SOME ASPECTS OF BEHAVIOUR AND ELECTROPHYSIOLOGY OF CHEMORECEPTION IN THE MOSQUITO, *ARMIGERES SUBALBATUS*

Thesis submitted to the University of Calicut
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CERTIFICATE

This is to certify that the thesis entitled "**Some aspects of behaviour and electrophysiology of chemoreception in the mosquito, *Armigeres subalbatus***" is a bonafide work of **Reena, K.**, conducted in the Department of Life Sciences under my guidance and supervision. This thesis has not previously formed the basis for the award of any other degree, diploma, associateship or any other similar title of any other University or Society.

15.2.1999. **(Dr. T. Ramakrishna)**

DECLARATION

This thesis entitled "**Some aspects of Behaviour and Electrophysiology of Chemoreception in the mosquito, *Armigeres subalbatus***" is being submitted by me to the University of Calicut in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Physiology in the Faculty of Science. The thesis is entirely the result of my work carried out in the Department of Life Sciences, under the guidance and supervision of **Dr. T. Ramakrishna**, Professor of Physiology, Department of Life Sciences, University of Calicut. This thesis or any part thereof has not been submitted for any other degree, diploma or associateship.

University of Calicut,
.02.1999.

REENA. K.

Dedicated to

Achan and Amma

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CONTENTS

PREFACE

Vector behaviour of mosquitoes has been a topic of interest for biologists, since mosquitoes were discovered as vectors of yellow fever. The knowledge about their role in transmission of malaria accelerated the investigations for the possible role in transmitting other communicable diseases. Thereafter voluminous literature has been published on various aspects of mosquitoes as vectors and their control. Since only the female mosquitoes act as vectors, most of the research has been conducted on various biological aspects of female mosquitoes. Even though chemoreceptor physiology in insects has became an active field of research, only very little is known about the sensory physiology of mosquitoes.

I have studied sensory physiology and behaviour of the mosquito, *Armigeres subalbatus* in some detail. I was inspired to work on this species because it is the most common and vicious man-biting mosquito of Kerala. From the literature survey it was clear that while several studies were carried out on the genus Aedes, Anopheles and Culex, little attention has been given to Armigeres. In this study, sensory physiological aspects have been given importance because, the major factor in host-vector interaction is based on chemoreception. Moreover, I was fortunate to be a student of Prof. Ramakrishna, who channalised me towards the fascinating field of sensory physiology.

The present study is divided into four chapters. In the first chapter, I have tried to give a general introduction about the topic. The second chapter explains the behavioural analysis using some plant extracts, organic as well as inorganic chemicals and natural products. The third chapter deals with the electrophysiology of chemoreception (labral and labellar response of male and female *A. subalbatus*

towards selected stimuli using electrophysiological techniques). The last chapter describes the summary and conclusions of the results obtained in the present study.

Besides contributing to the knowledge of sexually dimorphic behaviour in male and female *A. subalbatus* towards some of the attractants and repellents, this thesis embodies the first ever report of its kind on a basic mechanism of chemoreception in terms of "Across sensilla pattern", similar to the "across neuron pattern" reported among vertebrates. Hopefully, this might be a harbinger of further studies to explore similarities in basic mechanisms of chemoreception across different species.

Reena, K.

ABBREVIATIONS

ADP	-	Adenosine diphosphate
ANOVA	-	Analysis of variance
ATP	-	Adenosine tri phosphate
C I	-	Cell I
C II	-	Cell II
C III	-	Cell III
DDT	-	Dicholoro diphenyl trichloro ethane
DEET	-	N.N.diethyl 3-methyl benzamide
DEPA	-	N.N-diethyl-phenyl acetamide
h	-	Hour
Hz	-	Hertz
LD	-	Lethal dose
M	-	Molar
mv	-	Millivolt
ppm	-	Parts per million
R.H	-	Relative Humidity
S.D	-	Standard deviation
SEM	-	Standard error of mean
WHO	-	World Health Organisation
µv	-	Microvolt

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ABSTRACT

Chemoreception in *Armigeres subalbatus* was investigated through behavioural experiments and electrophysiological techniques. Behavioural experiments were carried out to analyse the olfactory response towards plant extracts, organic as well as inorganic chemicals and natural products. Twelve attractants and seven repellents were traced. Correlation between response to the tested stimuli and age of the mosquito was observed. Contact chemoreceptors of the labrum and labellum in males and females were investigated electrophysiologically, which showed that females possess contact chemosensilla on the labrum as well as labellum whereas in males only labellum possess contact chemosensilla. 0-24h aged mosquitoes (both the sexes) do not show any response to any of the stimuli tested. Across sensilla patterns in mosquitoes were analysed for the first time in mosquitoes and found that the pattern is different in males and females for the same chemical. Across sensilla pattern was compared to the across neuron pattern reported in the olfactory bulb and the nucleus solitarius in the brain stem of the vertebrates.

CHAPTER 1

General Introduction

Keen observers of nature were suspecting the role of blood sucking insects in causing disease, well before the scientific evidence was available. Mosquitoes were proposed as active agents in transmission of yellow fever pathogen by Nott in 1884 and Beauperthuy in 1853 (Prescott *et al.*, 1996). Their role as vectors of disease was confirmed scientifically only in the last quarter of 19th century. The crucial discovery was reported in 1878 by a Scottish doctor, Patrick Manson, who was working in China. He observed that mosquitoes acquired the nematode parasite *Wuchreria bancrofti*, by feeding on infected human beings. Even though he studied the growth and development of the nematode larvae in the mosquito, he disbelieved that the re-infection of human beings occurred when they drank water, in which infected mosquitoes were found. However, this investigation laid the foundation of medical entomology. In 1880, Laveran, an Algerian scientist first observed the exflagellation of male gametocytes of malarial parasite in a drop of human blood. In India, almost at the same time, Ronald Ross observed the production of male gametes within the stomach of mosquitoes that had a diet of blood from malaria infected patient. However, only in 1897 he could trace out the further development of this parasite in the stomach wall of mosquitoes (Prescott *et al.*, 1996).

Further studies unrefutedly illustrated the vector role of anopheles mosquito in transmission of malaria. Then onwards, mosquitoes had been declared as the most dreadful vector of diseases. In 1900, Walter Reed and his colleagues while working in Cuba, proved that the mosquito *Aedes aegypti* transmit yellow fever, a disease caused by an arbovirus. Graham in 1902, gathered evidence for the role of mosquitoes in the transmission of dengue fever, which is also a disease caused by a member of arbovirus (Prescott *et al.*, 1996). Later on, the vector role of mosquitoes

had been identified in more than 200 kinds of pathogenic arboviruses of humans and other animals (Clements, 1992).

Mosquitoes had been identified as vectors of three important types of pathogenic organisms viz. (1) plasmodium (malarial parasite), (2) arboviruses (yellow fever, dengue fever and the encephalitis parasite), (3) filarial worms (brugiean and bancroftian filarial parasite). Tropical third world countries like India are most affected by such vector borne diseases. WHO reports say that about 500-600 million people are infected every year by malarial parasites. Every year, it kills two to three million people globally (Collins and Besansky1994). Other mosquito borne diseases such as yellow fever, dengue, encephalitis and filariasis are also responsible for significant morbidity and mortality in humans (Klowden,1995).

The advent of various insecticides have given great hopes of controlling all vector borne diseases, especially malaria and filariasis. Public health workers in nineteen fifties thought that they were well on the way to wipe out not only malaria but also half a dozen or so major parasitic diseases that afflict human beings (Kolberg, 1994). These hopes had been belied when confronted with the hard facts of evolution, which enabled the emergence of insecticide resistant populations of mosquitoes. One of the most recent attempts to control mosquito borne diseases, is to develop transgenic mosquitoes which can modify wild population of mosquitoes in such a way that it could no longer transmit malaria and other mosquito borne diseases. However, development of a transgenic strain of mosquito demands a thorough knowledge of mosquito biology, population genetics and behaviour, for its successful field trials. Knowledge of host seeking and feeding behaviour of mosquitoes assumes significance in this context in understanding the transmission of vector borne diseases (Defoliart *et al.*, 1987).

Behavioural repertoires are the most amazing features of insects, especially the blood sucking insects. With a relatively simple nervous system and body size, they show surprisingly complex behavioural patterns. It is accepted that most of the insect behaviour are instincts, evolved through millions of years of selective pressure. When various environmental stimuli are received by the sensory receptors, they translate them into the nervous system signals and send to the central nervous system, where they get integrated. The resulting pattern of signals, triggers pre-programmed fixed action patterns residing in the central nervous system and is expressed as behaviour (Klowden 1995).

As a blood -sucking insects mosquitoes need to locate a host from the environment, where there are a lot of background disturbances. Mosquitoes and many other blood -sucking insects have evolved a strategy that make them attract not to the blood but to the organism containing blood. Instead of getting attracted to the blood itself, they get attracted to specific stimuli like CO_2, lactic acid, volatile fatty acids etc. released from the warm blooded animals (Sutcliffe, 1987). Since these stimuli get dispersed in down wind within a short period, movement along the gradient of these molecules are not feasible. It is believed that mosquitoes move along a series of odour islands present in the atmosphere to reach the host. However, mosquitoes do not show uniform spontaneous response to these stimuli throughout the day. Intensity of response to stimuli from the host reach the maximum at certain hours i.e., it follows a circadian rhythmicity with one or more peaks at dawn and dusk (Bildingmayer, 1974). The hours of peak activity will differ from species to species. So, while some species are active at twilight and at night, others are active during the day.

