or of the spoke-channel type, such as the pores of the complex double-walled. Olfactory sensilla may be hair shaped and up to 500µm long, such as the pheromone-sensitive sensilla trichodea of the moth ***Manduca sexta*** (Keil 1989), or they may be short pegs less than 10 µm in length, such as the grooved pegs (A3-type sensilla) of the Yellow fever mosquito ***Ae. aegypti*** (Mclver 1974; Cribb and Jones 1995). In mosquitoes some 90% of antennal sensilla have an olfactory function (Mclver 1982; Sutcliffe 1994). There are sensilla trichodea of various length, which contain receptor cells for compounds related to oviposition sites (Bentley et al. 1982), nectar sources (Davis 1977) and certain repellents (Lacher 1971; Davis and Rebert 1972). Receptor cells for behaviorally active host attractants, such as L-lactic acid, have been found only with the short A3-type sensilla, which belong to the double-walled type of multiporous sensilla (Bowen 1995; Cribb and Jones 1995). The sensilla coeloconica of ***Ae. aegypti*** on the other hand, are a porous pegs with an ultrastructure similar to known thermo/hygroreceptive units (Mclver 1973). This example shows that the old nomenclature may be misleading with regard to modality-specific structures and sensillar function. In addition to those on the antennae, olfactory sensilla are also found on the maxillary palps. The capitate pegs are a uniform population of single-walled multiporous sensilla that respond mainly to CO_2, one of the dendrites displays extensive lamellation as do those of other CO_2 sensilla (Mclver 1972). The different sensillum types show characteristic differences in sensillum numbers and distribution patterns between the sexes, between ***culicine*** and ***anopheline*** mosquitoes, and even more drastic differences between blood and nectar feeders (Mclver 1982).

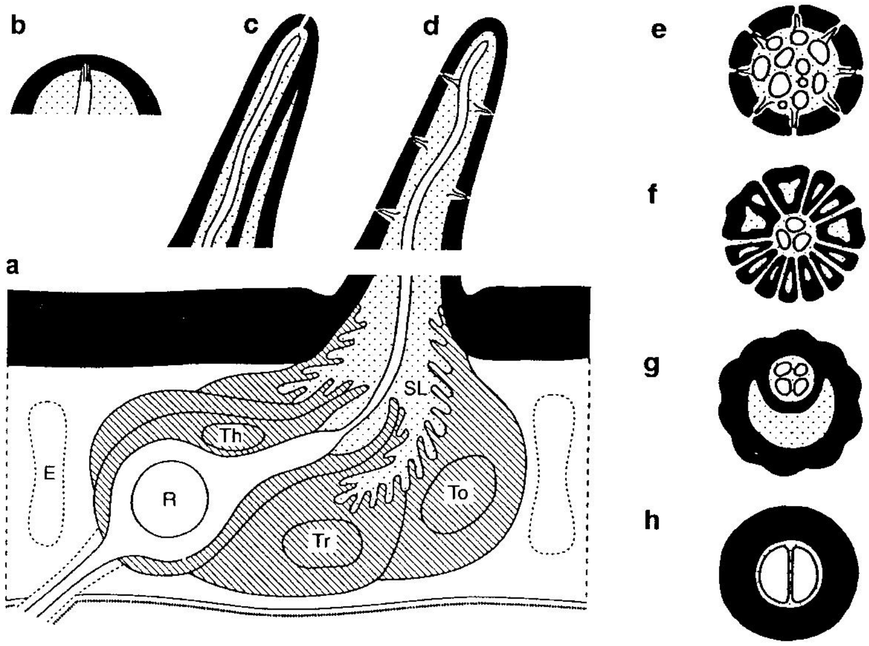

Figure (9) Schematic representation of insect sensilla. **A**: The cellular organization is rather uniform regardless of the specific receptor modality. One or several bipolar receptor cells (**R**) send an axon to the brain and a dendrite (with profiles in **b-h**) to the peripheral region of stimulus uptake which displays a specific cuticular apparatus. Three auxiliary cells (**Th, Tr, To**) surround the receptor cell(**s**) and border the sensillum lymph cavity (**SL**). The cuticle is black and undifferentiated epidermal cells (**E**)are shown as white shapes. Modality-specific specializations of the cuticular apparatus are shown in longitudinal section (**b-d**) and in cross-section (**e-h**). (**b**) Mechanosensitive campaniform sensillum. The dendrite displays a tubular body where it is compressed by deformations of the cuticle. (**c, g**) Gustatory sensillum. The dendrites of usually four taste receptor cells are exposed via terminal pore (**d, e**) Olfactory sensillum (single walled). The dendrites of several receptor cells responding to different odour qualities are accessible through wall pores. (**f**) double-walled olfactory sensillum with different structure of wall pores. (**h**) Poreless, thermo/hygrosensitive sensillum with two hygroreceptive dendrites. (Steinbrecht 1996).

5. 2 Materials and methods

5. 2. 1 Effect of ablation of some mosquito organs on repellency of Autan (Bayrepel) and an essential oil mixture

Two anthropophilic mosquitoes [(***Aedes aegypti*** , (Linnaeus) ; ***Anopheles stephensi*** , (Liston)] were used in this test that were carried out in the Institute of Zoology, Cell Biology and Parasitology at Heinrich Heine University (Düsseldorf : Germany). 250 female (15 days old) from the target species were taken and divided into five cages sized (48.5 × 40 × 30 cm), i. e. 50 female in each cage.

Ablation of mosquitoes organs : the five group of mosquitoes were treated as (group **1**: without antenna; group **2**: without maxillary bulbs; group **3**: without proboscis; group **4**: without frontal tarsus; group **5**: normal females as control). The organs of mosquitoes were ablated using micro shears under binocular after mosquito anesthetization.

Anesthetization of mosquitoes : the mosquitoes were anesthetized by exposing five individuals for 5 seconds (Tanaka 1985; El-Awami 1995) in a 50ml glass bottle to current of CO_2 gas released from other controlled glass bottle containing CO_2 dry ice. This treatment was done for a three minutes anesthetization period.

Repellents that were used in the experiment : a mixture from the best five oils in the repellency testes was used in this test. The mixture contained ***Litsea cubeba*** 1%, ***Melaleuca leucadendron*** 1%, ***Melaleuca quinquenervia*** 1%, ***Viola odorata*** 1%, ***Nepeta cataria*** 1% and was solved in the complex solvent composed by 20% Genapol , 10% PEG , 20% Ethanol and 50% water. Furthermore Bayrepel (1-piperidinecarboxylic acid, 2-(2-hydroxyethyl)-1-methylpropylester) was used in this experiment at a 20% concentration in the same solvent.

The test procedure : 100 µl of repellent material was embrocated on a $30cm^2$ exposure area of a human volunteer arm. The volunteer inserted his arm into the mosquito cage for three minutes every one hour. The number of biting mosquitoes and number of those only landing without biting were recorded in each exposure period in the tests. $30cm^2$ of a human arm was used uncovered and without any repellent as control for this experiment. After four hours the test was terminated. Each mosquito group was tested three times.

5. 2. 2 Morphology of mosquito sense organs

To study the morphology of mosquito sensilla that are located on the studied organs many photos were taken using a Scanning Electron Microscope with different magnifications using the same methods that were described in the chapters above.

5. 3 Results

5. 3. 1 Effects of ablation of organs on repellent sensation

Aedes aegypti : the percentages of biting mosquitoes in each group are presented in Figure 10. In the case of the mosquitoes without antenna the biting into the oil treated area was 3.33% and 0% in Bayrepel area, while it was 15.33% in control area. However, 12% of the mosquito group without maxillary bulbs fed in the oils mixture area and 5.33% in the Bayrepel area, while 46% of the mosquitoes were biting in the control area. In the case of the mosquito group without tips of the proboscis no biting was recorded in all types of treatment. In the group without the tip of the frontal tarsus and the control group the biting was recording only in the control treatment groups 26% and 27.33% respectively.

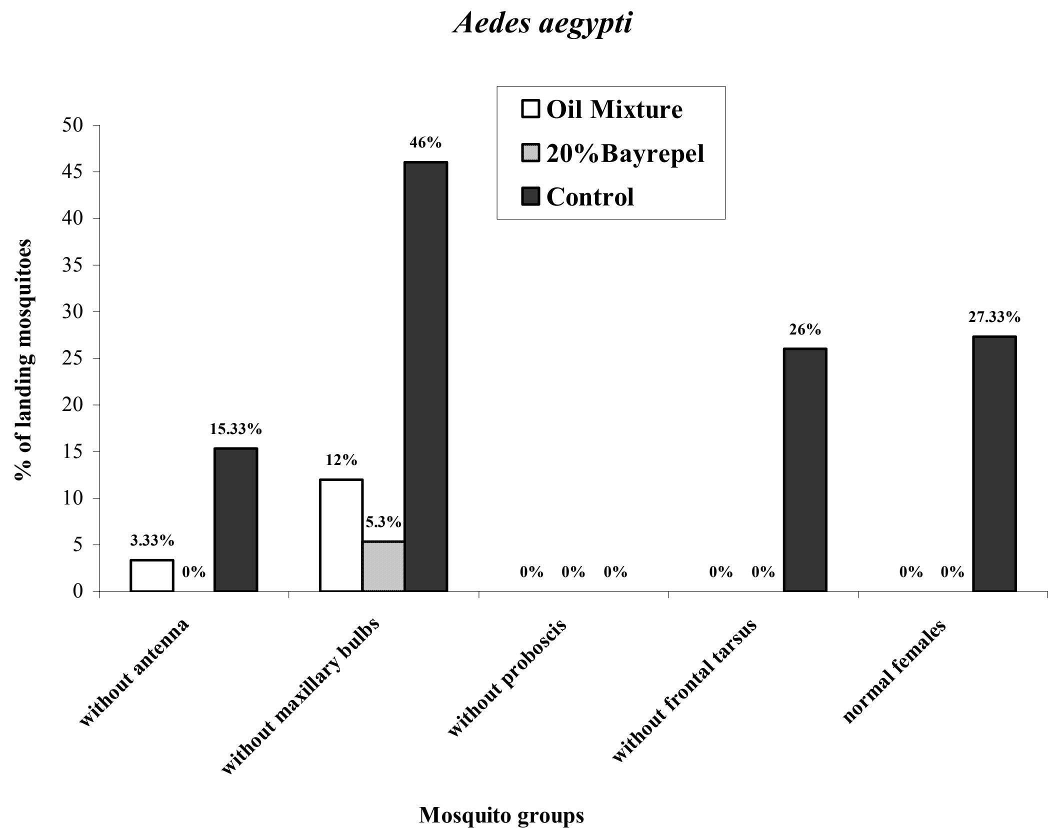

Figure (10) shows the percentages of biting of ***Aedes aegypti*** mosquitoes when human skin was treated with an oil mixture, Bayrepel or water (control).

Likewise the percentages of landing mosquitoes (landing without biting) on the treated areas are shown in Figure 11. 7.33% of mosquitoes without antenna were landing on oil mixture area and 4% on Bayrepel area while on the control area 47.33% were landing. 36.33% of mosquitoes without maxillary bulbs were landing on oil mixture area, 30% on Bayrepel area and 44% on control area. Moreover 9.33% of mosquitoes without the tip of the proboscis were landing on oil mixture area and 3.33% on Bayrepel area, while 30.66% were landing on control area. In the case of the mosquito group without the tips of the frontal tarsus 15.33% were landing on the oil mixture area and 12.66% on Bayrepel area, while 56% were landing on control area. But in the group of unchanged females only 2.66% were landing on the oil mixture area, 5.33% on Bayrepel area, but 66% were landing on the control area.

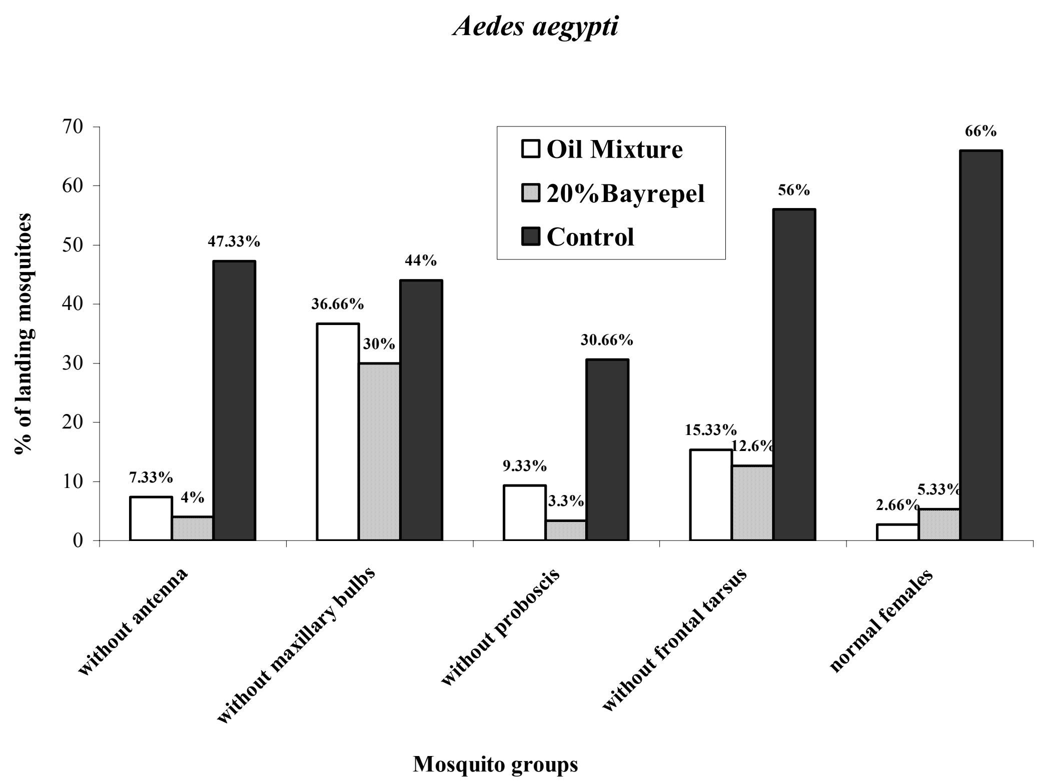

Figure (11) Percentages of landing of ***Aedes aegypti*** mosquitoes on treated human skin with oil mixture, Bayrepel or with water as control.

Anopheles stephensi : the percentages of biting mosquitoes on treated exposure areas are shown in Figure 12. In this species no biting mosquitoes were recorded in the oil mixture area and Bayrepel area. Thus the biting was only done in the control area in all mosquito groups except in the group without the tip of the proboscis. Biting was 0% in the control area, too.

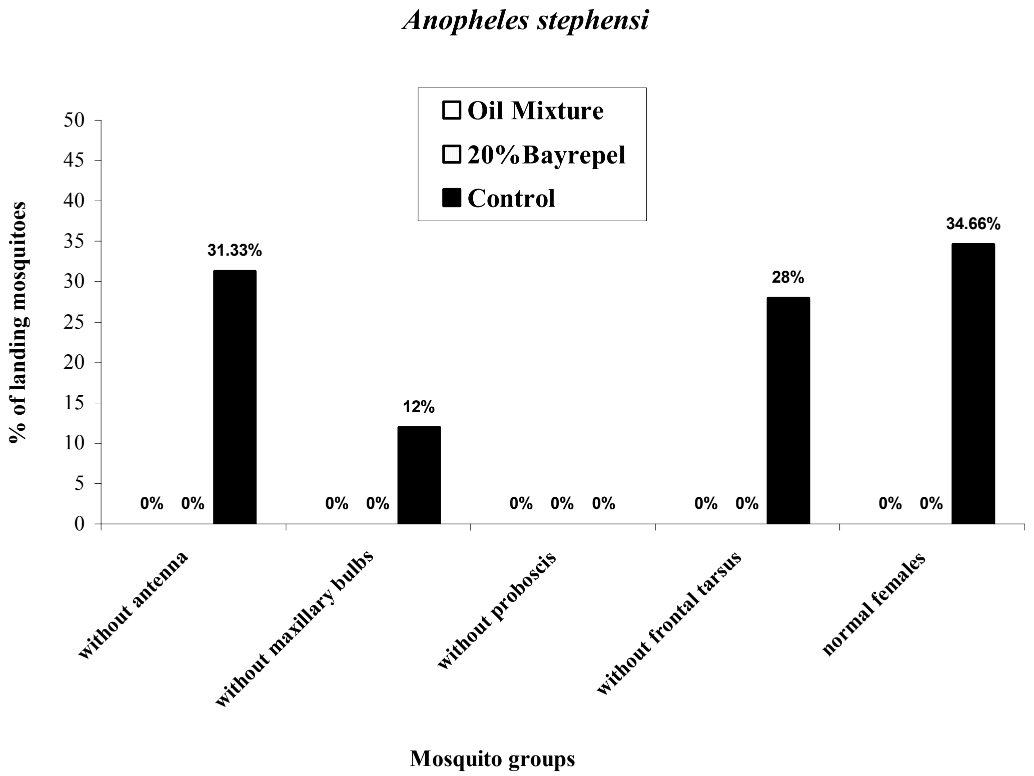

Figure (12) Percentages of biting ***Anopheles stephensi*** mosquito groups on human skin treated with oil mixture, Bayrepel or water as control.