Once activated by the host stimuli, it elicits a chain of behavioural stimulus-response events referred to as host seeking behaviour, which bring the mosquito in contact with a host. So, host seeking includes different patterns of behavioural

responses. Volatile compounds, among the prominent stimuli from the host, reach a wide range i.e., 20-35m away. This initiates an upwind zig zag movement towards a host (Gillies and Wilkes 1969). As they reach nearer the host, stimuli like visual cues and heat, guide the mosquito to the host. Once it alights on the host body, contact chemoreceptors get stimulated and another set of behavioural events are initiated usually. They are together called blood feeding behaviour.

Eventhough significant amount of work has been done in the field of host seeking and feeding behaviour of female mosquitoes, male mosquito has been virtually ignored by mosquito biologists. However, it is known that males can profoundly influence the host seeking and blood feeding behaviour of female mosquitoes. Females after mating, store sperm in their spermatheca, releasing a small quantity to fertilise the eggs as they are laid. At the time of mating, males transfer not only sperm but they also provide proteins from their accessory glands. This also contains some factors called mating factors, which prevent the female from remating (Craig, 1967) and also remove a physiological block that prevent female mosquitoes from ovipositing (Fuchs and Kang, 1978). These factors are also believed to inhibit the host seeking when female mosquito is carrying the fertilised eggs. Thus, in the absence of mating factors, unmated females, even though it carries eggs, may continue host seeking. It is therefore clear that, knowledge of male behaviour is also important to develop a better strategy for mosquito control.

For better understanding of this behavioural repertoire and its regulatory mechanisms in mosquitoes, their sensory reception, especially the chemosensory reception demands detailed understanding. The important chemosensory structures are the antennae and the contact chemoreceptors of the mouth parts and legs. As in all insects, mosquito antennae bear some of the most important sensory receptors which perceive odours. Labella and tarsi are generally accepted as the main

location of contact chemoreceptors in insects. These receptors are medium sized, curved hairs, each with a small papillae at its tip and with two cavities running throughout its length. At the base of the hair, is a sac containing several cells which produce the hair and its socket. Others are the neurones each with a distal fibre. Three or more fibres pass up through the thick walled cavity to, and into the pellicle, which is the receptor site of the sense organ.

1.1. SCOPE AND IMPORTANCE OF THE PRESENT STUDY

Eventhough, blood feeding behaviour of mosquitoes has been studied extensively, little attention has been given to the cues which are used by mosquitoes to locate and choose appropriate host plant in their environment. Behavioural assay with the plants available in our surroundings may suggest a possible role of these plants in feeding behaviour (attraction) or avoiding behaviour (repulsion) in this species. Moreover, electrophysiological studies have shown that the mosquito can distinguish between attractants and repellents and is even able to recognise small differences in concentration. From the earlier studies, it is clear that many synthetic as well as herbal attractants and repellents were traced for the mosquitoes which comes under the genus Aedes, Anopheles and Culex. No such investigation has been carried out in the genus Armigeres. As far as Kerala is concerned, *Armigeres subalbatus* is the most common and vicious man-biting mosquito (Reena and Ramakrishna, 1996). A possible suspicion over the vector status of this mosquito, with reference to Japanese encephalitis (which has been prevalent in Kerala, for the past few years) and dengue fever exists (Srinivas *et al.*, 1994; Pandian *et al.*, 1994).

Although the feeding behaviour of female mosquitoes which act as vectors has been studied extensively, almost nothing is known about the physiological mechanism underlying the feeding behaviour of male mosquitoes. Such studies

exclusively focused on the blood feeding female mosquitoes. Unlike other insects, there are remarkable differences between male and female mosquitoes in their feeding behaviour. Electron microscopic studies revealed the sexual dimorphism of sensilla in mosquitoes. So, this may be reflected in their behaviour also. Therefore, a comparative study in their behaviour related to chomoreception is of great significance. Moreover, it is found that mosquitoes have a delay period between their emergence and their first blood meal (Lehane, 1991), due to the lack of highly sensitive receptors soon after the emergence (Bowen *et al.*, 1994). Likewise, female mosquitoes that have fed to repletion will not engage in further host seeking behaviour (Edman *et al.*, 1975; Klowden, 1990). Hence an attempt has been made to observe the response of *A. subalbatus* at different ages, towards certain selected stimuli in order to establish the maturation process in this species.

1.2. OBJECTIVES

In the light of the earlier studies mentioned so far, further investigations are essential to explain the behavioural aspects of *A. subalbatus*. Hence the study is undertaken,

1. to assay the behavioural activities of *A. subalbatus* using different stimulants such as plant extracts, inorganic and organic chemicals and metabolic wastes.
2. to observe the olfactory response of *A. subalbatus* of different age groups to different stimuli and to correlate the response with the receptor maturation process.
3. to record the electrical activity of the labrum and labellum of male and female *A. subalbatus* using some selected stimulants and to compare the changes in the electrical activity between males and females.

CHAPTER 2

Behavioural Aspects of Chemoreception

2.1. FEEDING BEHAVIOUR : SOME GENERAL ASPECTS

2.1.1. PLANT FEEDING RESPONSE:

It is well known that female mosquitoes ingest blood to obtain protein for the development of ovary, but less well known is the fact that the mosquitoes of both sexes require plant juices as an energy source. Plant sugars are a major food resource for mosquitoes. Floral nectar is the best known source, but they also obtain sugars from extra floral nectaries, damaged fruits, damaged and intact vegetative tissues and honey dew (Hocking 1953, McCrae *et al.*, 1969; Joseph, 1970; Magnarelli, 1979a). These plant juices provide an important energy source during most of the adult life in both the sexes and they are the only food resource of males (Clements, 1992).

Little attention has been paid to the cues that mosquitoes use to locate and choose appropriate host plants in their environment. However, extensive literature is available regarding the response of mosquitoes to plant related compounds. Sugars derived from nectar and plant juices provide mosquitoes an energy source that is essential for flight, metabolic maintenance and certain aspects of reproduction and behaviour (Schaefer and Washino, 1970; Nayar and Sauerman, 1971, 1975; Magnarelli, 1978; Klowden, 1986). The successful location of sugar sources depends in part, on the ability of the mosquitoes to detect and orient to plant volatiles (Thorsteinson and Brust, 1962; Wenster, 1972; Vargo and Foster, 1982 Healy and Jepson, 1988; Jepson and Healy, 1988).

In 1955, Clements traced the sources of energy for flight in mosquitoes and found that females of *Culex pipiens* were able to use the blood as an energy source for flight. Thorsteinson and Brust (1962) studied the influence of flower scents on the aggregative behaviour of *Aedes aegypti.* McCrae *et al.*, (1969) observed the

activities of mosquitoes on nectar sources and found that floral nectar is the best known source of sugars for mosquitoes. Nectar feeding by mosquitoes has been observed in high arctic as well as in temperate and tropical regions. At Zika in Uganda, out of a total of 109 known species, 60 species in 10 genera were recorded feeding on nectar (Hocking, 1968). Records made over several seasons in the subarctic showed that the peaks of abundance of tundra and forest mosquitoes were synchronous with the peaks of nectar production in tundra and forest (McCrae *et al.*, 1969, Grimstad and Defoliart, 1974).

Plant juices were found to be important for the survival of mosquitoes. Females of *Aedes aegypti*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* lived much longer in the laboratory when provided with both sugar and blood meals than when given blood alone (Briegel and Kaiser, 1973; Harada *et al.*, 1976). Harada *et al.*, (1971, 1972) observed the survival and longevity of Culex mosquitoes fed with flowers of some nectar plants. In 1974, Harada *et al.*, observed the survival and longevity of adult Aedes mosquitoes fed on flowers of some nectar plants. Joseph (1970) studied the survival of fruit feeding mosquitoes. With *Culex tarsalis*, nectar feeding is particularly apparent during the autumn, at a time when there is almost no blood feeding, and the females are developing large fat bodies which provide an energy store during winter. In California, females of *Culex tarsalis* including the females in the diapausing component of the population continue to feed on nectar at a low rate throughout the winter, (Schaefer and Miura, 1972; Reisen *et al.*, 1986). In Japan, some females of three species of Culex, hibernating in caves, were found to contain nectar (Harada *et al.*, 1975). Magnarelli (1979a) analysed the diurnal nectar feeding of *Aedes contator* and *Aedes sollicitans*. Healy and Jepson (1988) observed the response of *Anopheles arabiensis* to *Achillea millefolium* flowers and isolated floral odour. An advanced bioassay for volatile plant odours and the location of floral nectar was conducted by

Jepson and Healy (1988). Smith and Gadawski (1994) observed the nectar feeding behaviour of male and female *Aedes provocans* in Canada. Bowen (1992a) described the electrophysiology of the terpene sensitive receptors in *Culex pipiens* and reported the results of behavioural assay that suggested a possible role of specific terpenes in the short distance feeding behaviour of this species.

Some studies have been conducted on the effect of sugar derivatives, fatty acids and amino acids in mosquitoes. Nayar and Sauerman (1974a) observed the long term regulation of sucrose intake by female *Aedes taeniorhynchus*. In Louisiana, Cupp and Stokes (1976) observed the feeding patterns of *Culex salinarius*. Jones and Madhukar (1976) studied the effect of sucrose on blood avidity in mosquitoes. Pappas and Larsen (1978) investigated the gustatory mechanism involved in sugar-feeding in *Culiseta inornata*. Amino acid requirements for mosquitoes were studied by Dadd (1978) and found that asparagine was essential in the case of *Culex pipiens* for optimal growth whereas the fatty acid, arachidonic acid was also found essential for the newly emerged *Culex pipiens* to fly and survive more than a few days (Dadd and Kleinjan, 1979). Dadd *et al.*, (1980) proved the requirement of arachidonic acid by studying two species of Culiseta reared on a synthetic diet. He also investigated the nucleotide, nucleoside and base nutritional requirements of *Culex pipiens* (Dadd, 1979). Day and Van Handel (1986) studied the differences between the nutritional reserves of laboratory maintained and field collected mosquitoes, while Friend *et al.*, (1989) studied the response of *Culiseta inornata* to water, sucrose and cellobiose. In 1992, Bowen analysed the pattern of sugar feeding in diapausing and non diapausing *Culex pipiens*.

2.1.2. BLOOD FEEDING RESPONSES:

Blood feeding behaviour of mosquitoes was also thoroughly investigated. Hosoi (1958) found out adenosine 5'-phosphate as a stimulating agent in blood for inducing gorging in the mosquitoes. Downe (1960) traced the blood meal sources

of some Aedes mosquitoes and observed the host preferences of these mosquitoes, while Service (1971) studied the feeding behaviour and host preferences of British mosquitoes. Feeding response in *Aedes aegypti* was observed by Galun *et al.*, (1963) by stimulating with adenosine triphosphate. In 1973, Jones and Pillitt studied the blood feeding behaviour of adult *Aedes aegypti* mosquitoes. Nayar and Sauerman (1975b) found that apart from the role of egg production, blood ingested by female mosquitoes was also useful as an energy source. They found that in the laboratory, blood-fed females survived longer than water fed females. Edman *et al.*, (1975) observed the blood feeding activity of partially engorged *Culex nigripalpus*. Friend and Smith (1977) found that the adenyl nucleotides are phagostimulants for nearly all groups of haematophagous insects. Friend (1978, 1981, 1985) conducted some laboratory studies and found that mosquitoes do not feed on diets that are at room temperature and covered by a membrane because a heat stimulus is necessary to attract them to the membrane and to induce probing. They concluded that both warmth and membrane were needed for mosquitoes to adopt the blood-feeding mode. Magnarelli (1979b) studied the blood feeding behaviour of mosquitoes on man, racoons and white footed mice. Galun *et al.*, (1985a) found that plasma factors that enhance the responsiveness of Culicines to ATP, are themselves sufficient to induce gorging. Engorgement response of anopheline mosquitoes to blood fractions was also studied by Galun *et al.*, (1985b). Edman and Spielman (1988) studied the physiology, ecology and behaviour of blood feeding mosquitoes. Foster and Eischen (1987) observed the frequency of blood feeding in relation to availability of sugar in *Aedes aegypti* and *Anopheles quadrimaculatus*. Gorging response of *Culex univittatus* to adenine nucleotides was studied by Galun and Friedman (1992).

Blood sucking mosquitoes usually have a delay period between their emergence from the egg and their first blood meal. The reasons for this are not

clear especially as other activities such as mating and dispersal commonly occur during this period. One reason for the delay may be that, after adult emergence, the female reproductive system of mosquitoes undergoes a maturation period lasting for several days (Lehane, 1991). Another reason for the delay in blood feeding may lie in the progressive thickening and hardening of the cuticle, which takes place during the teneral period (Lehane, 1991). This thickening may mean, as it does in mosquitoes (Bates, 1949) that for approximately first 24 hours after emergence, the mouth parts are insufficiently hard to permit efficient skin penetration.

As the post-emergence delay period progresses, or as the time since the blood meal lengthens, the mosquito becomes increasingly hungry for blood and more likely to begin host seeking. The feeding behaviour is not expressed immediately following adult emergence (Davis, 1984). Blood-feeding has been reported to be initiated between 24 to 72 hours after a female mosquito emerges (Seaton and Lumsden, 1941; Bishop and Gilchrist, 1946; Laarman, 1955). Bouts of activity will be mainly restricted to particular times of the day (Dethier, 1976) because activity in blood sucking insects occurs in set patterns during each 24h (circadian) cycle. The timing of these activity bouts is internally programmed and the time of day at which they occur is characteristic for each species. But species show variations in periodicity when collected from different habitats or at different times of the year (Lehane, 1991).

2.1.3. BEHAVIOUR TO SPECIFIC ATTRACTANTS

The location and selection of food and an oviposition site by a mosquito has been shown to be influenced, in part, by airborne chemical stimuli (Christophers, 1960; Gillett, 1961). Several investigators have isolated and identified various chemical substances that are to some extent either attractive or stimulating for feeding or oviposition (Gjullin and Johnsen, 1965; Gjullin *et al.*, 1965; Perry and

Fay, 1967; Ikeshoji *et al.*, 1967; Ikeshoji *et al*, 1979; Starrett and Osgood, 1973; Ikeshoji and Mulla, 1974; Bently *et al.*, 1982; Healy and Jepson, 1988; Bowen, 1992). Brett (1938) observed the relative attractiveness of *A. aegypti* to certain coloured clothes. Reeves (1951) conducted some field studies and found that carbondioxide is an important attractant for mosquitoes for host finding. Khan *et al.* (1965) screened humans for degrees of attractiveness to mosquitoes. In 1968, Acree *et al.* found that L. lactic acid isolated from humans acts as mosquito attractant. The role of lactic acid as mosquito attractant had been investigated by others (Smith *et al.*, 1969, 1970; Davis,1988; Kline *et al.*, 1990; Bowen *et al.*, 1994). Gillies and Wilkes (1969) observed that mosquitoes were attracted to calves at a distance between 15 and 80 meters on the basis of odour alone. Mayer and James (1969) found that mosquitoes are sensitive to very small change in carbondioxide levels; changes as small as 0.05% will elicit behavioural responses in a wind tunnel. A quantitative study of variation in mosquito response and host attractiveness was carried out by Khan *et al.* (1971). Behavioural experiments conducted by Bos and Laarman (1975) found that guinea pig lysine, cadaverine and estradiol as attractants of *Anopheles stephensi*. Bar-Zeev *et al.* (1977) studied the factors attracted to man in *Aedes aegypti.* Davies (1978) studied the preference of *Culex portesi* and *Culex taeniopus* to 20 animal species of Trinidad forest. Orientation of some Florida mosquitoes towards small invertebrates was observed by Edman in 1979. The role of carbondioxide in host finding by mosquitoes was thoroughly investigated by Gillies (1980).

An experimental evaluation of six different suction traps for attracting and capturing *Aedes aegypti* was devised by Kloter *et al.* (1983). Jaenson (1985) traced some male *Aedes diantaeus* attracted to mammals in Sweden. Takken and Kline (1989) observed that carbondioxide and 1-octan-3-ol are mosquito attractants. Kusakabe and Ikeshoji (1990) compared the attractancy of physical and chemical

stimuli to Aedin mosquitoes. Mosquito attraction to substances from the human skin was analysed by Schreck *et al.* (1990) and found that substances removed from the head and hands elicited the greatest attraction. Attraction of mosquitoes to diethyl methyl benzamide and ethyl hexanediol was studied by Mehr *et al.* (1990). They concluded that insect repellents can act as attractants when present in low concentration, deposits or residues. Ashida et al. (1990) studied the prophenoloxidase activation in *Aedes aegypti*. Field studies of Kline *et al.* (1990) analysed the potential of butanone, carbondioxide, honey extract, 1-octan 3-ol, L-lactic acid and phenol as attractants for mosquitoes. They also studied the interactive effects of 1-octan-3-ol and carbondioxide on mosquito surveillance (Kline *et al.*, 1991). The attractiveness of vertebrate hosts to *Culex pipiens* and *Aedes caspius* was observed by Braverman *et al.* (1991) in Israel and found that they have a wide range of mammalian and avian hosts such as calf, chickens, turkeys and sheep. Influence of human breath on selection of biting sites by *Anopheles albimanus* was studied by Knol *et al.* (1994). They concluded that the perception of exhaled breath guided the mosquitoes towards the head region of the host. Knol *et al.* (1994) devised an odour-baited trapping system for testing olfactory responses of *Anopheles gambiae* in a wind tunnel. Using an olfactometer bioassay, Eiras and Jepson (1994) traced the responses of female *A. aegypti* to host odours and convection currents.

2.1.3.1. Oviposition attractants:

Significant work has been carried out in the field of mosquito oviposition attractants. Christie (1958) analysed the oviposition behaviour of *Anopheles gambiae* and suggested some improved collecting methods of Anopheline eggs. Hudson and McLintock (1967) traced out a chemical factor that stimulates oviposition by *Culex tarsalis*. Substances that act as stimulants, such as egg

albumin, glutamic acid and inosine are typically of low volatility and are detected primarily in the water by contact chemoreceptors on the labella of the mosquito (Ikeshoji, 1968). Oviposition attractants, on the other hand, are sufficiently volatile to be detected by the olfactory receptors of the mosquito at a distance from the source (Davis, 1976). Ikeshoji (1968) isolated mosquito attractants for oviposition of *Culex pipiens* from field water. Ikeshoji and Mulla (1974) analysed the attractancy and repellency of alkyl carbonyl compounds for mosquito oviposition. Ikeshoji *et al.* (1967) studied the mosquito attractants and repellents which produce stimulative effects for oviposition in *Culex pipiens* in field water. In 1967, Perry and Fay correlated the chemical constitution and physical properties of fatty acid esters with oviposition response of *Aedes aegypti.* Their results showed that the methyl esters attracted more gravid females than did the ethyl esters of the same root compounds - propionic and butyric acids. They also found that ethyl and isopropyl acetate, methyl and ethyl propionate, and methyl and ethyl butyrate were said to be attractive for *A. aegypti* and *Culex salinarius.* Ikeshoji *et al.* (1979) found that 7, 11-dimethyl octadecane as an oviposition attractant for *Aedes aegypti* whereas 4-methyl cyclohexanol acts as oviposition attractant for *Aedes triseriatus* (Bently *et al.*, 1982). Daniel *et al.* (1987) isolated an oviposition attractant from natural breeding water of *Mansonia uniformis.* Beehler *et al.* (1993, 1994) showed that 3-methyl indole, Indole, 4-methyl phenol and phenol act as oviposition attractant of *Culex quinquefasciatus.*

2.1.4. BEHAVIOUR TO SPECIFIC REPELLENTS

Since the mosquito is unlikely to be eliminated in the near future, many investigators are now focussing on the mosquito repellents. In recent years, repellent devices have become common in urban and rural areas of India due to mosquito menace. Mosquito menace in India has now assumed alarming

proportions. Almost all parts of India suffer this problem. In cities, uninhibited growth and lack of proper development plans have compounded the problem. In spite of all control measures such as chemical, biological and integrated control, the mosquito menace is increasing day by day.