The landing behavior is presented in the Figure 13. In the case of the mosquitoes group without antenna 10% landing mosquitoes were recorded in oil mixture area, , 3.33% in the Bayrepel area and, 46% in the control area respectively. While in groups without maxillary bulbs the landing percentages were 0% in oil mixture area and in Bayrepel areas, while it was 21.33% in the control area. When the tip of the proboscis was removed, the percentage of landing was 5.33% in control and 1.33% in each of the other two treatments. However, the mosquito group without the tip of the frontal tarsus was landing at percentages of 12.66%, 16% and 66.66% on the oil mixture, Bayrepel and control areas, respectively. The percentages of landing in normal female without any chipped of organs was 2.66%, 0.66% and 49.33% in oil mixture, Bayrepel and control areas respectively.

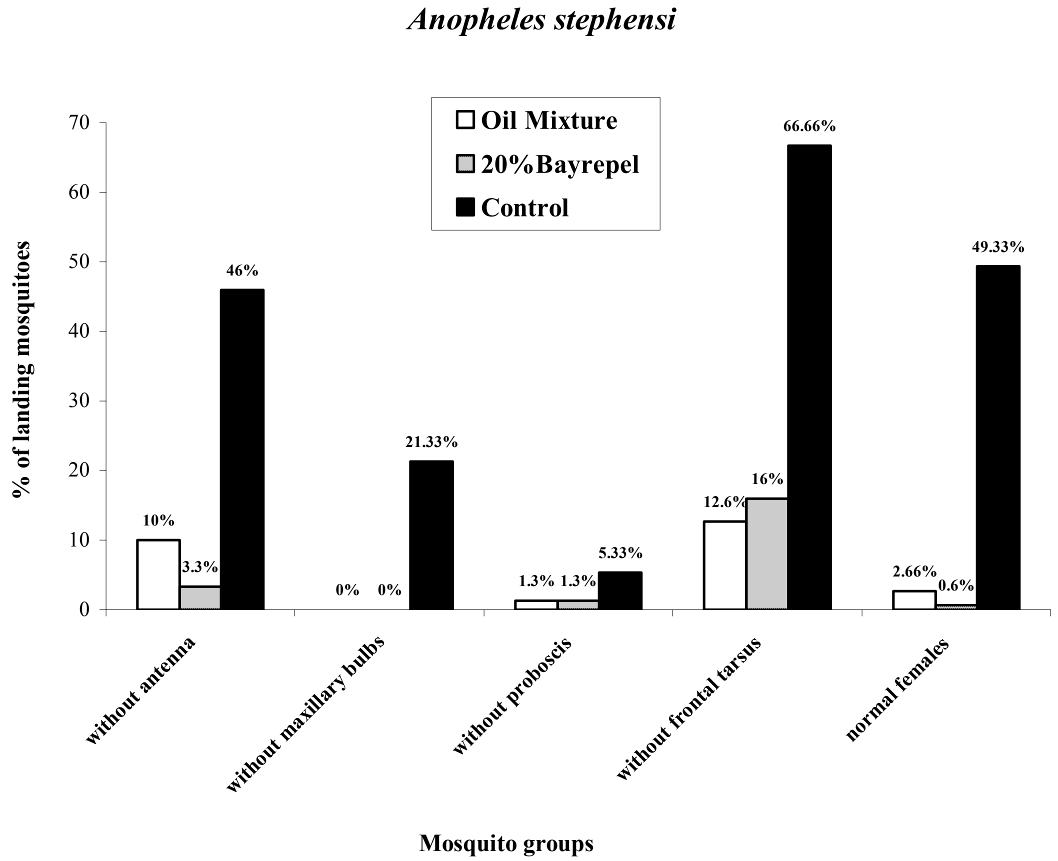

Figure (13) Percentages of landing of ***Anopheles stephensi*** mosquitoes on human skin treated with oil mixture, Bayrepel or water as control.

5. 3. 2 Morphology of mosquito sensilla

SEM micrographs were taken to study the mosquito organs to differentiate the typs and numbers of sense hairs that are located on the surface of these organs (antenna, maxillary bulbs, proboscis, and frontal tarsus). The micrographs are presented in Appendixes 4, 5, 6, 7 and 8 .

5 . 4 Discussion

5. 4. 1 Effect of organ ablation on the sensation of repellents

In the ***Aedes aegypti*** tests the increase of percentages of landing and biting mosquitoes in the mosquitoes groups that lacked some organs compared to normal mosquitoes became significantly clear. From Figures 10 and 11 the percentage of biting in oil mixture area was increased from 0% in normal females to 3.3% and 12% in the two mosquito groups without antenna and without maxillary bulbs, respectively. Likewise the landing percentages on oil mixture area were decreased in the normal females to 2.66%, while they were 7.33% in mosquitoes without antenna, 36.66% in the group without maxillary bulbs, 9.33% when the tip of proboscis was removed and 15.33% in the group of mosquitoes without the tip of frontal tarsus. However, in the Bayrepel tests the percentage of biting mosquitoes was 0% in all mosquito groups except in the group without maxillary bulbs where it was 5.33% . Here we can state, that the role of the maxillary bulbs in detecting the repellency effect of essential oils in ***Aedes aegypti*** mosquito is demonstrated. It will be justified when the great number and different kinds of sensilla on the maxillary bulbs were seen using the scanning electron microscope as presented in Appendix 5 .

On the other side the effects are not clear in the case of ***Anopheles stephensi***, because the results were balanced in all mosquito groups (see Figures 12 and 13). There is no clear differentiation in the results between normal female and other mosquito groups. Thus the responsible organs for repellent sensation in the ***Anopheles stephensi*** are surely different from those demonstrated in ***Aedes aegypti*** mosquitoes.

6 . Essential Oils As Mosquito Larvicides

6 . 1 Introduction

The materials extracted from various plant species have provided numerous beneficial uses ranging from pharmaceuticals to insecticides. Synthetic organic insecticides, although highly efficacious against target species such as mosquitoes, can be detrimental to a variety of animal life including man (Matsumura,1975). In addition to adverse environmental effects from conventional insecticides, most major mosquito disease vector and pest species have become physiologically resistant to many of these compounds (Brown,1986). These factors have created the need for environmentally safe, degradable and target-specific insecticides against mosquitoes. The search for such compounds has been directed extensively to the plant kingdom, whereas more than 2000 plant species are already known to have insecticide properties (Balandrin 1985, Rawls 1986, Sukamar et al., 1991). Historically, the commercial development of botanical insecticides is credited to a lady of Ragusa, Dalmatia, who noticed dead insects on a discarded bouquet of pyrethrin flowers. She began milling pyrethrum into powder and thus the pyrethrin industry was born (Sukumar et al.,1991). Since then, pyrethrins from ***Chrysanthemum*** flowers and many synthetic derivatives stand prominent as effective pesticides. The use of plant extracts, including allelochemical compounds such as essential oils, with known affects on insects, could be a useful complementary or alternative method to the heavy use of classical insecticides. This could improve the biodegradability of insecticide treatments and therefore decrease the quantity of toxic insecticide residues, increase insecticide selectivity and develop a better respect for the environment. This alternative strategy based on the identification of plant insecticidal molecules is not of recent. Origin humans have been traditionally using plants in order to protect crops (Regnault-Roger,1997). In the 19^{th} century, several active molecules were extracted from plants : nicotine, extracted from tobacco, appeared later to be toxic to mammals, rotenone from papilionidae, and pyrethrum from ***Chrysanthemum*** (***Compositeae***) which were chemically very unstable. The second world war, by upsetting economic and commercial trades, reduced the utilization of this first generation of plant insecticides. Consequently, petroleum-derived and chemical insecticides (carbamates, organochlorides, organophosphorous) were developed and have led to considerable ecological hazards. In the 1970s new pyrethroids were synthesized, enhancing the stability of the molecules, but they provoked insect resistance. Hence, over the last 15 years, research has been devoted to find other insecticidal molecules which could be extracted from plants (Arnason et al.,1989). Azadirachtin extracted from the tropical tree ***Azadirachta indica*** (***Meliaceae***) or neem, is one of the most representative compounds of this kind (Jacobson,1986 ; Saxena,1989). The most promising botanical groups are ***Meliaceae , Rutaceae, Asteraceae, Annonaceae, Labitae, Aristolochiaceae*** and ***Malvaceae*** (Regnault-Roger,1997). Some of them are characterized as aromatic plants.

One of the earliest reports of the use of plant extracts against mosquito larvae is credited to (Campbell et al.,1933), who found that plant alkaloids like nicotine, anabasine, methylanabasine and lupinine extracted from the Russian weed, ***Anabasis aphylla*** , killed larvae of ***Culex pipiens*** Linn., ***Cx. territans*** Walker, and ***Cx. quinquefasciatus*** Say. In a following study Holler (1940) noted that extracts from Amur cork tree fruit, ***Phellodendron amurense*** , induced mosquito larvicidal effect. Also the extracts deriveded from the male fern, ***Aspidium filix-mas*** , yielded a toxic constituent, filicin, a phloroglucinol propyl ketone, which was toxic to ***Cx. quinquefasciatus*** (Wilcoxon et al.,1940). Likewise Hartzell and Wilcoxon (1941) evaluated extracts from 150 species of plants for their toxicity to mosquitoes and found several to be very effective.

Ethanol extracts of 83 plant species belonging to the Asteraceae (Compositae) family, collected in the State of Minas Gerais, Brazil, were tested for larvicidal activity against the mosquito ***Aedes fluviatilis*** (***Diptera: Culicidae***). The extract from ***Tagetes minuta*** was the most active with a LC_{90} of 1.5 mg/l and LC_{50} of 1.0 mg/l. This plant has been the object of several studies by other groups and its active components have already been identified as thiophene derivatives, a class of compounds present in many ***Asteraceae*** species. The extract of ***Eclipta paniculata*** was also significantly active, with a LC_{90} of 17.2 mg/l and LC_{50} of 3.3 mg/l. Extracts of ***Achryrocline satureoides***, ***Gnaphalium spicatum***, ***Senecio brasiliensis***, ***Trixis vauthieri***, ***Tagetes patula*** and ***Vernonia ammophila*** were less active, killing more than 50% of the larvae only at the higher dose tested (100 mg/l) (Macêdo et al.,1997). Ten species of plants ***Amomum krevanh*** Pierre, ***Carthamus tinctorius*** L., ***Coriandrum sativum*** L., ***Eugenia caryophyllata*** Thunberg, ***Illicium vernum*** Hooker, ***Kaempferia galangal*** L., ***Murraya paniculata*** L., ***Myristica fragrans*** Houtt, ***Ocimum gratissimum*** L. and ***Spilanthes acmella*** Murr reported to possess carminative property, were screened for larvicidal potential against ***Culex quinquefasciatus***, marked larvicidal effects were seen with ***Kaempferia galangal***, ***Illicium vernum*** and ***Spilanthes acmella*** having LC_{50} values 50.54, 54.11 and 61.63 ppm respectively (Pitasawat et al., 1998). Methanol extracts of ***Abrus precatorius*** seed, ***Solanum suratense***, ***Solanum trilobatum*** and ***Leucas aspera*** leaves (Muthukrishnan et al.,1997) and ***Calophyllum inophyllum*** seed and leaf, and ***Rhinacanthus nasutus*** leaf (Pushpalatha and Muthukrishnan,1999) show significant larvicidal and growth regulatory activities at very low concentrations. Ethyl acetate fraction of ***Calophyllum inophyllum*** seed and leaf, ***Solanum suratense*** and ***Samadera indica*** leaf extracts and the petrol ether fraction of ***Rhinocanthus nasutus*** leaf extract, were evaluated against three mosquito species ***Aedes aegypti***, ***Anopheles stephensi*** and ***Culex quinquefasciatus***, the fecundity was decreased over the control ranged between 62.4 and 87.4% . and the sterility index of the mosquitoes reared in the media with the different extracts ranged between 82.8 and 93.3% at the concentration 50% (EC_{50}) (Muthukrishnan and Pushpalatha, 2001). Recently Jantan et al. (2003) evaluated of 17 methanol extracts and nine essential oils of Malaysian plants for their larvicidal activity against ***Aedes aegypti***. The essential oils of ***Cinnamomum impressicostatum*** Kosterm., ***C. microphyllum*** Ridl. and ***Curcuma domestica*** Valeton showed significant effect with LC_{50} values 13.7, 20.6 and 20.9 mg/ml respectively. Except for ***Zingiber cassumunar*** Roxb., the essential oils of the other species were also effective against the larvae with LC_{50} values less than 200 mg/ml of the methanol extracts, ***Garcinia praniana*** King, ***G. griffithii*** T. Anders, ***Labisia pumila*** var. ***alata*** Lindl., ***L. pumila*** var. ***pumila*** and ***Mitragyna speciosa*** Korth. showed relatively high activity with LC_{50} values ranged from 103 – 271 mg/ml. Generally the methanol extracts were less effective than the essential oils with most extracts exhibiting LC_{50} values greater than 500 mg/ml.

Since mosquitoes need water to breed, methods of prevention may include controlling water levels in lakes, marshes, ditches, or other mosquito breeding sites, eliminating small breeding sites if possible, and stocking bodies of water with fish species that feed on larvae. Both chemical and biological measures may be employed to kill immature mosquitoes during larval stages. Larvicides target larvae in the breeding habitat before they can mature into adult mosquitoes and disperse. Corbet et al. (2000) suggested that conventional larvicidal affect mosquito larvae in one or more of three possible modes: by physical flooding of the tracheal system, by toxicity especially by volatile components, and by interference with surface forces.

Larvicides include the bacterial insecticides ***Bacillus thuringiensis*** and ***Bacillus sphaericus***, the insect growth inhibitor methoprene, and the organophosphate insecticide temephos.

Mineral oils and other materials form a thin film on the surface of the water which cause larvae and pupae to drown. Liquid larvicide products are applied directly to water using back-pack sprayers and truck or aircraft mounted sprayers. Tablet, pellet, granular and briquet formulations of larvicides are also applied by mosquito controllers to breeding areas. source reduction aims to cut down opportunities for breeding, and can be as simple as turning over trapped water in a container to large-scale engineering and management of marsh water. Larviciding involves applying chemicals to habitats to kill pre-adult mosquitoes. Larviciding can reduce overall pesticide usage in a control program by reducing or eliminating the need for ground or aerial application of chemicals to kill adult mosquitoes. The application of easily degradable plant compounds is considered to be one of the safest methods of control of insect pests and vectors (Alkofahi et al.,1989).

Some essential oils and their components exhibited both a repellent and a larvicidal action, ***Ocimum*** volatile oils including camphor, cineole, methyl eugenol, limonene, myrcene and thymol, have repellent effects against mosquitoes, while ***O. basilicum*** exerted a larvicidal activity evaluated at EC_{50}=81ppm (Chokechaijaroenporn et al., 1994). Oil of peppermint ***Mentha piperita*** was examined as repellent and as larvicid against three mosquito species ***Aedes aegypti***, ***Anopheles stephensi*** and ***Culex quinquefasciatus***. As larvicid in a 3ml/m^2 of water surface area resulted in 100% mortality within 24h for ***Culex quinquefasciatus***, 90% for ***Aedes aegypti***, and 85% for ***Anopheles stephensi*** (Ansari et al.,2000) .

The present study tries to introduce first indications about the potentials when using essential oils as mosquito larvicides.

6 . 2 Materials and Methods

6 . 2 . 1 Screening of essential oils with respect to their larvicidal properties

The tests were conducted in the Institute of Zoology, Cell Biology and Parasitology of the Heinrich Heine University (Dusseldorf : Germany) at laboratory room temperature. The group of used essential oils are listed in Table 2 . In thes first phase the oils were tested against third instar larvae of ***Aedes aegypti*** mosquito (WHO 1981; Ansari et al. 2000; Rey et al. 2001) to detect their toxicity on mosquito larvae. Three replicates of each oil were prepared by dissolving the suitable amount of oil in tap water using acetone to make a 600 ml of 50 ppm oil solution. The solution was filled into three 500 ml glass beakers (200 ml for each). While only 2 ml acetone and 198 ml tap water were used in the control replicates (Xue et al. 2001). Ten 3^{rd} instar larvae of ***Aedes aegypti*** were transferred to each beaker. Number of dead larvae in each beaker was counted after 1, 12, and 24 hours of contact at room temperature. The larvae were considered dead, if they were immobile and unable to reach the water surface (Macêdo et al. 1997). The oils which failed to give 100% mortality after contact 24h were no more used, whereas the other oils that provided 100% mortality after contact less than 24h were selected and used for the next stages of the study.

6 . 2 . 2 Evaluation of selected oils against larvae of three mosquito species

These experiments were conducted following the results of the first stage aiming to calculate the LC_{50} of selected oils against the third instar larvae of the three mosquito species ***Aedes aegypti***, ***Anopheles stephensi***, and ***Culex quinquefasciatus*** . The trials were performed in the laboratory room at room temperature. Thirteen selected oils were targeted in this trials.

6 . 2 . 2 . 1 Preparation of the oil solution

Enough amount of target oil was dissolved in tap water using 2 ml of 100% acetone to produce a stock solution at 500 ppm. This solution was used to prepare the other serial solutions of target oil on concentrations such as 500, 100, 50, 10, or 1 ppm through dilution of the stock solution by tap water , three replicates of each concentration were made in addition to three control replicates containing 0 ppm oil, 2 ml 100% acetone and tap water.

6 . 2 . 2 . 2 Bioassay of oil solution

Each replicate containing 200 ml of the described oil solution was placed in a 500 ml glass beaker. Ten third instar larvae from target mosquitoes were transferred into each beaker (Mohtar etal.,1999). Thereafter the beakers were left on the laboratory table for 24 hours. The number of dead larvae in each beaker were counted after 1, 12, and 24 hours.

6 . 2 . 3 Persistence of oil toxicity under different circumstances

The thirteen oils which have been selected from results of the first stage were used in these trials to find out the survival of their toxicity during one month after preparation of the solution.

50 ppm oil solution were prepared by dissolving the calculated amount of target oil in tap water using 2 ml of 100% acetone. Thereafter this solution was divided into four glass wares. Two of these wares had fast plugs and the remained two stayed open without cover. Then, one of closed and one of opened wares were stored in a dark cabinet and the other two were placed on the laboratory table under laboratory conditions.

The bioassay of these four parts was conducted immediately and in a duration 1, 2, 3 weeks after solution preparation. At each time three replicates from each part were tested by transferring 100 ml from oil solution in each replicate into 500 ml glass beaker. Then 10 third instar larvae of ***Aedes aegypti*** were placed in each beaker , thereafter the replicates beakers were stored on a laboratory table for 24 hours under laboratory conditions. The number of dead larvae in each replicate was counted at 1, 2, 3, 6, 12, 24 hours after their contact with the oil solution.

6 . 2 . 4 Calculation of LC_{50}, and statistical analysis

Values of the LC_{50} (the concentration at which 50% of larvae were immobilized) were calculated by probit analysis using the PROBIT software SPSS . while RCBD ANOVA and LSD test were used to detect the significant differences between the treatments in all tests.

6 . 3 Results

6 . 3 . 1 Screening of the oils for larvicidal properties

Forty-one oils were screened for their toxicity to the 3rd instar of ***Aedes aegypti*** larvae in this phase of the study. The percentages of mortality were calculated for each oil after 1, 12, and 24 hours as shown in (Table 12).

Only thirteen oils induced 100% mortality after 24 hours or less. Thus these oils were selected for the following trials.

- The selected oils were :-
    1. Camphor (*Cinnamomum camphora*).
    2. Thyme (*Thymus serpyllum*).
    3. Amyris (*Amyris balsamifera*).
    4. Lemon (*Citrus limon*).
    5. Cedarwood (*Juniperus virginiana*).
    6. Frankincense (*Boswellia carteri*).
    7. Dill (*Anethum graveolens*).
    8. Myrtle (*Myrtus communis*).
    9. Juniper (*Juniperus communis*).
    10. Black Pepper (*Piper nigrum*).
    11. Verbena (*Lippia citriodora*).
    12. Helichrysum (*Helichrysum italicum*).
    13. Sandalwood (*Santalum album*).

Table (12). Percentages of mortality of ***Aedes aegypti*** third instar larvae in 50 ppm oils solutions after 1, 12, and 24 hours.

NO	Name of material	% of dead larvae *		
		After 1 h	After 12h	After 24h
1	Citronella (*Cymbopogon winterianus*)	6.67	43.3	60
2	Rosewood (*Aniba rosaeodora*)	0	33.3	60
3	Lavender (*Lavandula angustifolia*)	3.3	40	63.3
4	Camphor (*Cinnamonum camphora*)	93.3	100	100
5	Catnip (*Nepeta cataria*)	0	40	40
6	Geranium (*Pelargonium graveolens*)	0	63.3	73.3
7	Thyme (*Thymus serpyllum*)	36.7	100	100
8	Eucalyptus (*Eucalyptus globulus*)	10	16.7	16.7
9	Jasmine (*Jasminum grandiflorum*)	0	6.7	6.7
10	Broad-Leaved Eucalyptus (*Eucalyptus dives*)	43.3	83.3	96.7
11	Lemongrass (*Cymbopogon citratus.*)	0	0	0
12	Lemonscented Eucalyptus(*Eucalyptus citriodora*)	0	43.3	76.7
13	Fichtennadel (*Picea excelsa*)	60	90	96.7
14	Amyris (*Amyris balsamifera*)	0	90	100
15	Lemon (*Citrus limon*)	96.7	100	100
16	Narrow-Leaved Eucalyptus (*Eucalyptus radiata*)	6.7	46.7	50
17	Carotin oil (*Glycina soja*)	3.3	43.3	53.3
18	Cedarwood (*Juniperus virginiana*)	6.7	100	100
19	Frankincense (*Boswellia carteri*)	66.7	100	100
20	Dill (*Anethum graveolens*)	93.3	100	100
21	Myrtle (*Myrtus communis*)	86.7	96.7	100

22	Chamomile (*Anthemis nobilis*)	0	0	3.3
23	Cinnamon (*Cinnamomum zeylanicum*)	0	86.6	90
24	Juniper (*Juniperus communis*)	73.3	100	100
25	Sage (*Salvia sclarea*)	3.3	43.3	46.7
26	Peppermint (*Mentha piperita*)	0	36.7	53.3
27	Basil (*Ocimum basilicum*)	3.3	70	86.7
28	Cajeput (*Melaleuca leucadendron*)	0	3.3	3.3
29	Soya bean(*Glycina max*)	0	0	0
30	Rosemary (*Rosmarinus officinalis*)	6.7	10	16.7
31	Niaouli (*Melaleuca quinquenervia*)	13.3	13.3	30
32	Olive (*Olea europaea*)	0	26.7	43.3
33	Black pepper (*Piper nigrum*)	86.7	100	100
34	Verbena (*Lippia citriodora*)	63.3	100	100
35	Tagetes (*Tagetes minuta*)	0	3.3	3.3
36	Violet (*Viola odorata*)	3.3	86.7	86.7
37	Helichrysum (*Helichrysum italicum*)	6.7	100	100
38	Litsea (*Litsea cubeba*)	0	50	50
39	Sandalwood (*Santalum album*)	83.3	100	100
40	Galbanum (*Ferula galbaniflua*)	6.7	13.3	13.3
41	Chamomile, Roman (*Chamaemelum nobile*)	40	20	50

- each percentage was calculated for thirty larvae in three replicates.

6 . 3 . 2 Evaluation of selected oils against the larvae of three mosquito species

The thirteen selected oils from the last trails were evaluated here against the third instar larvae of the three mosquito species. The LC_{50} of these oils was calculated for 1, 12, 24 hours after application :

The LC_{50} after one hour of the thirteen selected oils against larvae of the three mosquito species are shown in (Figure 14). For ***Aedes aegypti*** larvae the LC_{50} ranged between 9.7 and 101.4 ppm . Using the overlapping of the standard errors of the LC_{50} values there are many significant differences between oils. Camphor (*Cinnamomum camphora*), amyris (*Amyris balsamifera*) and lemon (*Citrus limon*) differed significantly than others, while the differences between thyme (*Thymus serpyllum*), cedarwood (*Juniperus virginiana*), frankincense (*Boswellia carteri*), dill (*Anethum graveolens*), myrtle (*Myrtus communis*) and sandalwood (*Santalum album*) an one side were significant compared with juniper (*Juniperus communis*), black pepper (*Piper nigrum*), verbena (*Lippia citriodora*) and helichrysum (*Helichrysum italicum*) on the other side. The other comparisons were not significant. Against ***Anopheles stephensi*** the LC_{50} ranged from 50.2 to 101.4 ppm. Statistically the differences were significant between thyme, amyris and cedarwood on one side and the other oils on the other side. However no differences remained to other comparisons. In the case of ***Culex quinquefasciatus*** the LC_{50} values varied from 10 to 101.4 ppm. The overlapping was absent between dill and other oils: thus between camphor, thyme, lemon, frankincense, myrtle, juniper, pepper and sandalwood on one side and amyris, cedarwood, verbena and helichrysum on the other side.

As shown in Figure 14 the effects were very similar against the three mosquito species in thyme, verbena, and helichrysum. While the toxicity varied between the mosquito species in other tested oils, ***Anopheles stephensi*** was more resistant than others mosquito species in camphor, lemon, frankincense, dill, myrtle, juniper, papperblack, and sandalwood from thirteen tested oils, whereas ***Culex quinquefasciatus*** surpassed only in amyris and cedarwood. Thus ***Aedes aegypti*** shared the top with ***Anopheles stephensi*** in cases of juniper and black papper.

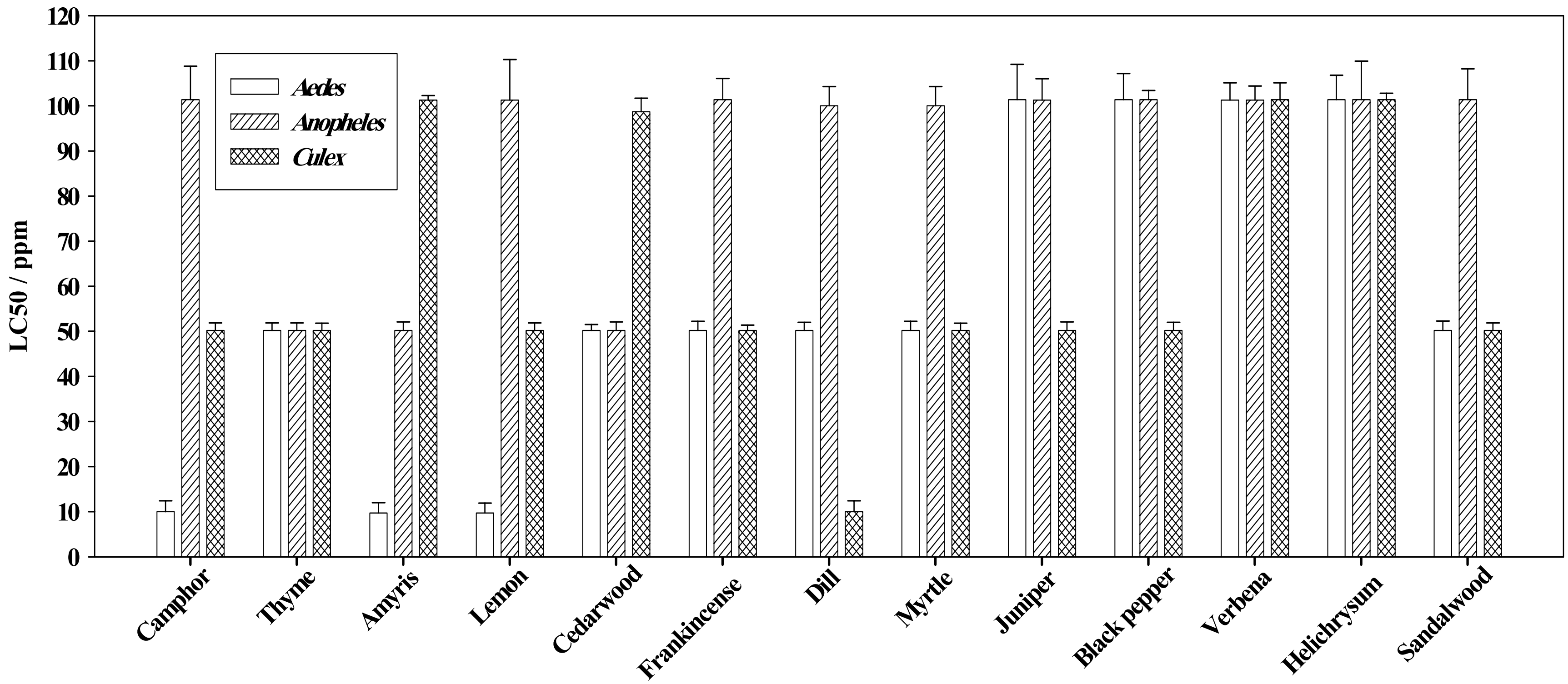

Figure (14) The LC_{50} values by ppm of the selected oils after one hour contact with third instar larvae of the three mosquito species.

The LC_{50} after 12 hours of the thirteen selected oils against larvae of the three mosquito species are shown in Figure 15. In ***Aedes aegypti*** tests the LC_{50} values ranged from 1 to 101.4 ppm .Thyme differed significantly from others oils. Also camphor, amyris, lemon, cedarwood, frankincense, and sandalwood differed significantly with dill, myrtle, juniper, black pepper, verbena, and helichrysum. Thus there are significant differences among dill, myrtle, juniper and black pepper on one side and verbena and helichrysum on the other side. LC_{50} values of the thirteen oils after 12 hours against ***Anopheles stephensi*** larvae varied from 9.7 to 101.4 ppm. As seen in Figure 15 there are no overlappings between the standard error limits of thyme, amyris, cedarwood on one side and other oils on the other. Also the sandalwood standard error value has no overlappings with others. The ***Culex quinquefasciatus*** larvae were more sensitive. Thus the LC_{50} values of tested oils ranged between 1 and 50.2 ppm. The significant differences appeared among thyme on one side and the other oils on the other. While amyris, cedarwood, dill, myrtle, black pepper and sandalwood differed significantly with camphor, lemon, frankincense, juniper, verbena, and helichrysum.

It appears in Figure 15 that, ***Anopheles stephensi*** is the most resistant one in camphor, thyme, lemon, frankincense, dill, myrtle, juniper, black pepper, and sandalwood. While ***Culex quinquefasciatus*** was more weak than other species in dill, myrtle, black pepper, verbena, and helichrysum. ***Aedes aegypti*** was with ***Anopheles stephensi*** most resistant in verbena and helichrysum. On the other hand, the three species were similarly resistant in amyris and cedarwood.