Smoke coils and mats containing pyrethroids are used widely to protect humans from the bites of endophilic mosquitoes (Feles *et al.*,1968; Ansari, 1990). In 1946, Ribbands observed the repellency of pyrethrum and lethane sprays to mosquitoes. In British Guina, Symes and Hadaway (1947) conducted initial experiments in the use of DDT against mosquitoes. The mode of action of repellents against blood sucking flies was analysed by Hocking and Khan in 1966. Feles *et al.* (1968, 1971) evaluated the smoke of insecticidal coils against mosquitoes. They measured the repellency in terms of percentage and found that a piece of a coil (0.25 gm) containing 0.5% pyrethrins repelled 42% of test mosquitoes. Hudson and Esozed (1971) analysed the effect of smoke from mosquito coils on *Anopheles gambiae* and *Mansonia uniformis*. In China, Li *et al.* (1974) studied the repellency of mosquitoes towards the chemical quwenling but Schreck (1977, 1985) reported that both quwenling and DEET (N,N- diethyl 3-methyl benzamide) were ineffective in repelling *Anopheles albimanus*. Several studies have been conducted to observe the sensitivity of mosquito species to the repellent DEET (Rutledge *et al.*, 1978, 1983; Curtis and Hill, 1988; Das *et al.*, 1988; Schreck and McGovern, 1989; Kuthiala *et al.*, 1992; Francis *et al.*, 1993) Experiments of McGovern *et al.* (1978) proved that alicyclic amides act as repellents for *A. aegypti* and *Anopheles quadrimaculatus*. McGovern and Schreck (1988) also found that monocarboxylic esters of aliphatic diols that contained an alicyclic group in acyl portion of the molecules were effective repellents for *A. aegypti*, *A. quadrimaculatus* and *Anopheles albimanus* when sprayed on clothes. Personal protection is also offered by the topical application of

repellents such as permethrin against *Aedes taeniorhynchus* (Schreck and Kline, 1989).

The efficacy of fabrics impregnated with N,N-diethyl-phenylacetamide (DEPA), was studied in the laboratory and field against *A. aegypti* and *C. quinquefasciatus* (Rao *et al.*, 1991). 2% pyrethrum extract in kerosene brought major reduction in numbers of *Anopheles culcifacies* and *A. stephensi* (Sharma *et al.*, 1992). In a laboratory study, the irritancy of bendiocarb, lambda cyhalothrin and DDT to *A. gambiae* was evaluated using WHO conical exposure chambers and excito-repellency test boxes (Evans, 1993). Coleman *et al.* (1993) conducted laboratory evaluation of the repellents lactone CIC-4 and piperidine compounds against four anopheline species. Similar studies had been conducted in *Culex pipiens* (Coleman *et al.*, 1994) to evaluate the efficacy of these repellents.

In New Guinea, smoke coils containing pyrethroids were widely used against mosquitoes (Charlwood and Jolley, 1984). Although mosquito repellents containing pyrethroids generally are considered safe and there are no serious complaints of toxic reactions from their use, prolonged exposure to coil smoke may be harmful (Liu *et al.*, 1987). Through the laboratory experiments, Ameen *et al.*, (1993) evaluated eleven smoke producing repellents against *Culex quinquefasciatus*. Snow *et al.* (1987) studied the indigenous smoke producing mosquito repellents. Pandian *et al.* (1989) found that smoke of herbal leaves seems to be an effective mosquito repellent and this has distinct advantages over chemical repellents because it does not leave poisonous residues and does not pollute the environment.

Curtis *et al.* (1990) and Ansari *et al.* (1990) reviewed the currently available synthetic and natural repellents. New restrictions on the use of chemicals for insect control have stimulated investigations on the insecticidal properties of plant

materials (Supavaran *et al.*, 1974). Biologically active plant extracts are therefore being studied for their potential efficacy to minimise the extent of pollution and to reduce the cost. The smoke of the leaves *Vitex negundo* and *Leucas aspera* are more toxic to *C. quinquefasciatus* than the synthetic mosquito mats which contain 4% d-allethrin (Pandian *et al.*, 1994). *V. negundo* was effective against larvae of *C. quinquefasciatus* at 120 ppm concentrations (Kalyansundaram and Babu, 1982). Petroleum ether extracts of *L. aspera* was tested for the larvicidal efficacy of *C. quinquefasciatus* (Kalyansundaram and Das, 1985). Kumar and Dutta (1987) studied the larvicidal activity of plant oils extracted from *Cymbopogon nardus, Lavendula officinalis, Mentha arvensis, Ricinus communis, Eucalyptus globulus, Eugenia caryophyllus* etc. in acetone against the larvae of *A. stephensi*, resulted in complete mortality at 250 ppm. Extracts of *Anacardium occidentale, Agave americana, Allium sativum, Coriandrum sativum, Nerium oleander, Spatodea campanulata, Tibouchina scrobiculata and Vernonia salzmanni* also showed larvicidal properties against *Aedes fluviatilis* (Consoli *et al.*, 1988). Thangam and Kathiresan (1988) studied the larvicidal action of 17 extracts of mangrove plants against *A. stephensi*. Sujatha *et al.*(1988) evaluated certain plant extracts known to contain toxic principles, against mosquitoes. Shahab *et al.* (1990) studied the toxicity and teratogenic effect of Peucedanum (Soya) extract against the larvae of *Culex fatigans*. Schreck and Leonhardt (1991) evaluated the efficacy of quwenling, derived from extracts of the lemon eucalyptus plant (*Eucalyptus maculata citriodon*) against *Anopheles albimanus, A. quadrimaculatus, Aedes aegypti, A. albopictus* and *A. taeniorhynchus*. The crude extract of *Ageratum conyzoides* was found to suppress the population of *A. stephensi* at higher dosages (Saxena and Saxena, 1992). Laboratory evaluation of Pittendrigh and Zacharuk (1992) found Xanthan gum, to be a potential mosquito control agent against *Aedes atropalpus*. Watanable *et al.* (1993) isolated a new mosquito repellent from the essential oil of

Eucalyptus camaldulensis against *A. aegypti*. Achary *et al.* (1993) investigated the efficacy of Ipomoea leaf extract for the control of *C. quinquefasciatus* population. Alkaloids isolated from *Annona squamosa* have shown larvicidal growth regulating and chemosterilant activities against *A. stephensi* at 50-200 ppm concentration (Saxena *et al.*, 1993). Sharma *et al.* (1993) found that 2% neem oil mixed in coconut oil, when applied to the exposed body parts of human volunteers, provided complete protection for 12 hours from the bites of all anopheline mosquitoes. Kamal and Mangala (1993) isolated rotenoids from *Indigofera tinctoria* and assessed their bioefficacy against the larvae of *A. stephensi*. Mwaiko and Savaeli (1994) extracted lemon peel oil and observed larvicidal efficacy against *C. quinquefasciatus*, while Perich *et al.* (1994) examined the toxicity of the extracts from three species of Tagetes against *A. Stephensi* and *A. aegypti*. Pupicidal effect of *Ocimum sanctum* on *A. aegypti* was observed by Kumari *et al.* (1994). Sharma *et al.*, (1993) evaluated the repellent action of neem oil (extracted from the seeds of *Azadirachta indica*). They found that kerosene lamps containing 1% neem oil provide economical personal protection from mosquito bites. Rao *et al.* (1995) developed the combined use of neem and water management for the control of culicine mosquitoes in rice field. Reena and Ramakrishna (1997) reported three herbal repellents against *A. subalbatus*.

2.1.5. SIGNIFICANCE OF BEHAVIOURAL STUDIES DEALING WITH ATTRACTANTS/REPELLENTS:

Though it is known that plant sugars are the only food resource for the male mosquito, little attention has been paid to identify such attractants. Moreover, these attractants can be used to develop new mosquito traps for a better strategy of mosquito control.

A recent study by the Vector Control Research Centre at Pondicherry has shown that the insecticide impregnated coils and mats generally contain synthetic pyrethroids like bioallethrin. They have also found that on an average, a person inhales 6-8 litres of air per minute. If one sleeps with the mat or coil on, overnight (6 hours) one may inhale about $3m^3$ of air containing 1.5 mg of chemical. This value is much below the LD_{50} value. However, continuous inhalation and long term exposure are known to induce repetitive activity in various parts of the nervous system (Mohan, 1990). Pyrethroids in coils or mats may continue as a standard method of mosquito protection as it has become a dire necessity. There is, therefore, need for a safer protection (Sharma and Ansari, 1994). Various plant products have been tried recently with a good degree of success as protectants against a number of insect pests. However, herbal repellents against blood sucking insects have not been thoroughly investigated even though many plant extracts act as repellents, larvicides or insecticides due to their strong odoriferous nature. Repellency is another mode of insect control through natural plant products, which could induce toxic effects to the target organism prior to their coming closer to the compound (Rajini *et al.*, 1993).