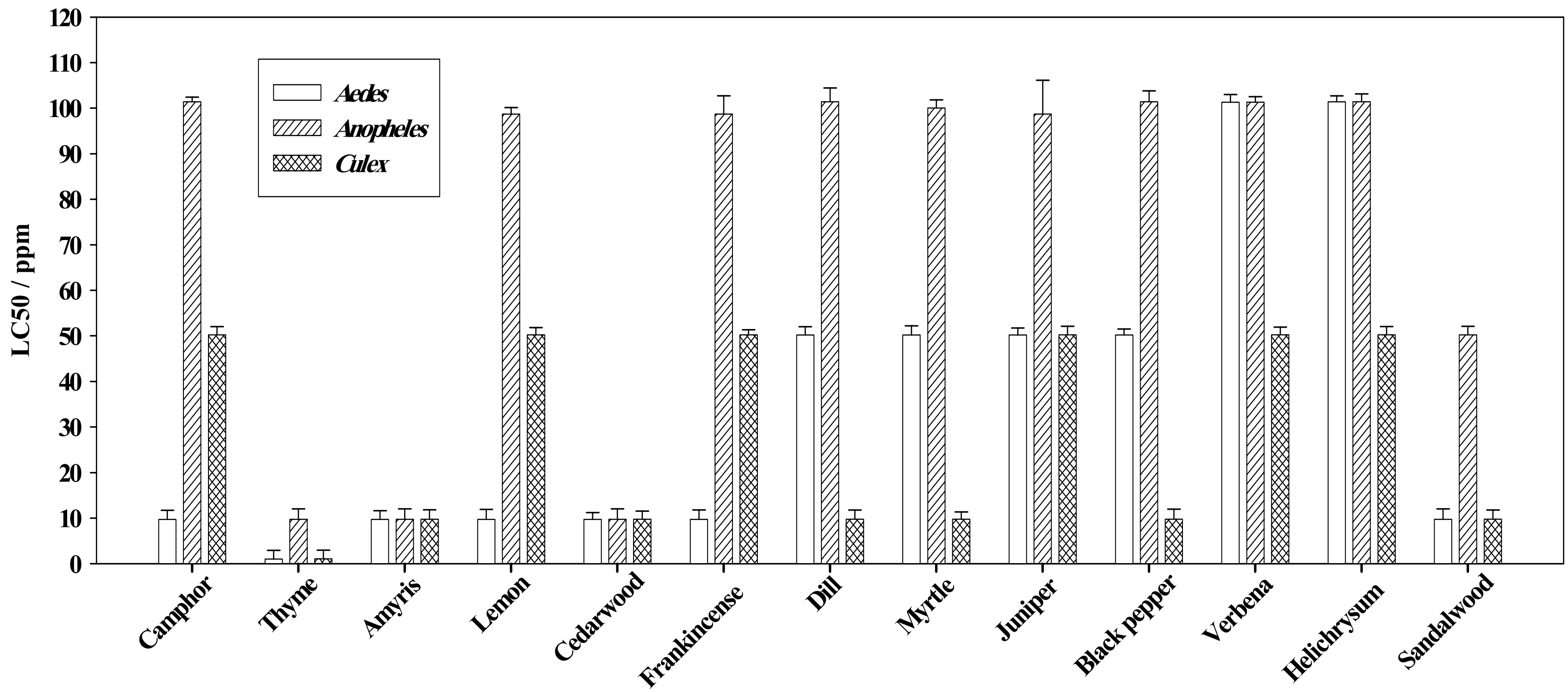

Figure (15) The LC_{50} values by ppm of the selected oils after 12 hours contact with third instar larvae of the three mosquito species.

The LC_{50} after 24 hours of the thirteen tested oils against the larvae of the three mosquito species are shown in Figure 16 . The ***Aedes aegypti*** values varied from 1 to 101.3 ppm. The differences were significant between thyme, amyris, and cedarwood on one side and the other oils on the other. While camphor, lemon, frankincense, and sandalwood differed with dill, myrtle, juniper, black pepper, verbena, and helichrysum. Thus there are significant differences among dill, myrtle, juniper, and black pepper on one side and verbena and helichrysum on the other. The variations were not very sharp between many oils in the case of ***Anopheles stephensi***,. The LC_{50} values of the thirteen tested oils ranged between 9.7 and 101.4 ppm, and the significant differences appeared between thyme, amyris, cedarwood, and verbena on one side and the other oils on the other. Also sandalwood differed with camphor, lemon, frankincense, dill, myrtle, juniper, black pepper, and helichrysum. In the case of ***Culex quinquefasciatus*** larvae the LC_{50} value varied from 1 to 50.2 ppm and the significant differences appeared between thyme, amyris, and dill on one side and the other oils on the other. While cedarwood, myrtle, juniper, black pepper, helichrysum, and sandalwood differed from camphor, lemon, frankincense, and verbena.

In Figure 16 the view has changed in many oils compared to the last two figures. ***Aedes aegypti*** was the most resistant one in verbena, while ***Anopheles stephensi*** stays more resistant than others in camphor, thyme, amyris, lemon, frankincense, dill, myrtle, juniper, black pepper, and sandalwood. The LC_{50} values of ***Culex quinquefasciatus*** decreased in most oils compared with the last two figures.

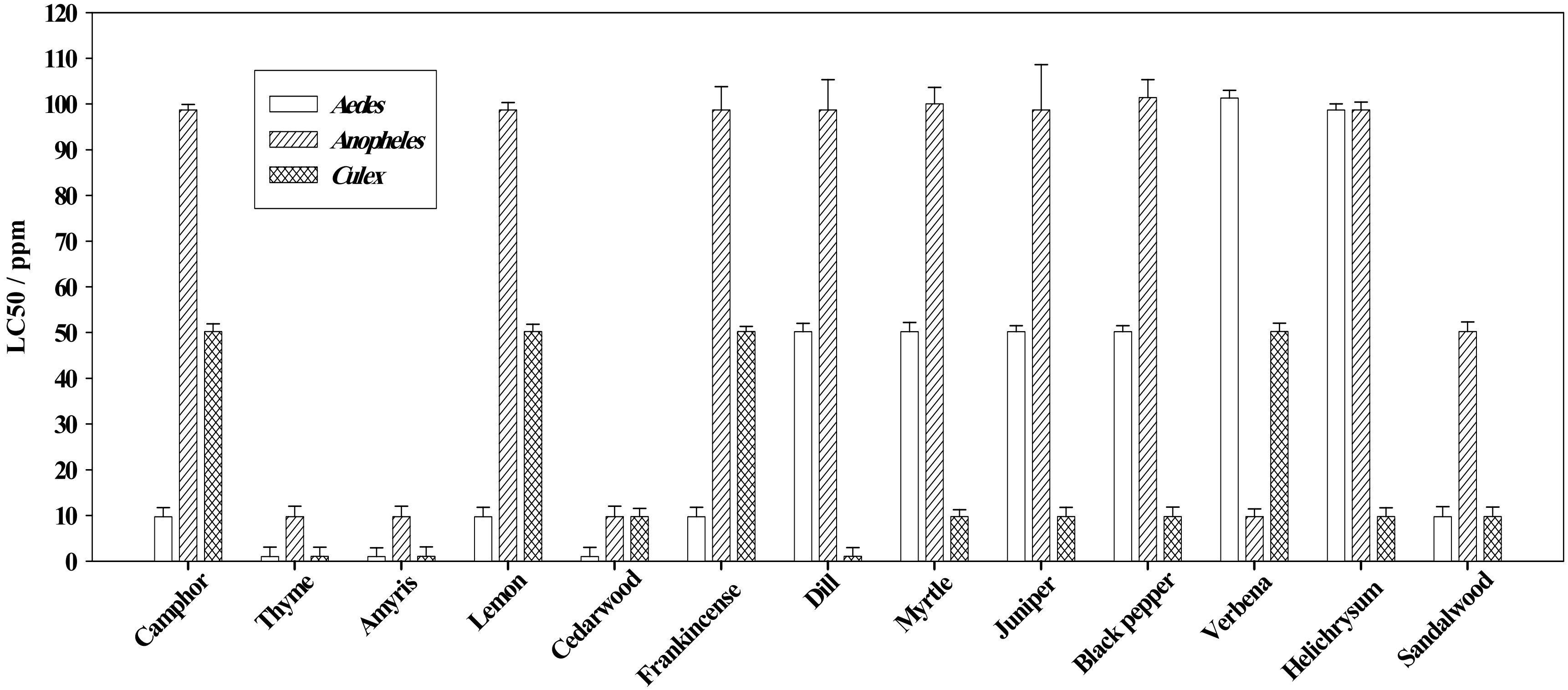

Figure (16) The LC_{50} values by ppm of the selected oils after 24 hours contact with third instar larvae of the three mosquito species.

6. 3. 3 Persistency of oil toxicity under different circumstances

The percentages of dead larvae in each replicate was calculated for 1, 2, 3, 6, 12, 24 hours after contact with the oil solution and the mean values of dead larvae percentages for each treatment were placed in a table for each storages method. The bioassay was conducted immediately, one, two, and three weeks after the preparation of solution.

The mean values of percentages of dead larvae in the 50 ppm solution of tested oils stored in dark opened place are shown in Table 13 . Under these condition the toxicity decreased rapidly with time. Only Camphor (*Cinnamomum camphora*) and Thyme (*Thymus serpyllum*) reserved a good value of their toxicity until after two weeks and they lost their toxicity at the fourth evaluation after three weeks. While the other tested oils failed quickly in the second bioassay after one week. Statistical analysis shows significant differences between camphor on one side and amyris (*Amyris balsamifera*), lemon (*Citrus limon*), cedarwood (*Juniperus virginiana*), frankincense (*Boswellia carteri*), dill (*Anethum graveolens*), myrtle (*Myrtus communis*), juniper (*Juniperus communis*), black pepper (*Piper nigrum*), verbena (*Lippia citriodora*), helichrysum (*Helichrysum italicum*), and sandalwood (*Santalum album*) on the other side. Also thyme (*Thymus serpyllum*) differed with juniper, verbena and helichrysum. ($F=2.9$, $df=12$, $P>0.05$, $LSD=26.03$). Likewise the first bioassay immediately after preparation differed significantly from the following bioassays. There is significant difference between the second bioassay after one week and the fourth bioassay after three weeks. ($F=60.7$, $df=3$, $P>0.05$, $LSD=11.45$).

Table (13). The percentages of dead third instar larvae of ***Aedes aegypti*** mosquitoes in 50 ppm solutions of selected oils that were stored in open wares in the dark for one month after the preparation of the solution.

Time (hours)	**Immediately after preparation**												
	The selected oils												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	100	70	50	60	80	90	60	60	50	40	20	10	20
2	100	80	70	80	80	100	90	80	50	80	20	20	40
3	100	80	80	80	80	100	100	100	70	100	20	20	50
6	100	100	100	100	100	100	100	100	100	100	30	50	100
12	100	100	100	100	100	100	100	100	100	100	30	80	100
24	100	100	100	100	100	100	100	100	100	100	30	90	100
Time (hours)	**After one week**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	30	20	0	0	0	0	0	0	0	0	0	0	0
2	70	60	0	0	0	0	0	0	0	0	0	0	30
3	90	70	0	10	0	0	0	0	0	0	0	0	30
6	100	100	0	10	0	0	0	0	0	0	0	0	40
12	100	100	20	20	30	0	0	0	0	0	0	0	40
24	100	100	20	20	30	0	0	0	0	0	0	0	50
Time (hours)	**After two weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	30	10	0	0	0	0	0	0	0	0	0	0	0
2	30	10	0	0	0	0	0	0	0	0	0	0	0
3	60	10	0	0	0	0	0	0	0	0	0	0	10
6	70	20	10	0	10	0	0	0	0	0	0	0	40
12	80	40	10	10	10	0	0	0	0	0	0	0	40
24	100	70	20	10	10	0	0	0	0	0	0	0	40
Time (hours)	**After three weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	10
6	0	0	0	0	0	0	0	0	0	0	0	0	10
12	0	0	0	0	0	0	0	0	0	0	0	0	10
24	0	0	0	0	0	0	0	0	0	0	0	0	10

* each average calculated from three replicates.

The percentages of dead larvae in the 50 ppm oil solution stored in dark closed places are shown in Table 14 . Many oils reserved their toxicity until the fourth bioassay under these storages condition. Camphor, thyme, lemon, and sandalwood remained high toxic until fourth evaluation after three weeks. While the amyris and cedarwood had good effects until the second bioassay after one week, but their toxicity decreased to unacceptable rates after two weeks. While the remaining oils unfortunately showed only low immediate toxicity or they lost their effects during the second evaluation. In statistical analysis it appeared that camphor, thyme and lemon had significant differences with other oils, also amyris, cedarwood and sandalwood differed from verbena and helichrysum ($F=7.31$, $df=12$, $P>0.05$, $LSD=28.79$). Likewise there are significant differences among bioassays when comparing the first bioassay immediately after the preparation with the following bioassays (F=20.84, df=3, P>0.05, LSD=14.74).

Table (14). The percentages of dead third instar larvae of ***Aedes aegypti*** mosquitoes in 50 ppm solutions of selected oils that were stored in closed wares in the dark for one month after the preparation of the solutions.

Time (hours)	Immediately after preparation												
	The selected oils												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	100	70	50	60	80	90	60	60	50	40	20	10	20
2	100	80	70	80	80	100	90	80	50	80	20	20	40
3	100	80	80	80	80	100	100	100	70	100	20	20	50
6	100	100	100	100	100	100	100	100	100	100	30	50	100
12	100	100	100	100	100	100	100	100	100	100	30	80	100
24	100	100	100	100	100	100	100	100	100	100	30	90	100
Time (hours)	**After one week**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	80	20	10	30	60	0	0	0	0	0	0	0	0
2	90	80	70	50	70	0	10	0	0	0	10	0	40
3	100	80	90	60	70	0	20	0	0	0	20	0	50
6	100	100	90	70	70	0	20	0	0	0	30	0	60
12	100	100	90	100	80	0	20	0	0	0	30	0	60
24	100	100	100	100	80	0	20	0	0	0	30	0	60
Time (hours)	**After two weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	80	50	10	60	10	0	0	0	0	0	0	0	0
2	100	60	20	70	30	0	0	0	0	0	0	0	0
3	100	60	40	90	30	0	0	0	0	0	0	0	0
6	100	80	40	100	30	0	0	0	0	0	0	0	10
12	100	90	40	100	30	0	0	0	0	0	0	0	30
24	100	90	40	100	30	0	0	0	0	0	0	0	70
Time (hours)	**After three weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	80	50	0	20	0	0	0	0	0	0	0	0	0
2	80	100	0	30	0	0	0	0	0	0	0	0	0
3	90	100	0	50	0	0	0	0	0	0	0	0	30
6	100	100	0	100	0	0	0	0	0	0	0	0	50
12	100	100	0	100	0	0	0	0	0	0	0	0	60
24	100	100	0	100	0	0	0	0	0	0	0	0	70

* each average calculated from three replicates.

The mean values of percentages of dead larvae in tested oil solutions stored in light opened places are shown in the Table 15. No oil was remains effective under these conditions until fourth bioassay after three weeks the best one here was camphor, it remained toxic until the third evaluation after two weeks. While thyme, lemon and sandalwood stays effective until the second test after one week, they lost later their toxicity. The toxicity of other oils decreased rapidly after the preparation of the solution. The ANOVA shows significant differences between camphor and all other oils except thyme. While thyme has significant differences with verbena and helichrysum (F=2.83, df=12, P>0.05, LSD=27.32). Also the differences appeared between first evaluation immediately after preparation and all following tests (F=55.6, df=3, P>0.05, LSD=12).

Table (15). The percentages of dead third instar larvae of ***Aedes aegypti*** mosquitoes in 50 ppm solutions of selected oils that were stored in the light in opened bottles during one month after the solutions preparations.

Time (hours)	Immediately after preparation												
	The selected oils												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	100	70	50	60	80	90	60	60	50	40	20	10	20
2	100	80	70	80	80	100	90	80	50	80	20	20	40
3	100	80	80	80	80	100	100	100	70	100	20	20	50
6	100	100	100	100	100	100	100	100	100	100	30	50	100
12	100	100	100	100	100	100	100	100	100	100	30	80	100
24	100	100	100	100	100	100	100	100	100	100	30	90	100
Time (hours)	**After one week**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	40	40	0	0	0	0	0	0	0	0	0	0	0
2	80	40	0	20	0	0	0	0	0	0	0	0	20
3	80	40	0	20	0	0	0	0	0	0	0	0	30
6	100	80	0	40	0	0	10	0	0	0	0	0	50
12	100	90	10	50	0	0	10	0	0	0	0	0	60
24	100	90	20	60	0	0	10	0	0	0	0	0	60
Time (hours)	**After two weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	30	0	0	0	0	0	0	0	0	0	0	0	0
2	90	0	0	0	0	0	0	0	0	0	0	0	0
3	90	0	0	0	0	0	0	0	0	0	0	0	0
6	90	0	0	0	0	0	0	0	0	0	0	0	40
12	90	10	0	0	0	0	0	0	0	0	0	0	40
24	100	40	10	30	0	0	0	0	0	0	0	0	40
Time (hours)	**After three weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0	0

* each average calculated from three replicates.

When the oil solutions were stored in the light closed places some oils stayed toxic until the fourth bioassay. As shown in Table 16 camphor, thyme, lemon, and sandalwood stayed effective until the fourth evaluation after three weeks. Cedarwood was toxic in the third test after two weeks and lost its toxicity in the following test. The other oils were toxic in the first bioassay immediately after preparation, thereafter they lost their toxicity in the following tests (except verbena: its weak toxicity was presented since the first evaluation). Statistically there are significant differences between camphor, thyme and lemon on one side and amyris, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood on the other. Also cedarwood differed significantly from camphor, frankincense, dill, myrtle, juniper, black pepper, verbena, and helichrysum. Sandalwood differed from camphor, thyme, lemon, verbena, and helichrysum. Thus amyris is significant different from verbena ($F=7.96$, $df=12$, $P>0.05$, $LSD=25.64$). Likewise the first bioassay differed from all following tests. Also the second evaluation after one week differed with the fourth bioassay after three weeks. ($F=29.11$, $df=3$, $P>0.05$, $LSD=13.13$).

Table (16). The percentages of dead third instar larvae of ***Aedes aegypti*** mosquitoes in 50 ppm solutions of selected oils that were stored in the light in closed bottles during one month after the preparation of the solutions.

Time (hours)	Immediately after preparation												
	The selected oils												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	100	70	50	60	80	90	60	60	50	40	20	10	20
2	100	80	70	80	80	100	90	80	50	80	20	20	40
3	100	80	80	80	80	100	100	100	70	100	20	20	50
6	100	100	100	100	100	100	100	100	100	100	30	50	100
12	100	100	100	100	100	100	100	100	100	100	30	80	100
24	100	100	100	100	100	100	100	100	100	100	30	90	100
Time (hours)	**After one week**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	60	50	20	40	60	0	0	20	20	0	0	0	10
2	90	60	50	60	70	0	0	20	20	0	0	0	40
3	100	80	60	60	70	0	0	20	20	0	0	0	40
6	100	100	60	70	80	0	0	20	30	0	0	0	50
12	100	100	70	100	80	0	0	20	30	0	0	0	60
24	100	100	70	100	80	0	0	20	30	0	10	0	60
Time (hours)	**After two weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	90	20	0	40	30	0	0	0	0	0	0	0	0
2	100	50	0	50	40	0	0	0	0	0	0	0	0
3	100	70	0	60	60	0	0	0	0	0	0	0	0
6	100	70	0	80	60	0	0	0	0	0	0	0	20
12	100	80	0	80	70	0	0	0	0	0	0	0	40
24	100	100	30	100	70	0	0	0	0	0	0	0	60
Time (hours)	**After three weeks**												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	40	20	0	10	0	0	0	0	0	0	0	0	0
2	60	30	0	30	0	0	0	0	0	0	0	0	0
3	80	40	0	50	0	0	0	0	0	0	0	0	20
6	90	100	0	60	0	0	0	0	0	0	0	0	20
12	100	100	0	90	0	0	0	0	0	0	0	0	50
24	100	100	0	100	0	0	0	0	0	0	0	0	80

* each average calculated from three replicates.

6. 4 Discussion

6. 4. 1 Effect of the exposure time on the LC_{50} values

The exposure time was very important for the values of LC_{50} for the tested oils. In the most cases the LC_{50} values had an inverse relation with time, thus they decreased more after 12 and 24 hours than after 1 hour. In Figures 14, 15, 16 the LC_{50} of thyme against ***Aedes aegypti*** and ***Culex quinquefasciatus*** decreased from 50.2 ppm after one hour to only 1 ppm after 24 hours. Also the LC_{50} of amyris against ***Culex quinquefasciatus*** was 101.3 ppm after one hour, 9.7 ppm after 12 hours and only 1 ppm after 24 hours. Moreover, there are many examples for this relation . Therefore the calculating time of LC_{50} is very important before comparison of potential larvicidal materials.

6. 4. 2 The variation of oil toxicity among the mosquito species

In the last three Figures 14, 15, 16 many variations in oil toxicity between the three tested mosquito species appeared. Generally the LC_{50} of many oils was high in case of ***Anopheles stephensi*** compared to other mosquito species except some irregular cases. ***Aedes aegypti*** comes in as the second for most tested oils and at the end there is ***Culex quinquefasciatus***. These variations are not abnormal, since they correspond to many previous studies. Minijas and Sarda (1986) showed that crude extracts containing saponin from fruit pods of ***Swartzia madagascariensis*** produced higher mortality in larvae of ***Anopheles gambiae*** than in larvae of ***Aedes aegypti***, and no mortality was induced in larvae of ***Culex quinquefasciatus***. Also different susceptibilities occurred with petroleum-ether extracts of ***Acorus calamus, Ageratum conyzoides, Annona squamosa, Bambusa arundanasia, Madhuca longifolia*** and ***Citrus medica*** in trials against larvae of the three species of mosquitoes (Sujatha et al., 1988). Similarly when extracts of the pond weeds ***Myriophyllum*** and ***potamogeton*** were assayed against larvae of ***Anopheles occidentalis*** and ***Culex pipiens***, ***Culex pipiens*** showed more resistance to both extracts (Graham and Schooley, 1984). Such a differential species susceptibility was also noticed by Dhillon et al. (1982), when algal toxins were assayed against ***Aedes aegypti, Culex quinquefasciatus*** and ***Culiseta incidens***.

Our study also fits with that of Novak (1985), when several volatile oils were assayed against the larvae of ***Anopheles claviger*** and ***Aedes cantans*** showing that anophelines were less sensitive than aedines.

6. 4. 3 Effect of the storage method and the store time on the persistence of toxicity

The toxicity of selected oils were affected significantly by methods and periods of storage. Immediately after preparation most oils were high toxic. Thus one week after the preparation of the solutions the results varied depending on storing circumstances. In Tables 13, 14, 15 and 16 the percentages of dead larvae in some oil solutions remained high until the fourth evaluation after three weeks especially when the solutions were stored in fast closed ware in light or in dark places. Tables 14 and 16 show the percentages of dead larvae in oil solutions stored in closed dark and light places respectively. The oils of camphor, thyme, lemon, and sandalwood stayed high toxic until fourth test, whereas in the open wares no oil stayed effective until fourth test as seen in Tables 13 and 15 . Statistical analysis gave significant differences between the solutions that were stored in closed wares and the same solutions stored in opened wares. While there are no differences between oil solutions that were stored in dark and/or light places ($F=8.5$, $df=3$, $P>0.05$, $LSD=7.72$). On the other hand all comparisons between series testes differed significantly ($F=138.4$, $df=3$, $P>0.05$, $LSD=7.23$).

Light and storing circumstances have often been forgotten or underestimated factors in the study of insecticides. In previous studies little attention has been paid to its role in plant-insect reactions. The activation of plant secondary substances by light, and their subsequent photosensitizing effects on insects, especially mosquito larvae, is an important factor contributing to the enhancement of toxicity. Polyacetylenes and thiophenes, that occur in certain plants of the ***Asteraceae*** family, show the greatest potential as photoactive pest control agents (Sukumar et al., 1991). Some times the activity increased with light, indicating a phototoxic action of alpha-terthienyl. Berberine, an isoquinoline alkaloid present in many different families, is also photoactivated. Larval, pupal and adult survival of ***Aedes atropalpus*** was affected by berberine treatment with toxicity of the alkaloid increasing after exposure to light. Philogene et al. (1984) speculated that the fluorescent nature of berberine could be the reason for its photodynamic activity. Rose bengal, a xanthene-derivative, also causes enhanced mortality in mosquito larvae by photosensitized oxidation reactions. Its primary mode of action depends on the absorption of visible light energy, causing photo-oxidative toxicity (Pimprikar et al., 1979). Also in many cases the bioactivity of plant derivative compounds decreased when it was exposed to the light as that happened in case of azadirachtin Its antifeedant activity decreased rapidly in sunlight (Stokes and Redfern, 1982). Barnby et al. (1989) reported that tetrahydroazadirachtin photo-degraded by 15% following exposure to ultraviolet radiation, resulting in a significant decrease in biological activity.

In our study the light was not effective on oil toxicity, since the activity was similar in the both cases (light and dark). While the solutions that were stored in closed (dark or light) wares stayed toxic until the last bioassay. The solutions that were stored in opened wares (dark or light) mostly lost their toxicity after storing for a short time. Therefore we can conclude that the volatile compounds in the tested oils played a major role to make appear the high toxicity in the first test. They are rapidly evaporated when they are stored in opened wares.

7. General Discussion

Our results and many previous literatures undoubtedly show that the essential oils and other secondary plant metabolites have many different effects on insects like toxicity, repellency, and feed deterrents. These effects came from plant defense mechanisms against phytophagous insects. Thus many workers tried to use these characters of these compounds for insect management in many different directions especially in agriculture and in the public health field.

7. 1 Repellency Effect of Essential oils

Essential oils are common used as repellents to protect people and domestic animals from hematophagous arthropods. But their usage confronted some difficulties since their volatility perhaps plays a central role in repellency. Zhu et al . (2001) said that essential oils or semiochemicals with low volatility may allow a longer repelling period. Therefore the use of gas chromatography retention times of such chemicals may be a useful parameter for developing effective and long-term repellents against insects such as the Formosan subterranean termite.

The oils that were used in this study were analyzed using MS/GC to get the retention times and molecular weight of mine constituents of each oil. These results were compared with results of oils as repellents and as larvicides.

The percentages, molecular weights, and retention times of main constituents of the essential oils group comparative with their repellency and toxicity effects against adults and larvae of ***Aedes aegypti*** mosquito respectively are shown in Table 17 .

Except for some cases the relation between the repellency effect of essential oils and their retention times was absent. In Table 17 the highest repellency was obtained by rosewood 89.2%, whereas his retention time was only 10.42 minutes. On the other side the lowest repellency was that of jasmine (only 13.5%) while the retention time was 11.64 minutes. Also in the carotin oil trials the retention time was 26.73 minutes while the repellency was only 16.2%. There are many further examples in Table 17 that evidenced inaccuracy of Zhu hypothesis for the use of the relation between the oil volatility and their repellency.

Table (17) Percentages, Molecular weights, and retention times of main constituents of the essential oils group comparative with their repellency and toxicity effects. (from G C results)

NO	Name of material *	M. C.	% M. C.	M. W.	R. T.	% R	% M
1	Citronella	citronellal	32	154.252	11.621	75.7	60
2	Rosewood	linalol	79	154.252	10.42	89.2	60
3	Lavender	Linalyl acetate	36.6	196.289	10.22	24.3	63.3
4	Camphor	Cineole	32.4	154.252	8.4	32.4	100
5	Catnip	Nepetalactone	93.66	166.218	17.07	83.8	40
6	Geranium	citronellal	22.66	154.252	13.69	78.4	73.3
7	Thyme	Thymol and carvacrol	20.17	150.22	16.15	56.7	100
8	Eucalyptus	Cineol	94.21	154.252	8.43	56.7	16.7
9	Jasmine	Benzyl acetate	24.46	150.177	11.64	13.5	6.7
10	Broad-Leaved	Cineol	46.88	154.252	14.48	18.9	96.7
11	Lemongrass	Citral	39.86	152.236	14.85	70.3	0
12	Lemonscented *Eu.*	Cineol	73.13	154.252	11.716	59.4	76.7
13	Fichtennadel	camphene	28.38	136.236	15.12	21.6	96.7
14	Amyris	Caryophyllene,	19.69	204.355	22.82	29.7	100
15	Lemon	Limonene	79.27	136.236	8.4	67.6	100
16	Narrow-Leaved *Eu.*	Cineol	80.12	154.252	8.42	64.9	50
17	Carotin oil	$	71.23	126	26.73	16.2	53.3
18	Cedarwood	cedrol	11.21	222.37	22.17	37.8	100
19	Frankincense	Monoterpene hydrocarbons	64.49	136	6.14	75.7	100
20	Dill	Carvone	20.5	150.22	14.25	78.4	100
21	Myrtle	myrtenol	40.07	152.236	8.39	56.7	100
22	Chamomile	Esters of angelic acids	41.63	129	8.83	64.9	3.3
23	Cinnamon	L. Eugenol	30.85	164.204	14.96	70.3	90
24	Juniper	Monoterpene	35.69	105	6.07	43.2	100

25	Sage	Linalyl acetate	72.13	196.289	14.44	45.9	46.7
26	Peppermint	Menthol	48.05	154	12.42	59.4	53.3
27	Basil	Methyl chavicol	58.77	154	10.34	81.1	86.7
28	Cajeput	Cineol	84.49	154.252	8.25	43.2	3.3
29	Soya bean	$	$	$	$	54	0
30	Rosemary	Camphene	33.46	136.236	8.34	43.2	16.7
31	Niaouli	Cineol	63.1	154.252	8.43	75.7	30
32	Olive	$	$	$	$	67.6	43.3
33	Black Pepper	Monoterpenes	23	136	6.99	64.9	100
34	Verbena	Citral	30.98	152.236	8.31	70.3	100
35	Tagetes	Tagetones	35.69	152.236	8.47	83.8	3.3
36	Violet	Parmone	49.89	192.3	25.92	67.6	86.7
37	Sandalwood	Santalols	26.12	220	23.07	59.4	100
38	Litsea	Citral	37.99	152.236	14.65	73	50
39	Helichrysum	Nerol	28.55	154.252	17.19	43.2	100
40	Galbanum	myrcene	65.07	136.236	7.12	70.3	13.3
41	Chamomile	Esters of angelic and tiglic acids	38.79	156	8.81	70.3	50

M. C. = main constituent ; **%M. C**. = percentage of main constituent in the oil ; **M. W**. = Molecular weight ; **R. T**. = retention time ; **%R** = repellency against ***Aedes aegypti*** ; **%M** = percentage of mortality against larvae of ***Aedes aegypti*** . * see (Appendix 9) : $ = This oil is not volatile oil, therefore they not analyzed by the same method on the GC.

7. 2 Toxicity Effect of Essential Oils

Many plant essential oils produce toxic action as ovicidal, larvicidal, pupicidal, and adulticidal effects, most behaving as general toxicants. The differential responses induced by phytochemicals on various species of mosquitoes were influenced by extrinsic and intrinsic factors depending on the species of plant, the parts of the plant, the solvents used for extraction, the geographical location, where the plants were grown, and the methods employed for evaluation. Some workers explained the differences of plant essential oils toxicity among insect species as basis of metabolic factors. To investigate the mechanisms of toxicity, Yu (1987) conducted a comparative study between larvae of a generalist insect, the fall armyworm ***Spodoptera frugiperda*** (***Lepidoptera***: ***Noctuidae***) and a semispecialist, the velvetbean caterpillar ***Anticarsia gemmatalis*** (***Lepidoptera*** : ***Noctuidae***). The results showed that the midgut microsomes included cytochrome P_{450} monooxygenases, metabolized allelochemicals such as terpenes and that the monooxygenase activity towards these allelochemicals as generally higher in the generalist than in the semispecialist insect, and that this played an important role in the detoxication of plant toxins.

In usage of plant essential oils as larvicides we can not ignore the physical effect of oils. Since the oil make a film on the water surface, it will kill the mosquitoes larvae by flooding their tracheal systems. This mode of killing is not a result of chemical toxicity but it comes from the physical effects of oil, that mean any oil toxic or not toxic will inducing the same action. Therefore if we want to test the oil toxicity against mosquitoes larvae, the oil must be dissolved in the water.

7. 3 The relation between repellency effect and toxicity

In this study there was no relation seen between repellency and toxicity effects of essential oils, since in Table 17 some examples show high toxicity with low repellency such as camphor, thyme, broad-leaved, fichtennadel, amyris, cedarwood, myrtle, juniper, sandalwood, and helichrysum. And some oils displayed high repellency with low toxicity as in rosewood, catnip, lemongrass, niaouli, tagetes, and galbanum. When some oils exposed semi-equal values in both two effects such oils of geranium, frankincense, dill, cinnamon, sage, peppermint, and basil. therefore we can averment that the toxicity effect was not the source of repellency effect as some people think, but there are other inducements of the oils for their repellency effect. This conclusion is in agreement with Regnault-Roger (1997).