The plant kingdom which is a rich source of effective chemicals still lies unexplored. Only some reports are available on the use of bioactive substances from plants for mosquito management. Because of the availability of large number of medicinal plants in Kerala, a search for herbal repellents against mosquitoes seems to be worthwhile. The earlier studies dealing with repellents were mainly against the mosquitoes belonging to the genus Aedes, Anopheles and Culex. No such work has been carried out on *Armigeres subalbatus*, which is the most common and vicious man-biting mosquito of Kerala (Reena and Ramakrishna, 1996).

Mosquitoes use air-borne chemical cues to guide them to resources such as blood-meal hosts, plants and oviposition sites. For this, it is necessary to understand about what chemical signals a mosquito can detect and at what air-borne concentrations such compounds are effective. Such studies have helped to clarify the role of lactic acid, ammonia, carbondioxide, octenol, phenols, temperature and humidity in the attraction of mosquitoes to blood-meal hosts. Egg raft, pheromone, indoles, cresols, methyl cyclohexanol, 2-butoxy ethanol and fatty acid esters have been examined with respect to oviposition site location and selection in mosquitoes. However, plant volatiles have received less attention. Information from such studies can also be useful in the design of both attractants and more effective repellents, for a better strategy of mosquito control.

In the light of the above mentioned studies, an attempt has been made in this chapter,

1) to trace attractants and repellents among plant extracts, natural products, organic chemicals and inorganic chemicals of *A. subalbatus.*

2) to formulate a new oviposition attractant for *A. subalbatus.*

3) to study age related responses of *A. subalbatus* to different attractants/ repellents.

2. 2. MATERIALS AND METHODS

2.2.1.INSECTS

Morphologically, *Armigeres subalbatus* is the largest of the mosquitoes available in Kerala. It has a length of 4±0.5 cm and a width of 1±0.3 cm. On the basis of bigger size, *A. subalbatus* can easily be identified without any difficulty. These mosquitoes of both sex (Plate-1) were collected from different places of Kozhikode and Malappuram districts and the identification was confirmed by the Vector Control Research Center, Pondichery. *A. subalbatus* is classified as follows.

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family:Culicidae
Genus: Armigeres
Species: subalbatus

2.2.2. STUDY AREA

A detailed survey was carried out for the habitats of *A. subalbatus* in Kozhikode and Malappuram Districts of Kerala by observing the abundance. Five sites were selected for the collection of mosquitoes. The sites include water tanks, drainage, septic tanks, bath rooms and living rooms. These five sites were,

1) Premises of Students' hostel, Calicut university.

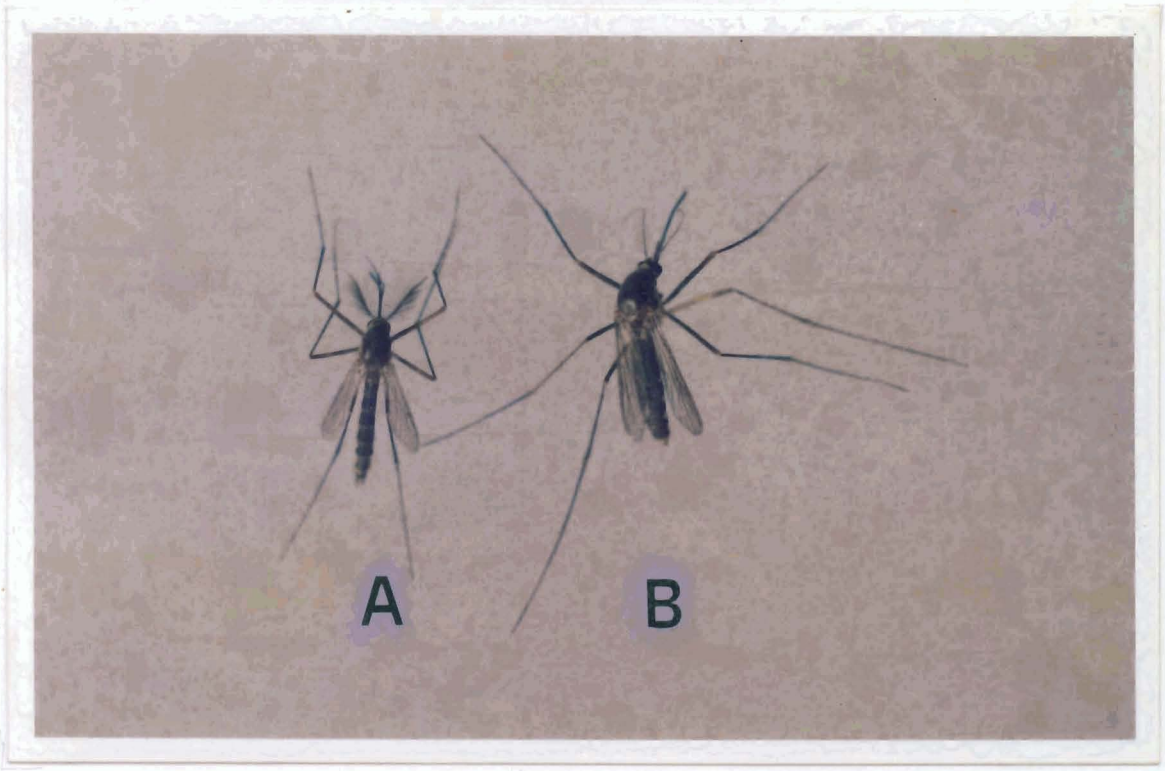

PLATE - 1. Adult *Armigeres subalbatus.*

A - Male B - Female

2) Premises of a pond and waist pit behind a house at Quilandy, Kozhikode District

3) Premises near the drainage canal of housing colony, Calicut.

4) Premises of a hospital, Nadakav, Calicut.

5) Premises of animal house, Dept. of Life Sciences, Calicut University.

Eggs, larvae and adults were collected from these sites using different methods for the study.

2.2.3. SAMPLING OF EGG POPULATION

Eggs of A. subalbatus were found in all the habitats mentioned above. This species usually breed in foul smelling waters. They deposit their eggs on the upper surface of the floating vegetation, on the walls of man made containers, on mud, debris or other wet surfaces on the water's edges. The eggs will be in sticky masses glued to the wet surfaces. The eggs were collected for culturing A. subalbatus in the laboratory.

2.2.4. SAMPLING OF ADULT POPULATION

Only very few mosquito species commonly rest in man made shelters. Most of them rest in natural shelters such as amongst vegetation, in hollow trees, animal burrows and crevices in the ground etc. So search for out door resting mosquitoes frequently proved not only time consuming but also unrewarding (Bown and Bang, 1980). Adult *A. subalbatus* were collected from the field using sweep nets from the sites mentioned above. Collections were

made during the peak hours of biting activity of these mosquitoes i.e., at 6 am - 7 am and 6 pm - 7 pm., since it is a crepuscular biter (Pandian, 1994). Blood fed resting females were collected from indoors especially from walls, ceilings and clothing etc. The most efficient method of collecting resting adults was to place the mouth of a polythene bag over them with out disturbing the insect, and once they are trapped the mouth of the bag is to be tied up.

2.2.5. MASS CULTURING

2.2.5.1. Mosquito Cage

A. subalbatus were cultured and maintained in a cage, composed of mosquito net and perplex sheet in wooden frame. The roof of the cage was made up of mosquito net, with an opening on the top for the collection of mosquitoes. The opening of the top was covered with polythene bag. The cage was divided into two chambers by fixing a wooden partition. The top portion of the cage was made without any partition for the free flying of the mosquitoes between the chambers (Fig.1).

2.2.5.2. Rearing

The eggs collected in the filter paper from the field were transferred into a beaker filled with water. This beaker was kept inside the cage. The larvae were hatched out after two to three days. These larvae were fed with yeast granules and dog biscuits. This was given for about four to six days. by that time the larvae entered into pupal stage. No feed was provided during the pupal stage since it is a non-feeder. Adults emerged within two to three days into the cage and were

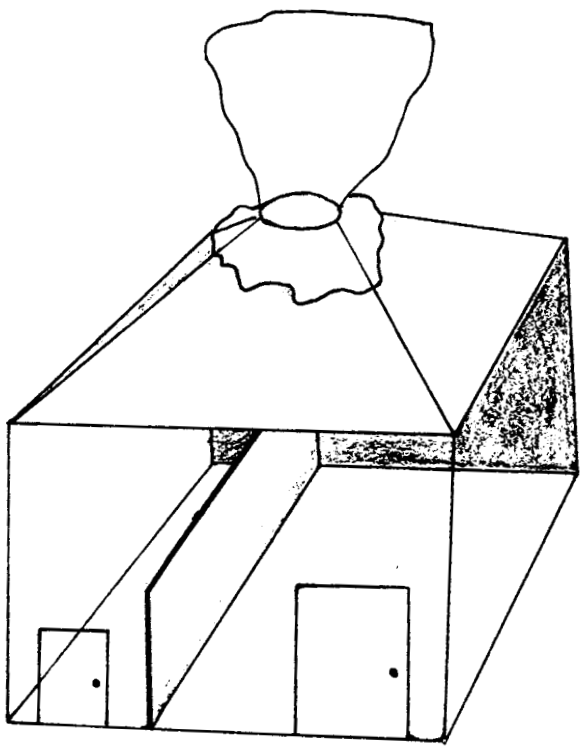

FIGURE-1. Mosquito culturing cage.

maintained on a diet of honey, 15% sucrose solution and water. Three cotton balls soaked in this diet were kept in a petridish inside the larger chamber of the cage. The female mosquitoes were also provided with blood meal periodically by introducing a rat into the small chamber of the cage, since blood meal is essential for the production of eggs (Clements, 1992). This colony was maintained till the experiments were over. Since *A. subalbatus* breeds in fowl smelling water (Pandian, 1994), a filter paper lined plastic pot with fowl smelling water was kept inside the cage for egg collection (Fig. 2). This filter paper strip with eggs were again immersed in the beaker with water and the cycle was repeated and the colony was maintained at a temperature of 28±8°C, at 75-80% relative humidity under 12 hours light and 12 hours dark cycle. Field-collected adult mosquitoes were introduced into the cage for egg production in large numbers.

2.2.6. APPARATUS AND EXPERIMENTAL PROCEDURE

Mosquitoes to be tested for their response to air-borne stimuli were introduced into a thirteen centimetre long and eight centimetre diameter glass cylinder , covered with mosquito- net at both ends (fig-3). Detailed procedure of stimulus delivery has been described previously (Bowen, 1992; Reena and Ramakrishna, 1997). Carrier air stream was directed into the cylinder by switching on a small fan, kept one meter away from one end of the cylinder. Stimulus air stream was given by keeping a cotton ball of uniform size, soaked in the stimulant and kept in between the fan and the cylinder. The carrier air stream was given continuously throughout the entire experiment. The stimulus air stream was given for one minute and at the end of that time responding mosquitoes were counted. The cotton soaked in the stimulant was then removed and the carrier air stream was allowed to flow through the cylinder for two minutes before the next

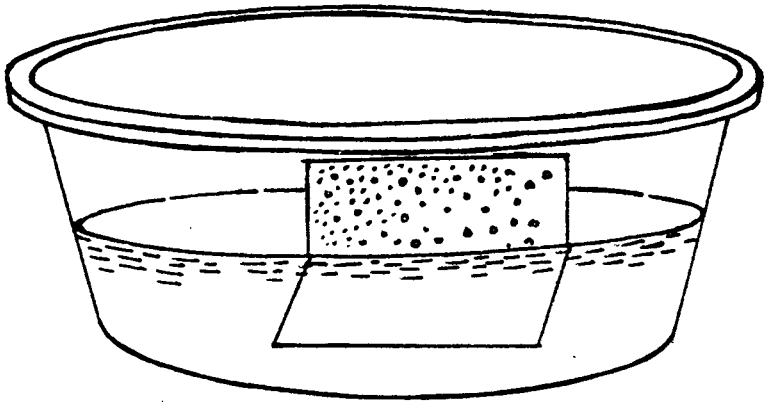

FIGURE-2. Set-up for egg collection.

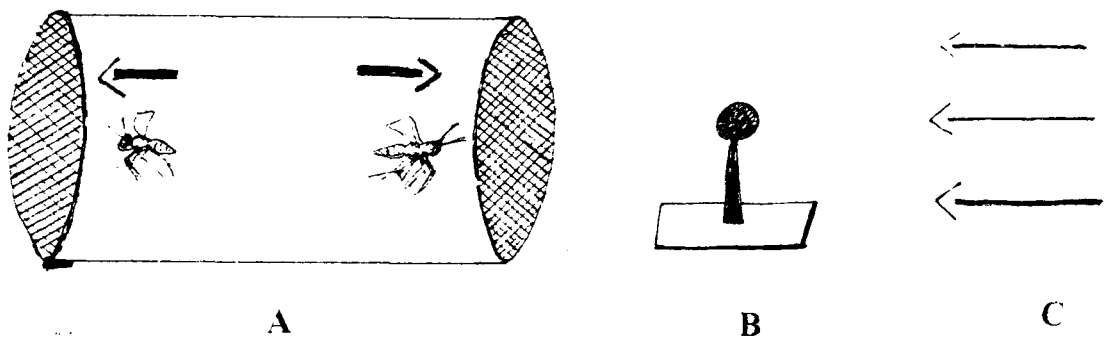

FIGURE-3. Behavioural set up to test odour peference (attractants/ repellents) with artificial carrier air-stream.

A- glass cylinder B- cotton ball soaked in stimulant
C- air stream
→ attracted mosquitoes ← repelled moquitoes

stimulus was given, to clear the cylinder from the volatiles and to allow the mosquitoes to adapt to odour-free condition. Behavioural responses were measured by the method followed by Reena and Ramakrishna (1997), by observing the mosquitoes probing on either side of the screens or those making short flights from the sides of the cylinder towards the screen and then subsequently probing, were counted as responders. Sustained flight activity was not a behavioural option in this set up. There are three types of responses viz. attraction, repulsion and no response. If the mosquitoes fly against the stimulus air stream and probed on the net, the stimulus was counted as attractant. If the mosquitoes move along the stimulus air stream flow, that stimulus was counted as repellent. The non-responding mosquitoes in the presence of stimulus air stream were immobile except for occasional grooming movements and were treated as non-responders. Behavioural responses were assessed by this experiment.

In order to confirm the results obtained through the above method, tests were repeated without carrier air stream (fig-4). Cotton soaked in the stimulus was kept one centimetre away from one end of the screen. If the mosquitoes fly away from the stimulus end where the soaked cotton was kept, to the opposite side, they are considered to have shown repellent behaviour to that given stimulus, and if the mosquitoes fly towards the stimulant, they are considered to have shown attractant behaviour.

Experiments were conducted at dawn (5-7 am) and dusk (5-7 p.m.) hours of the day since *A. subalbatus* is a crepuscular biter, and this is the time of the day during which they exhibit intensive swarming and feeding activities. The assays were conducted in light under ambient room conditions (73% R.H at 20-28°C).

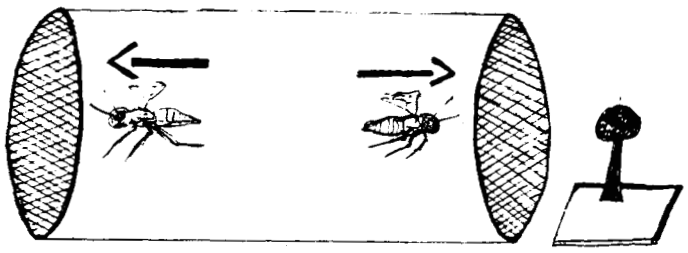

FIGURE-4. Behavioural set up to test odour preference (attractants/ repellents) without artificial carrier air-stream.

A- glass cylinder B- cotton ball soaked in stimulant
→ attracted mosquitoes ← repelled moquitoes.

Mosquitoes were tested in groups of ten and the experiments were repeated ten times with each stimulant.

2.2.7. STIMULI USED

Tested stimuli were classified into four categories viz. plants, natural products, organic and inorganic chemicals.

2.2.7.1. Plants

Tested plants were classified into three divisions : ornamental plants , medicinal plants and common plants. Names of the plants were listed in the tables-1, 2 and 3. All those selected plants have some odour. Fresh leaves of the plants were crushed in a mixer without adding water and the extract was filtered through a sieve and stored in the refrigerator. A cotton ball of uniform size was soaked in the extract just before the commencement of the experiment. Around 3 ml. of plant extract was needed to soak the cotton.

2.2.7.2. Natural Products

Name of the natural products tested were listed in the table 4. These stimulants were taken in 100% concentrations.

TABLE – 1.

Plant extract (Ornamental plants) tested to study behavioural response.

No	Ornamental plants	Response	
		Male	Female
1	*Fittonia verschaffeltii*	NR	NR
2	*Odontonema strichum*	NR	NR
3	*Pachystache lutea*	NR	NR
4	*Celosia cristata*	NR	NR
5	*Amaranthus caudatus*	NR	NR
6	*Gompherana globosa*	NR	NR
7	*Cananga odorata*	NR	NR
8	*Allanta cathartica*	NR	NR
9	*Tabenaemontana coronaria*	NR	NR
10	*Plumeria rubra acutifolia*	NR	NR
11	*Rauwolfia tetraphylla*	NR	NR
12	*Tagetes erectus*	NR	NR
13	*Michellia champaka*	NR	NR
14	*Lantana camara*	NR	NR
15	*Jasminum sambac*	NR	NR
16	*Chyrasethamum intybus*	NR	NR
17	*Ixora chinensis*	NR	NR
18	*Hibiscus rosa sinesis*	NR	NR
19	*Catharanthus roseus*	NR	NR
20	*Impatiens balsamina*	NR	NR

NR: No Response

TABLE – 2.

Plant extract (Common plants) tested to study behavioural response.

No	Common plants	Response	
		Male	Female
1	*Eupotorium odoratum*	NR	NR
2	*Acacia auriculiformia*	NR	NR
3	*Anacardium occodentale*	NR	NR
4	*Brassica olaracea var. Capituta*	NR	NR
5	*Cucurbita pepo*	NR	NR
6	*Casia fistula*	NR	NR
7	*Magnifera indica*	NR	NR
8	*Annona muricata*	NR	NR
9	*Annona squamosa*	NR	NR
10	*Annona cherimola*	NR	NR
11	*Clerodendrum viscosum*	NR	NR
12	*Momardica charantia*	NR	NR
13	*Allium sepa (Allium)*	NR	NR
14	*Lycopersium esculentum*	NR	NR
15	*Moringa oleifera*	NR	NR
16	*Clerodendron paniculatum*	NR	NR
17	*Carica papaya*	NR	NR
18	*Brassica Juncea*	NR	NR
19	*Piper betle*	NR	NR
20	*Nicotina tabaccum*	NR	NR

NR: No Response

TABLE - 3

Plant extracts (Medicinal plants) tested to study behavioural response.