7. 4 Conclusions

The plant essential oils supply a wide promising field in many different ways industry, pharmacy, alternative medicine, integrated pest management, etc. In our field control of medical insects the plant secondary metabolites especially the essential oils play as source of plant derivatives insecticides that will be effective and environmentally friendly. Thus, this study offered useful materials for the control of blood sucking insects as repellents and/or as insecticides.

8. Abstract

The use of plant derived material such as secondary metabolites to control domestic and agricultural pests was widespread in ancient cultures and increased too in last few years with an increase of the knowledge about harmful use of chemical products against the pests. In the present study groups of essential oils were targeted to expose their effects on the three mosquito species: Yellow Fever Mosquito ***Aedes aegypti*** (L.), Malaria mosquito ***Anopheles stephensi*** (Liston), and ***Culex quinquefasciatus*** (Say), a vector of filariasis and encephalitis. All these species belong the order of ***Diptera*** and the family of ***Culicidae***. The group tested contained 41 plant essential oils presented in Table 2 . They were evaluated in the laboratory as repellents against mosquito species by use of the direct test on the human subjects to calculate the protection time, percentage of repellency, percentage of landing mosquitoes, and percentage of biting mosquitoes. At first the oils were screened at a 20% dilution. The best five oils according to result of the first test were:

1- Litsea (*Litsea cubeba*).
2- Cajeput (*Melaleuca leucadendron*)
3- Niaouli (*Melaleuca quinquenervia*)
4- Violet (*Viola odorata*)
5- Catnip (*Nepeta cataria*)

These oils were selected to test them by use of three different formulations:

1- Ethanol alone .
2- A complex formulation containing 20% Genapol, 10% PEG, 20% Ethanol, 50% water .
3- Ethanol containing 5% Vanillin.

Then they were mixed together to produce low concentrations with high repellency. As shown in Table 7 , the repellents trials resulted that some essential oils induced a good repellency effect especially when tested with a formulation that contained fixation material like Genapol, PEG, or Vanillin. Thus the formulation 2 induced a higher repellency effect than others. Some oil mixtures at low concentrations induced a better repellency effect than all products that contained only a single oil. Apparently there is a synergistic effect by these essential oils.

To find out which sense organ of the mosquito is responsible for the repellency effect of the essential oil mixture containing ***Litsea cubeba*** 1%, ***Melaleuca leucadendron*** 1%,

Melaleuca quinquenervia 1%, ***Viola odorata*** 1%, ***Nepeta cataria*** 1% this mixture was tested against five groups of mosquitoes (group **1**: without antenna; group **2**: without maxillary bulbs; group **3**: without proboscis; group **4**: without frontal tarsus; group **5**: normal females as control), belonging two anthropophilic species ***Aedes aegypti*** and ***Anopheles stephensi***, while Bayrepel was used in this experiment at a 20% concentration in the same solvent, and water as control. The result was that the maxillary bulbs is the important organ to note the repellents in ***Aedes aegypti*** mosquitoes. In ***Anopheles stephensi*** mosquitoes the responsible organ, however, was not found in this study. Many (SEM) micrographs were taken to illustrate the large number and different types of sensilla that are located on the surface of studied organs.

Thirteen oils were selected as good larvicides, when the oils were tested for their larvicidal activity on the third instar larva of ***Aedes aegypti*** (Camphor ; Thyme ; Amyris ; Lemon ; Cedarwood ; Frankincense ; Dill ; Myrtle ; Juniper ; Black Pepper ; Verbena ; Helichrysum ; Sandalwood). These thirteen oils were tested against the three mosquito species with a series of concentrations to calculate their LC_{50} after 1, 12, 24 hours. Moreover, the persistency of these thirteen oils in their solutions was evaluated under different conditions for one month. The result of this trials demonstrated that many essential oils have strong larvicidal effects with long persistency, especially when they were stored in closed wares at dark places.

The life cycles of the three subjected mosquito species were studied under different conditions (in laboratory room condition ranging between 19^0 and 22^0C ; in incubator adjusted at $28C^0$) . The samples were examined three times daily to record every change of behavior especially the dates of molting were recorded. The differences were extremely clear in life cycle of the three species between the two environments. The life cycle of individuals that had been reared in stable conditions in the incubator was shorter than those that were reared at room conditions. Moreover, micrographs of all mosquito stages were taken in their habitats.

Zusammenfassung

Der Einsatz pflanzlicher Inhaltsstoffe zur Schädlingsbekämpfung in der Nutzviehzucht und in der Landwirtschaft, war in alten Kulturen weit verbreitet. Heute macht man sich dieses alte Wissen zunutze und erforscht in zunehmendem Maße die Möglichkeiten, die pflanzliche sekundäre Metaboliten bieten können.

In der vorliegenden Arbeit wurden die Effekte verschiedener ätherischer Öle auf drei Stechmückenarten untersucht: 1. Gelbfiebermücke ***Aedes aegypti*** (L.), 2. Malariamücke ***Anopheles stephensi*** (Liston) und 3. ***Culex quinquefasciatus*** (Say) (Überträger der Filariasis und Encephalitis). Diese Arten gehören zur Ordnung der **Diptera** und hier zur Familie der **Culicidae**. Getestet wurden 41 pflanzliche ätherische Öle, welche in Tabelle 2 aufgelistet sind. Sie wurden im Labor als Repellentien gegen die genannten Mückenarten ausgetestet. Die Bestimmung der Wirksamkeit erfolgte direkt am Menschen über die Zeit der Schutzwirkung, den Prozentsatz der Mückenlandungen und den Prozentsatz der Stiche. Die Öle wurden zuerst in einer 20% igen Verdünnung getestet, wobei folgende fünf am wirksamsten waren: 1-Litsea (*Litsea cubeba*), 2-Cajeput (*Melaleuca leucadendron*), 3-Niaouli (*Melaleuca quinquenervia*), 4-Violet (*Viola odorata*), 5-Katzenminze (*Nepeta cataria*). Diese fünf Öle wurden in verschiedenen Formulierungen weitergetestet:

1. Ethanol
2. 20% Genapol, 10% PEG, 20% Ethanol, 50% Wasser
3. Ethanol mit 5% Vanillin

Tabelle 7 ist zu entnehmen, dass einige ätherische Öle einen großen repellenten Effekt zeigten, insbesondere dann, wenn Formulierungen verwendet wurden, die Fixantien wie Genapol, PEG oder Vanillin enthielten. So bewirkte Formulierung 2 die höchste repellente Wirkung von allen. Einige Öl-Mischungen zeigten bessere Ergebnisse als Produkte, die nur ein Öl enthielten. Offenkundig besteht ein synergistischer Effekt zwischen diesen Ölen.

Um zu bestimmen, welche Sinnesorgane der Mücken für den repellenten Effekt der Öl-Mischung, bestehend aus *Litsea cubeba* 1%, *Melaleuca leucadendron* 1%, *Melaleuca quinquenervia* 1%, *Viola odorata* 1% und *Nepeta cataria* 1%, verantwortlich ist, wurde diese Mischung gegen fünf Gruppen von Mücken getestet, Gruppe1: ohne Antennen; Gruppe2: ohne maxillary bulbs (Maxillartaster); Gruppe3: ohne Proboscis; Gruppe4: ohne Frontal-Tarsus; Gruppe5: normale Weibchen als Kontrolle.
Die verwendeten Mücken gehörten zu den Arten *Aedes aegypti* und *Anopheles stephensi.* Derselbe Versuch wurde auch mit Bayrepel (20%) im selben Lösungsmittel und mit Wasser als Kontrolle durchgeführt. Verantwortlich für den repellenten Effekt waren im Falle von *Aedes aegypti* die Maxillari taster. Das ursächliche Organ bei *Anopheles stephensi* konnte in dieser Studie nicht ausgemacht werden. Um die große Anzahl der verschiedenen Typen von Sensillen auf den untersuchten Organen zu illustrieren, wurden rasterelektronenmikroskopische Aufnahmen angefertigt.

Dreizehn Öle zeigten gute larvizide Wirkung auf das dritte Larvenstadium von *Aedes aegypti*: Camphor, Thyme, Amyris, Lemon, Cedarwood, Frankincense, Dill, Myrtle, Juniper, Black Pepper, Verbena, Helichrysum und Sandalwood. Diese dreizehn Öle wurden an den drei Stechmückenarten in verschiedenen Konzentrationen getestet, um ihren LC_{50} nach 1, 12 und 24 Stunden zu bestimmen. Weiterhin wurde die Haltbarkeit der Öle unter verschiedenen Bedingungen über einen Zeitraum von einem Monat untersucht, wobei sich herausstellte, dass viele ätherische Öle ihre starke larvizide Wirkung über einen langen Zeitraum beibehalten, insbesondere dann, wenn sie in geschlossenen Gefäßen, sowohl in der Dunkelheit als auch im Licht, aufbewahrt wurden.

Der Lebenszyklus der drei Stechmückenarten wurde unter dem Einfluss verschiedener Temperaturen analysiert (im Labor zwischen 19°C und 22°C und im Inkubator bei 28°C). Dabei zeigte sich ein deutlich verkürzter Entwicklungszyklus bei den Mücken, die im Inkubator bei 28°C gehalten wurden.
Des Weiteren wurden photographische Aufnahmen von allen Lebensstadien in ihrem entsprechenden Habitat angefertigt. Somit würden eine Reihe von praktisch verwertbaren Ergebnissen erzielt.

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APPENDIXES

Appendix (1) Light microscope micrographs of life cycle stages of ***Aedes aegypti*** mosquitoes.

1- The eggs of ***Aedes aegypti*** mosquitoes on wet white filter paper.

2- The first larval stage in its habitat (water).

3- The second larval stage in its habitat (water).

4- The third larval stage in its habitat (water).

5- The fourth larval stage in its habitat (water).

6- The pupa stage in its habitat (water).

7- The female of the ***Aedes aegypti*** mosquito.

8- The male of the ***Aedes aegypti*** mosquito.


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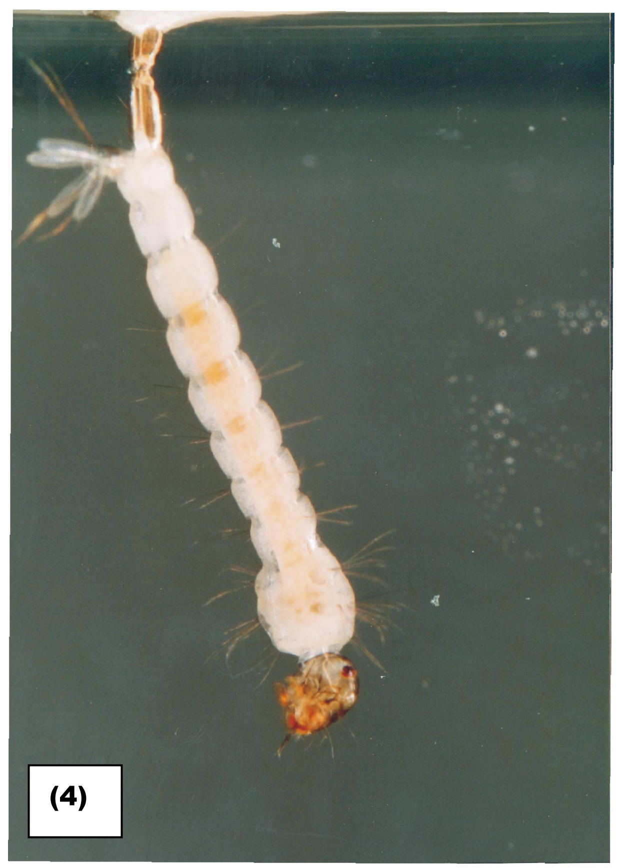
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Appendix (2) Light microscope micrographs of life cycle stages of ***Anopheles stephensi*** mosquitoes.

1- The eggs of ***Anopheles stephensi*** mosquitoes on the water surface of a Petri dish.

2- The first larval stage in its habitat (water).

3- The second larval stage in its habitat (water).

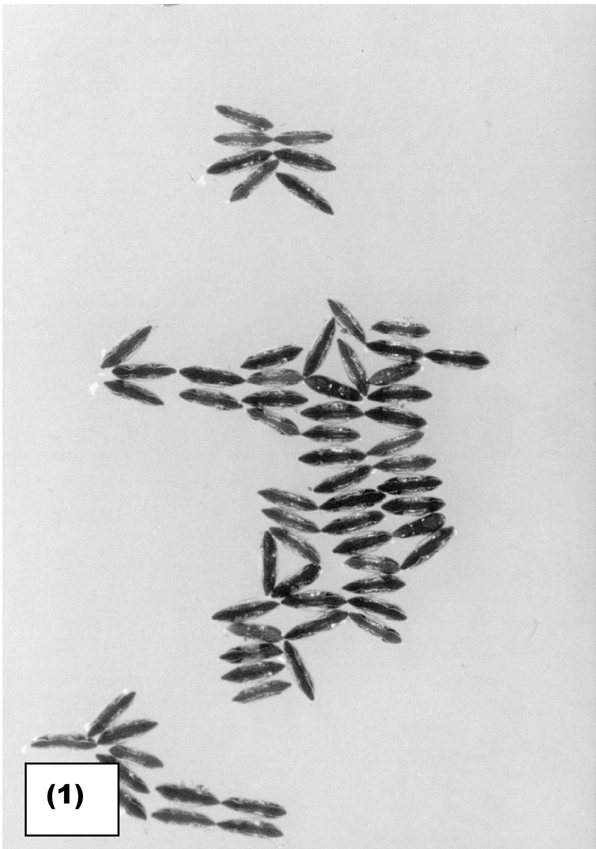
4- The third larval stage in its habitat (water).

5- The fourth larval stage in its habitat (water).

6- The pupa stage in its habitat (water).

7- The female of the ***Anopheles stephensi*** mosquito.

8- The male of the ***Anopheles stephensi*** mosquito.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Appendix (3) Light microscope micrographs of life cycle stages of ***Culex quinquefasciatus*** mosquitoes.

1- The eggs raft of ***Culex quinquefasciatus*** mosquitoes on the water surface of a Petri dish.

2- The first larval stage in its habitat (water).

3- The second larval stage in its habitat (water).

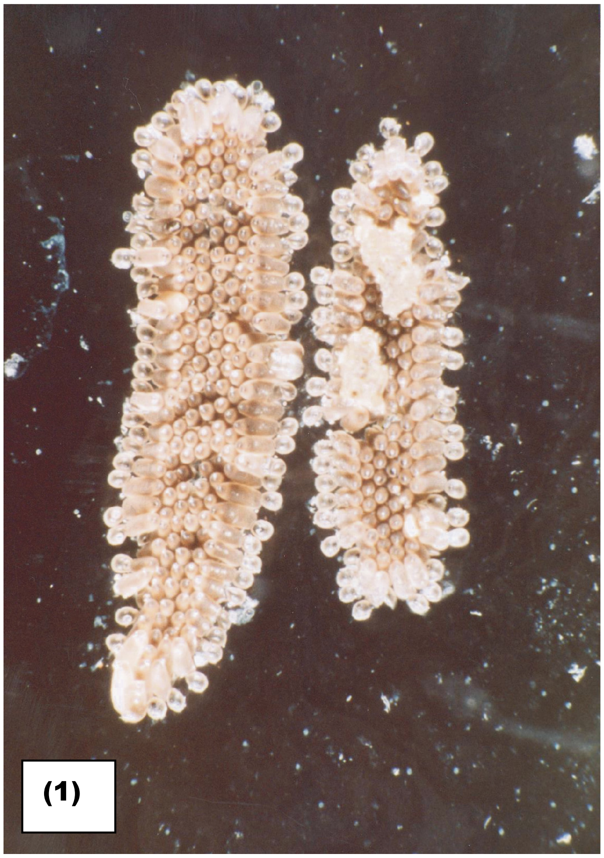
4- The third larval stage in its habitat (water).

5- The fourth larval stage in its habitat (water).

6- The pupa stage in its habitat (water).

7- The female of the ***Culex quinquefasciatus*** mosquito.