No	Medicinal plants	Response	
		Male	Female
1	*Coleus ambonicos*	NR	NR
2	*Azadirachta indica*	R	R
3	*Mentha piperita*	NR	NR
4	*Piper nigrum*	NR	NR
5	*Ocimum sanctum*	R	R
6	*Zingiber officinale*	NR	NR
7	*Curcuma domestica*	NR	NR
8	*Murraya koenigii*	NR	NR
9	*Leucas aspera*	R	R
10	*Eugenia aromaticum*	NR	NR
11	*Vitex negundo*	R	R
12	*Coriandrum sativum*	NR	NR
13	*Premna latifolia*	R	NR
14	*Rauvolfia sarpantina*	NR	NR
15	*Phyllanthus emblica*	NR	NR
16	*Myristica fragrans*	NR	NR
17	*Ricinus communis*	NR	NR
18	*Cymbopogon citratus*	R	R
19	*Psidium gujava*	NR	NR
20	*Mimosa pudica*	NR	NR

NR : No Response R: Repulsion

2.2.7.3. Organic and Inorganic chemicals

Ten inorganic chemicals (table -5) and 25 organic chemicals (table-6) were tested to observe the olfactory response. 100% concentrated solutions were taken for the assay.

2.2.7.4. Mosquitoes

The mosquitoes used for the behavioural assay were reared in the laboratory. The experiments were conducted in the mosquitoes of three age groups. Newly emerged mosquitoes were tested for their response. They were categorised into three groups ie. 0-48 h, 48-96 h and above 96 h. Each group of mosquitoes was exposed to all the stimuli tested and the percentage of response was calculated for attractants, repellents and noresponse categories. Males and females were tested separately.

2.2.9. OVIPOSITION ATTRACTANT

Different combinations of stimuli were made to trace the best oviposition attractant for *A. subalbatus* as follows.

1. Yeast : 0.1gm yeast granules were dissolved in two litres of tap water
2. Humus : 10gm of organic material rich humus were added to two litres of tap water and mixed thoroughly.
3. Acetone : To two litres of tap water, 0.5ml acetone was added.
4. Arecanut husk: To two litres of tap water, 25g dried arecanut husk was soaked and kept for three days before the commencement of the experiment.
5. Mixture of arecanut husk, acetone and yeast : 25g of dried arecanut husk was soaked in two litres of tap water in which 0.05g of yeast granules were

TABLE - 4
Natural products tested to study behavioural response.

No	Natural products	Response	
		Male	Female
1	Honey	A	A
2	Blood	NR	NR
3	Sweat	A	A
4	Urine	A	A

A: Attraction NR : No Response

TABLE - 5
Inorganic chemicals tested to study behavioural response.

No	Inorganic chemicals	Response	
		Male	Female
1	Boric acid	NR	NR
2	Ammonia	A	A
3	Amonium chloride	A	A
4	Potassium permanganate	NR	NR
5	Magnesium sulphate	NR	NR
6	Manganese chloride	NR	NR
7	Barium chloride	NR	NR
8	Potassium dichromate	NR	NR
9	Sodium chloride	NR	NR
10	Calcium chloride	NR	NR

A: Attraction NR : No Response

TABLE – 6
Organic Chemicals tested to study behavioural response.

No	Organic Chemicals	Response	
		Male	Female
	Hetrocyclic		
1	1,4, Dioxan	A	A
2	Pyridine	NR	NR
	Hydro carbon		
3	Chlorofom	NR	NR
4	Toluene	NR	NR
	Aldehyde		
5	Formaldehyde	NR	NR
6	Benzaldehyde	NR	NR
	Ketone		
7	Acetone	A	A
8	Menthone	NR	NR
	Alcohol		
9	Propan-ol	NR	NR
10	Methanol	NR	NR
11	Isobutyl alcohol	NR	NR
12	Butan-1-ol	A	A
13	Amyl alcohol	A	A
14	Phenol	A	A
	Ether		
15	Petroleum ether	A	A
16	Diethyl ether	NR	NR
	Acid		
17	Lactic acid	A	A
18	Citric acid	NR	NR
19	Acetic acid	R	R
	Amine		
20	Methyl amine	NR	NR
21	Benzyl amine	NR	NR
	Esters		
22	Ethyl acetate	NR	NR
23	Methyl butyrate	NR	NR
	Amide		
24	Urea	NR	NR
25	Thio urea	NR	NR

A: Attraction R: Repulsion NR : No Response

dissolved. This mixture was kept for three days for the decaying of arecanut husk. To this, 0.5ml of acetone was added.

6. Tap water : Two litres of clean tap water.

Each one of the above preparations was taken in separate plastic troughs of eight litre capacity with 45cm diameter at the mouth. The filter paper (length 10cm, width 8cm) stained gray black was stuck on the walls of the plastic troughs in such a way that half of the width of the filter paper was immersed in the stimulant (Plate-2).

These six troughs were kept at selected location. The eggs deposited on the filter paper strips were removed after 48h for enumeration. The experiment was repeated ten times. The mean number of eggs were calculated. The trough from which maximum number of eggs were obtained was found out and that combination of stimulants was considered as the best oviposition attractant.

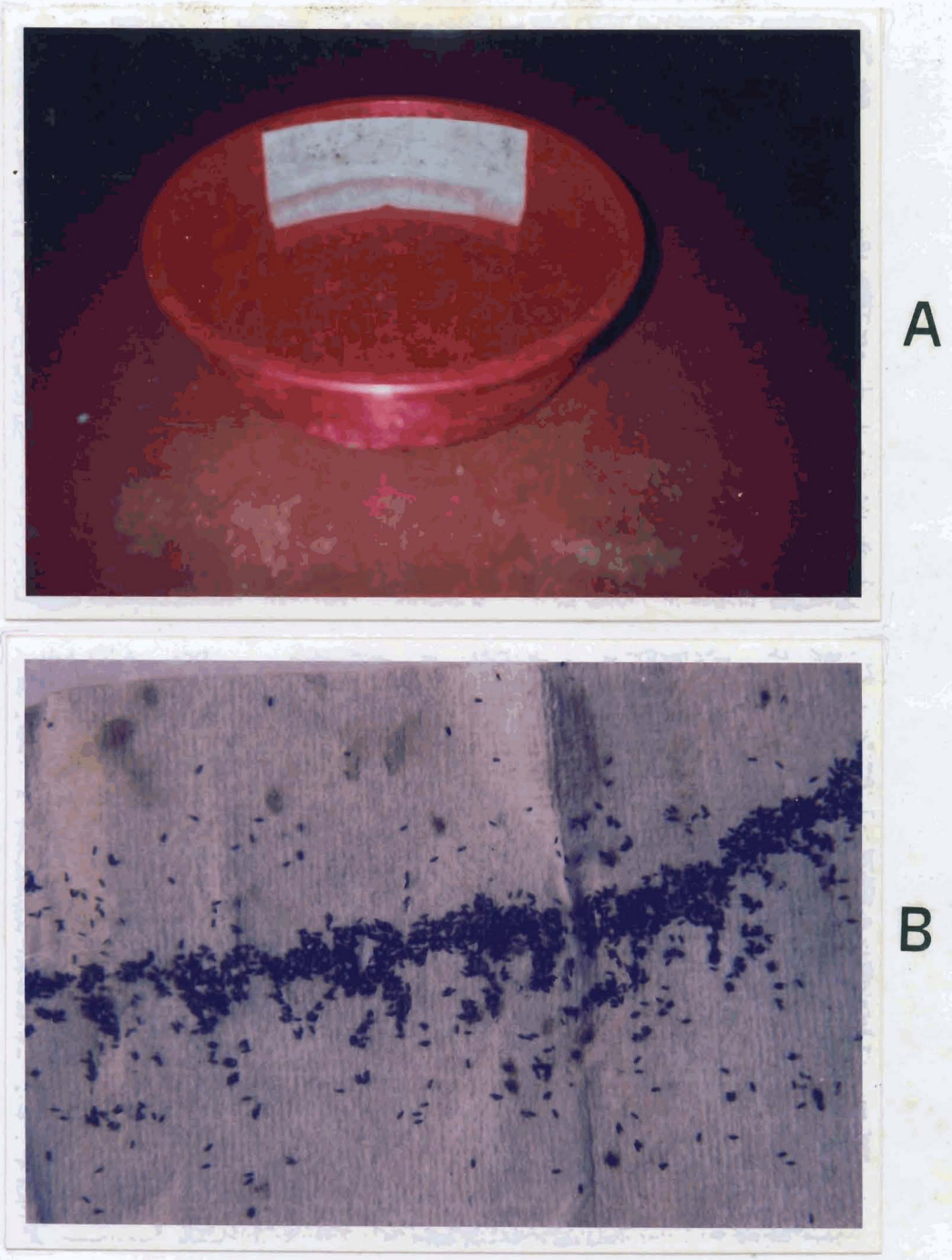

PLATE – 2. **A - Experimental set up for tracing oviposition attractant.**
B - Eggs collected in the filter paper.

2.3. RESULTS

2.3.1. ATTRACTANTS OF *A. SUBALBATUS*

Table 1, 2 and 3 summarise the names of the plants tested for the behavioural assay. As shown in the table, both the sexes of *A. subalbatus* has no attraction towards any of the plant extract tested in all the three age groups (0.48h, 48-96h and >96h).

The results of the behavioural assay using different groups of natural products are summarised in table-4, inorganic chemicals in table 5 and organic chemicals (heterocyclics, hydrocarbon, aldehydes, ketone, alcohol, ether, acids, amines, esters and acids) in table 6. Percentage of male and female *A. subalbatus* from different age groups (0-48h, 48-96h and >96h) showing attractive behaviour towards natural products are shown in table 7, inorganic chemicals in table 8 and organic chemicals in table 9.

In the case of 0-48h aged females, the order of preference as attractant is Acetone > 1,4 Dioxan > Honey > Butan-1-ol > Phenol > Petroleum ether > Amyl alcohol > Ammonia > Ammonium Chloride > Urine > Lactic acid > Sweat (Fig. 5).