8- The male of the ***Culex quinquefasciatus*** mosquito.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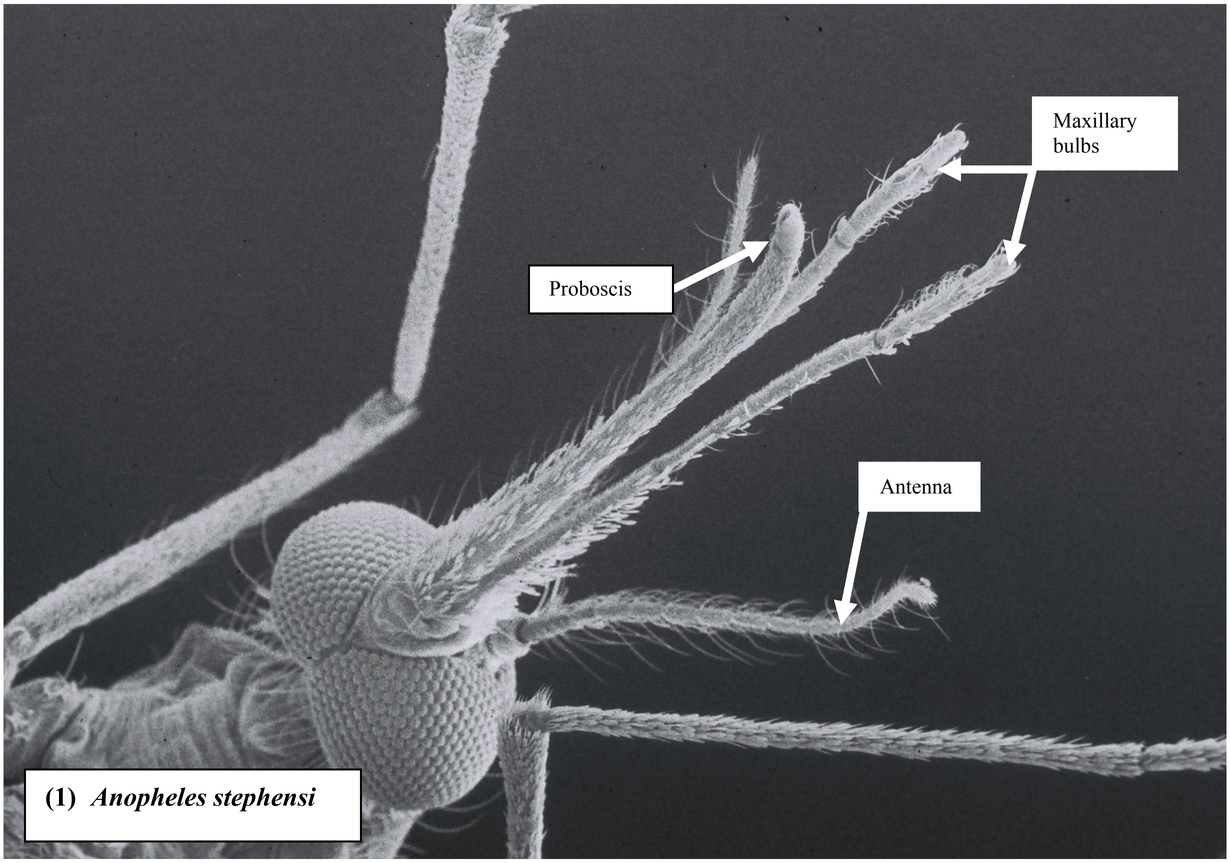
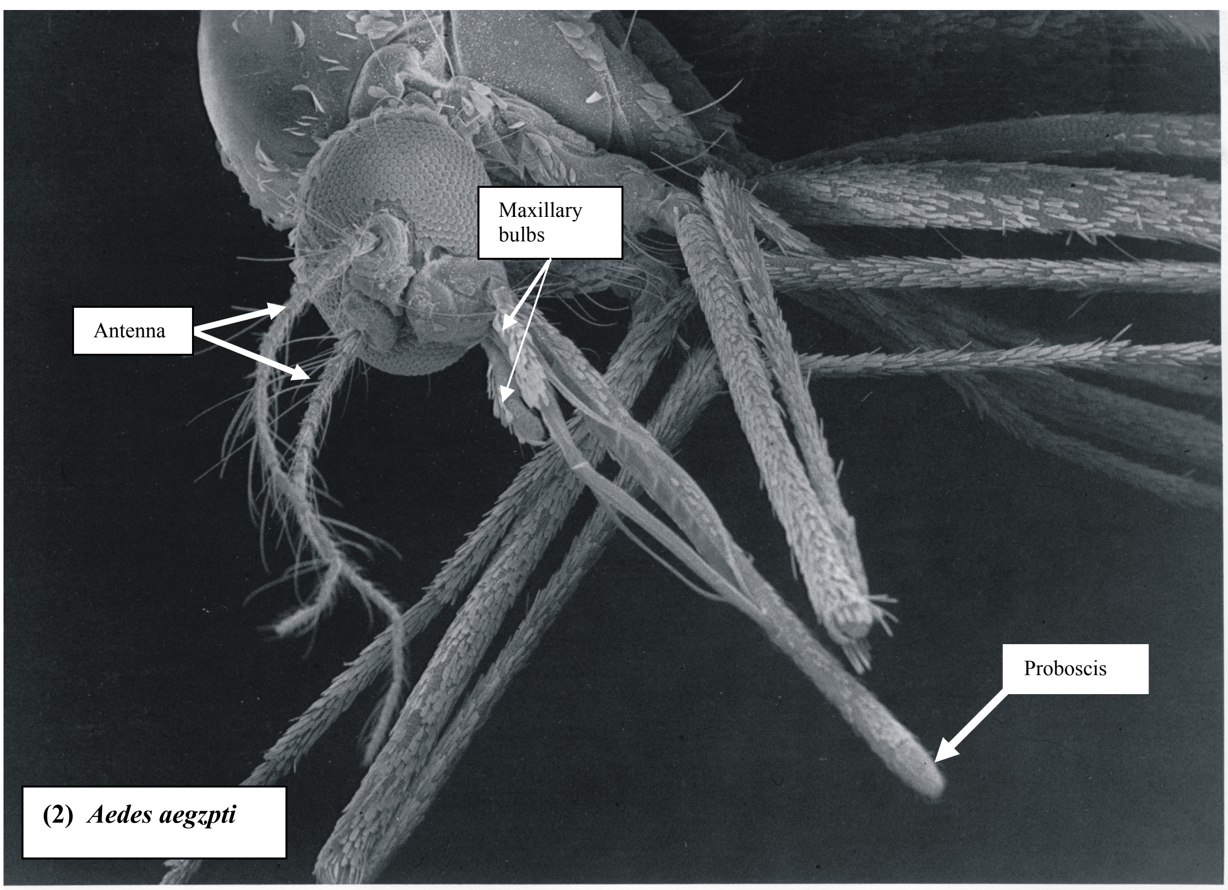
Appendix (4) Scanning electron microscope (SEM) micrographs of ***Aedes aegypti*** and ***Anopheles stephensi*** females (the head and their sense organs) .

(1) Head of ***Anopheles stephensi*** female showing the antenna, maxillary bulbs and proboscis. X 40

(2) Head of ***Aedes aegypti*** female showing the antenna, maxillary bulbs and proboscis. x 35


Maxillary bulbs
Proboscis
Antenna
(1) Anopheles stephensi


Maxillary bulbs
Antenna
Proboscis
(2) Aedes aegzpti

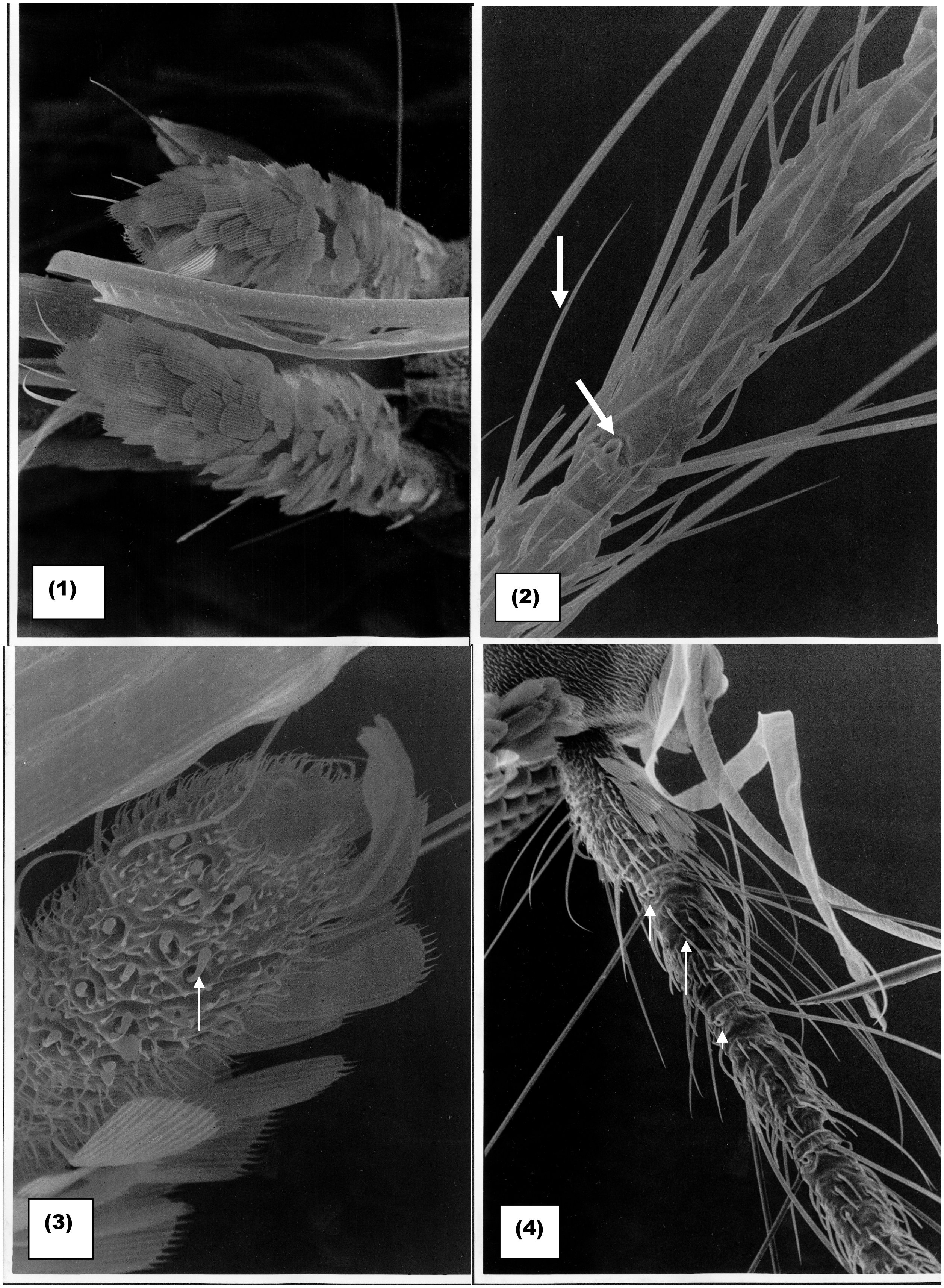
Appendix (5) Scanning electron microscope micrographs (SEM) of the antenna and maxillary bulbs of ***Aedes aegypti*** females and the sensilla (cuticular sense organs) that are dispersed on their surface .

(1) The position of two maxillary bulbs. x 302

(2) Some sense organs located on the middle segments of the antenna. x 695

(3) Some types of sensilla located on maxillary bulbs. x 770

(4) Some sensilla on the basal segments of the antenna. x 381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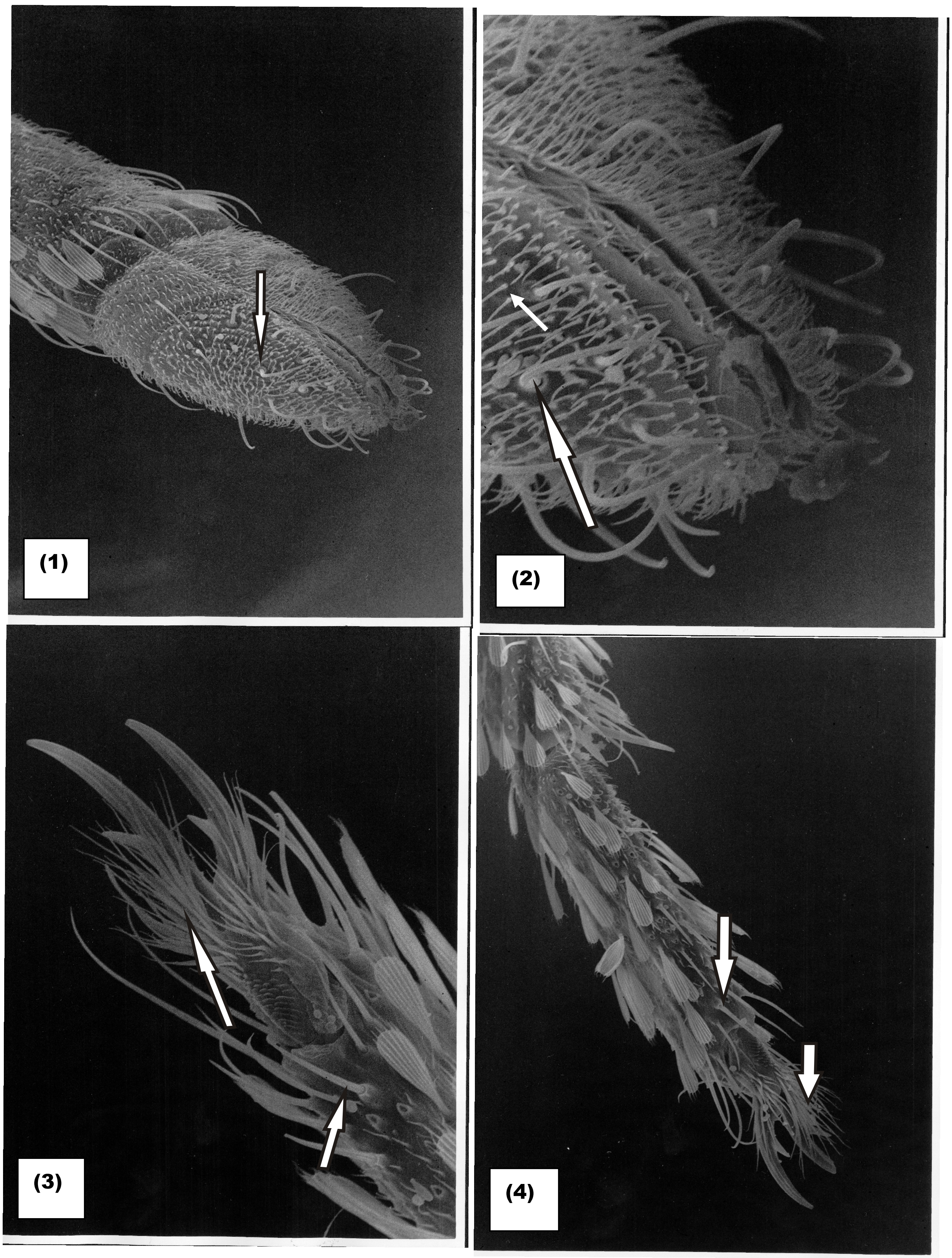
Appendix (6) Scanning electron microscope micrographs (SEM) of the proboscis and the frontal tarsus of ***Aedes aegypti*** females and the sensilla (cuticular sense organs) that dispersed on their surface .

(1) The tip of the proboscis of a female. x 385

(2) Some types of sensilla on the surface of the proboscis tip. x 1320

(3) Sensory hairs on the tip of the frontal tarsus. x 751

(4) Tip of the frontal tarsus. x 392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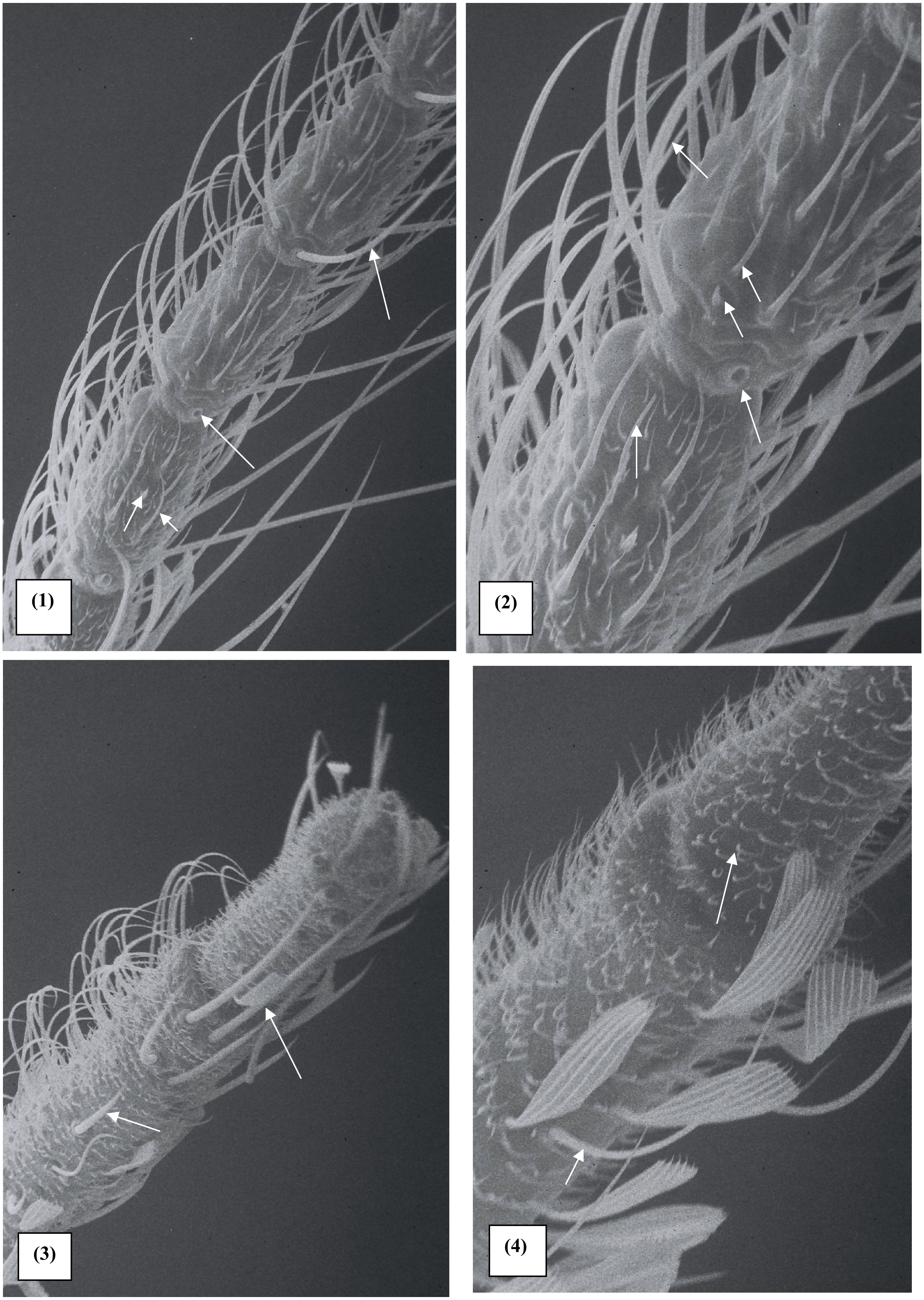
Appendix (7) Scanning electron microscope micrographs (SEM) of the antenna and the maxillary bulbs of ***Anopheles stephensi*** females and some sensilla (cuticular sense organs) that are located on their surface .

(1) Sensory hairs located on the antenna x 420

(2) Some different types of sensory sensilla dispersed on the antennal surface. x 830

(3) Long sensory hairs on the maxillary bulbs. x 415

(4) Two types of sensilla located on the maxillary bulb surface. x 810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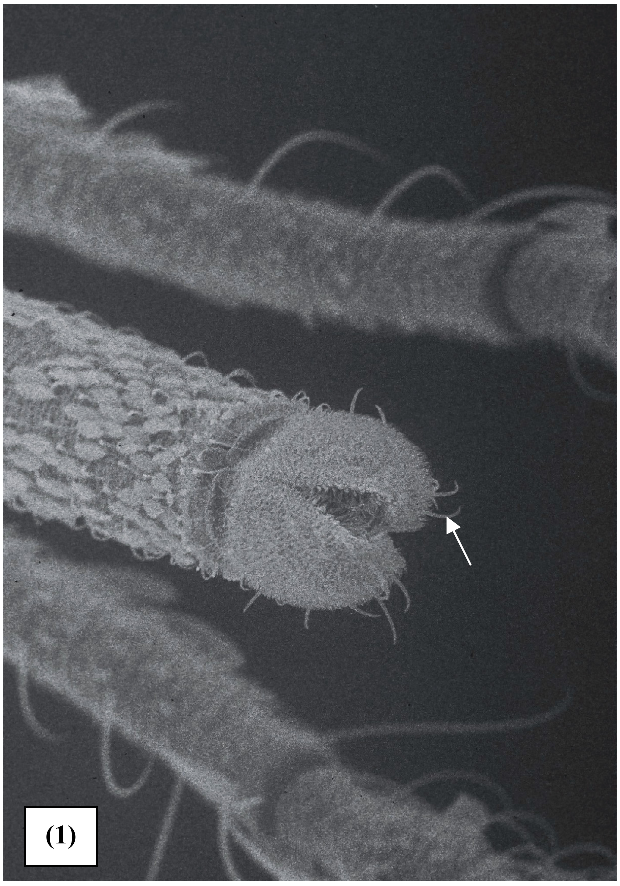
Appendix (8) Scanning electron microscope micrographs (SEM) of the proboscis and the frontal tarsus of ***Anopheles stephensi*** females and the sensilla (cuticular sense organs) that are dispersed on their surface .

(1) The tip of the proboscis of the female. x 314

(2) Some types of sensilla on the surface of the proboscis tip. x 670

(3) Sensory hairs on the tip of the frontal tarsus. x 627

(4) Tip of the frontal tarsus. x 1170



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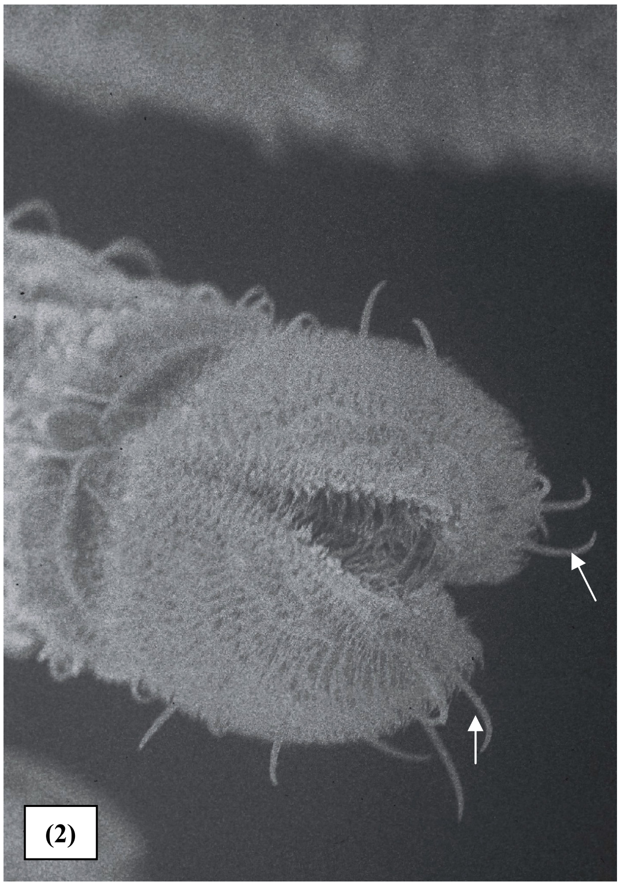

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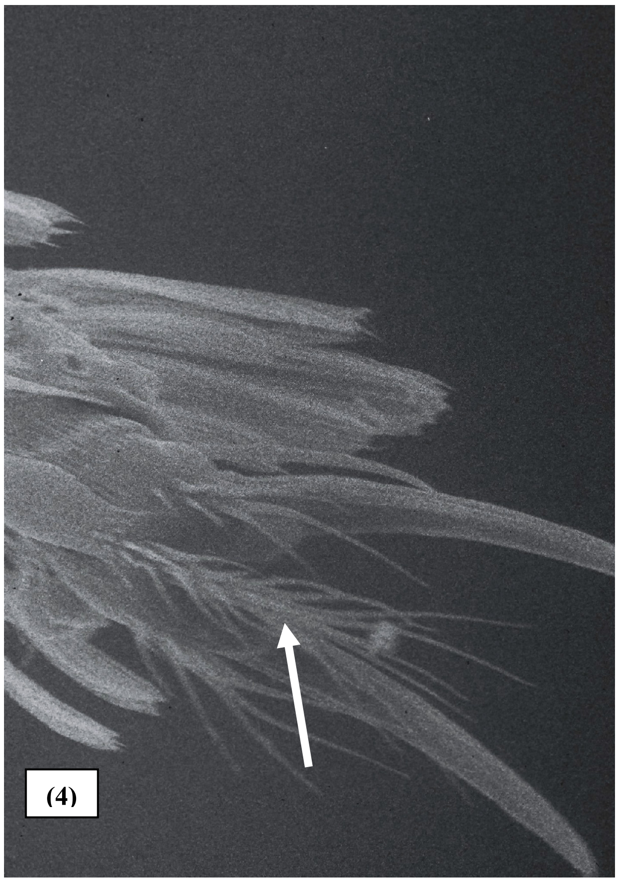

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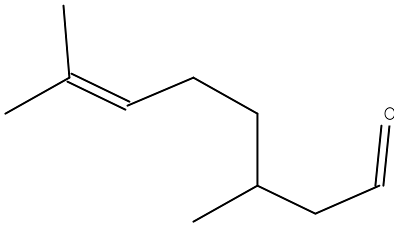
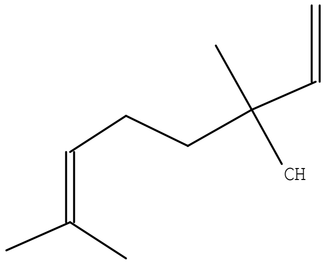
Appendix (9) Molecular structures of main constituents of some plant essential oils that are included in the study, DEET and Bayrepel, Chapman and Hall/CRC(2004).

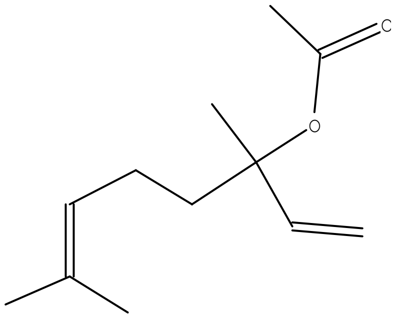
1- citronellal



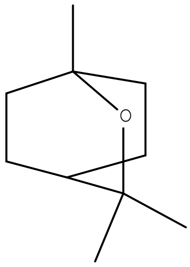
2- linalool



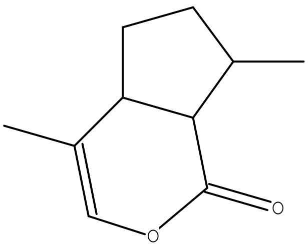
3- Linalyl acetate



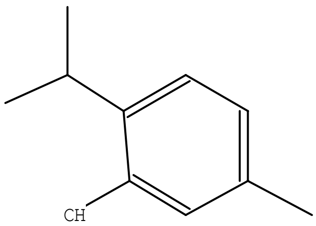
4- Cineole



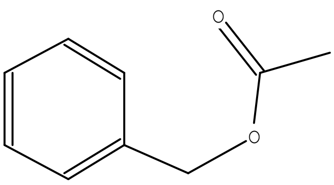
5- Nepetalactone



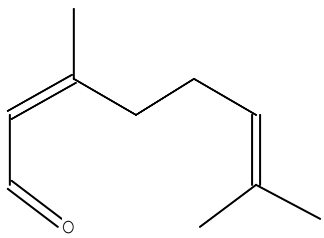
6- Thymol



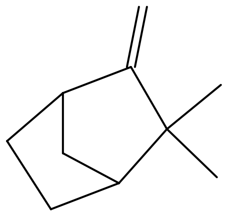
7- Benzyl acetate



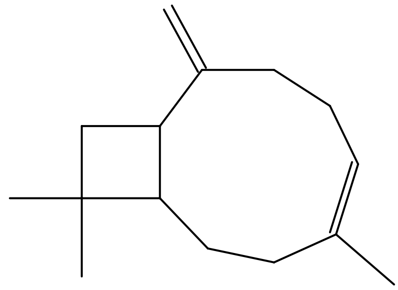
8- Citral



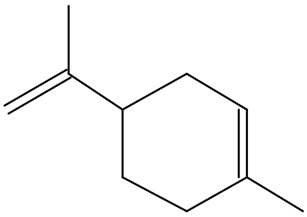
9- Camphene



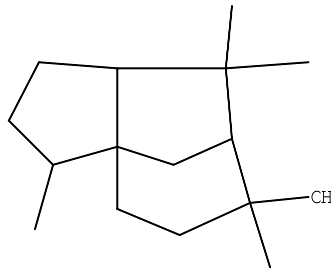
10- Caryophyllene



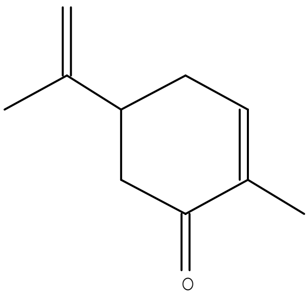
11- Limonene



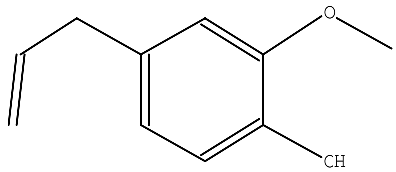
12- Cedranol



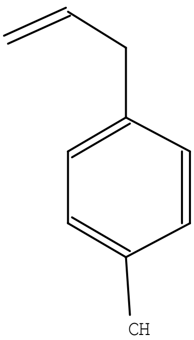
13- Carvone



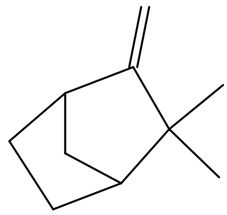
14- Eugenol



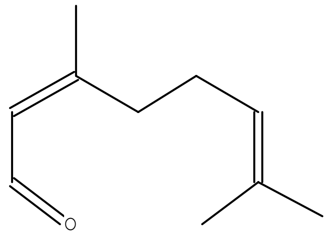
15- chavicol



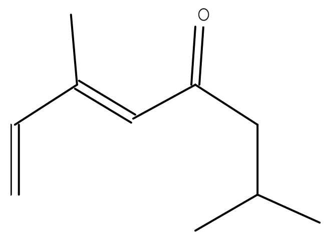
16- Camphene



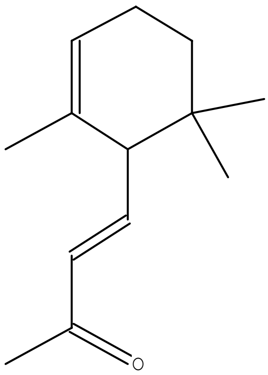
17- Citral



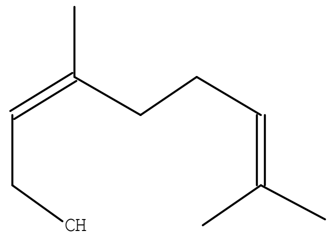
18-Tagetones



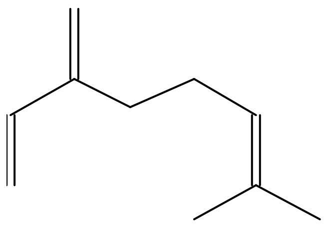
19 – Parmone



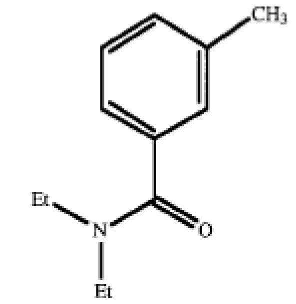
20- Nerol



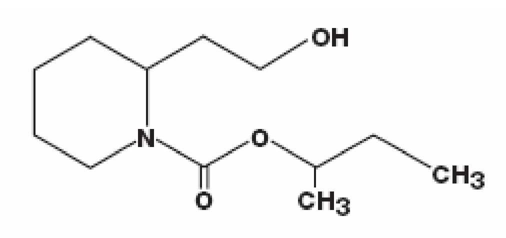
21- myrcene



22- DEET



23- Bayrepel



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(Abdelkrim M. A. Elourfi)