In the case of 0-48h aged males, the order of preference as attractant is Acetone > Honey > Butan-1-ol > 1,4 Dioxan > Ammonium chloride > Amyl alcohol > Ammonia > Phenol > Petroleum ether > Lactic acid > Urine > Sweat (Fig. 6).

In the case of 48-96 hr aged females, the order of preference as attractant is Acetone > Honey > Lactic acid> Phenol > 1,4 Dioxan > Butan -1-ol > Ammonia > Petroleum ether > Sweat > Amyl alcohol > Ammonium chloride > Urine (Fig. 7).

TABLE - 7

Percentage of mosquitoes showing attractant behaviour towards natural products

Sl. No	Name of the natural products	Age of Females (in hours)			Age of Males (in hours)		
		0-48	48-96	>96	0-48	48-96	>96
1	Honey	73	81	91	81	89	98
2	Sweat	43	69	83	60	62	60
3	Urine	53	61	74	60	69	61

TABLE - 8

Percentage of mosquitoes showing attractant behaviour towards inorganic chemicals

Sl. No	Name of the inorganic chemicals	Age of Females (in hours)			Age of Males (in hours)		
		0-48	48-96	>96	0-48	48-96	>96
1	Ammonia	62	70	69	64	66	65
2	Ammonium chloride	61	65	61	67	70	71

TABLE -9

Percentage of mosquitoes showing attractant behaviour towards organic chemicals

Sl. No	Name of the organic chemicals	Age of Females (in hours)			Age of Males (in hours)		
		0-48	48-96	>96	0-48	48-96	>96
1	Acetone	79	85	91	89	94	97
2	1,4 Dioxan	73	75	77	68	71	73
3	Phenol	69	78	86	64	70	70
4	Petroleum ether	62	69	65	63	65	69
5	Lactic acid	51	79	93	61	64	61
6	Butan-1-ol	70	72	72	72	76	73
7	Amyl alcohol	62	65	61	64	65	67

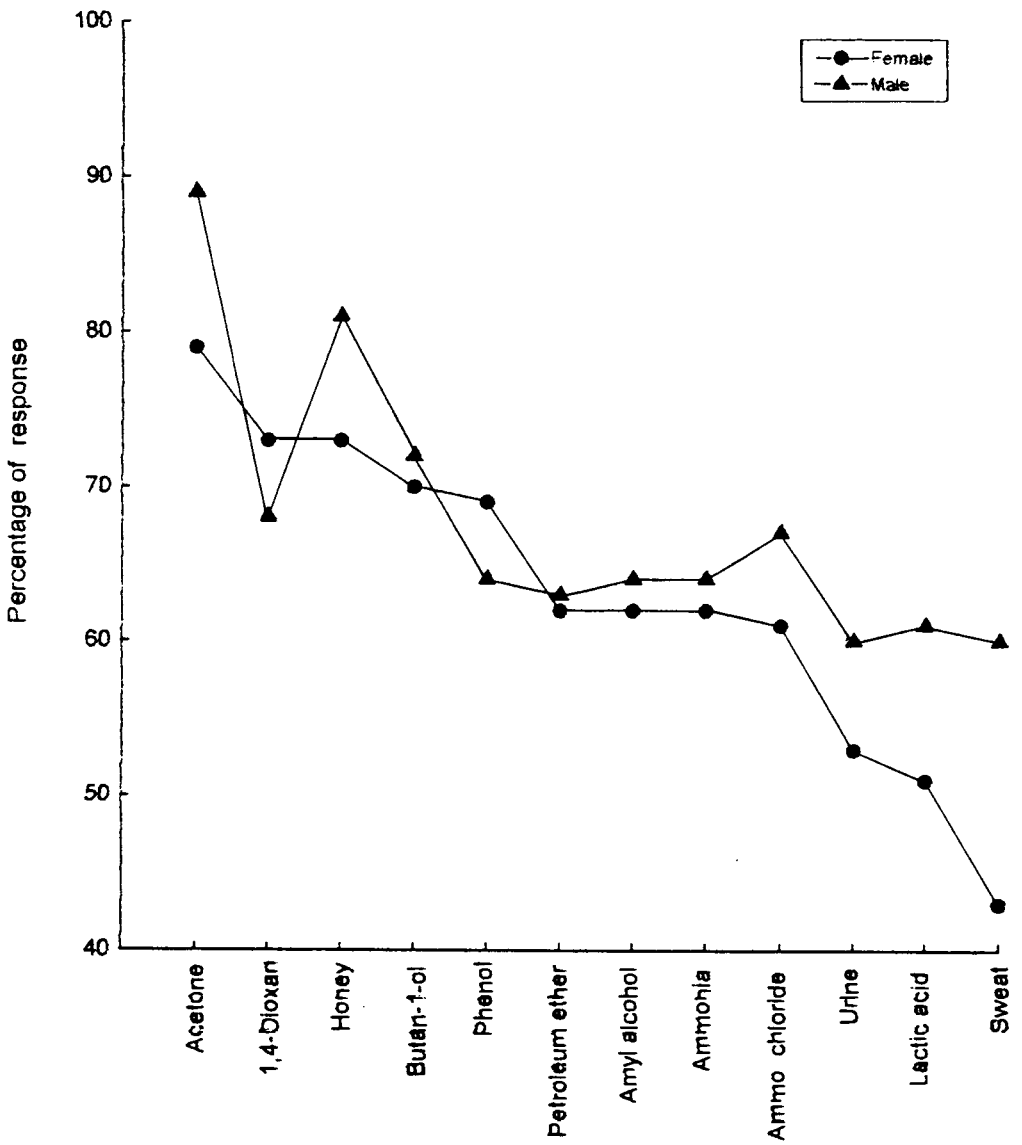

FIGURE -5. Response of *A. subalbatus* (0-48h age) towards attractants. Female preferences in descending order and male's response to the same chemical.

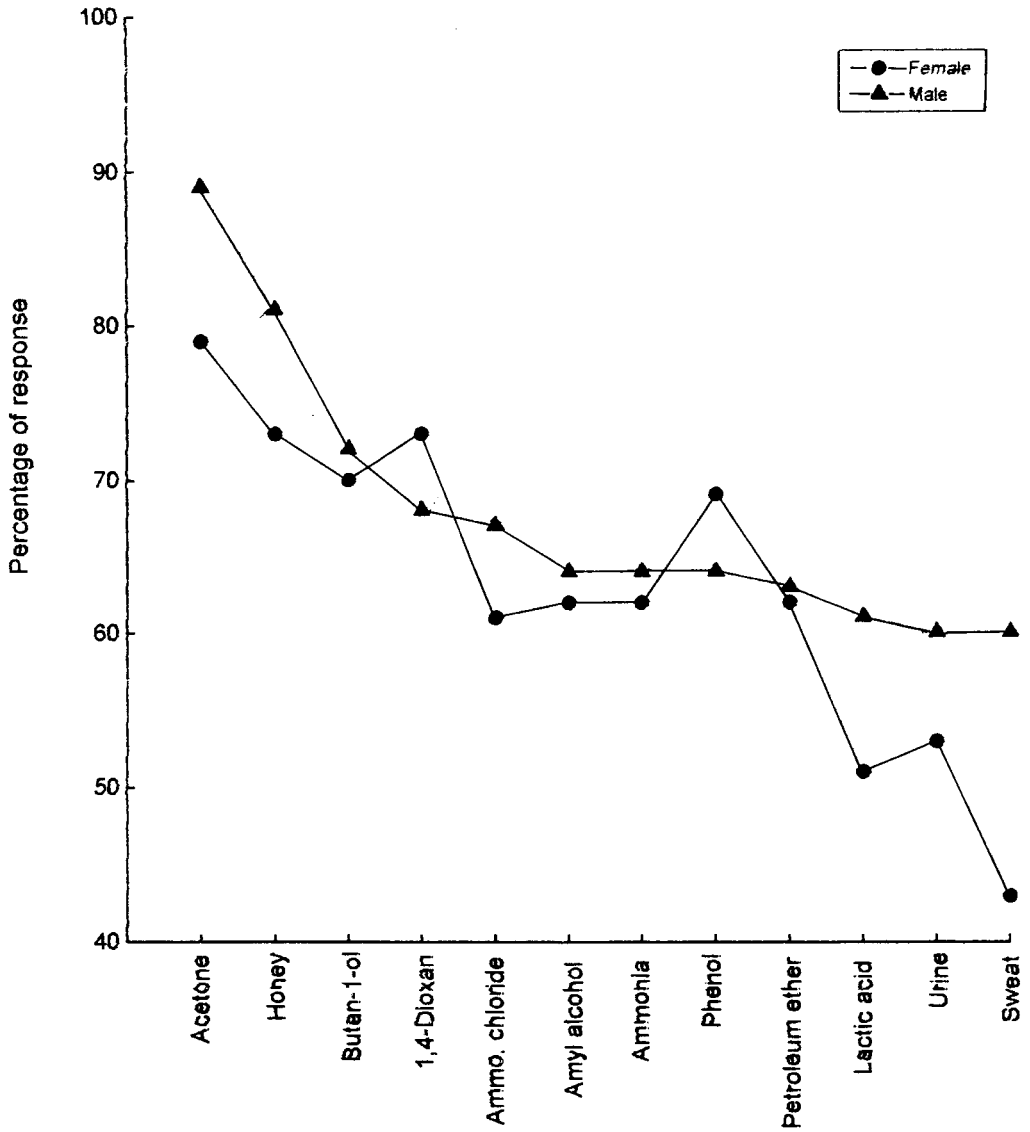

FIGURE-6. Response of *A. subalbatus* (0-48h age) towards attractants. Male preferences in descending order and female's response to the same chemical.

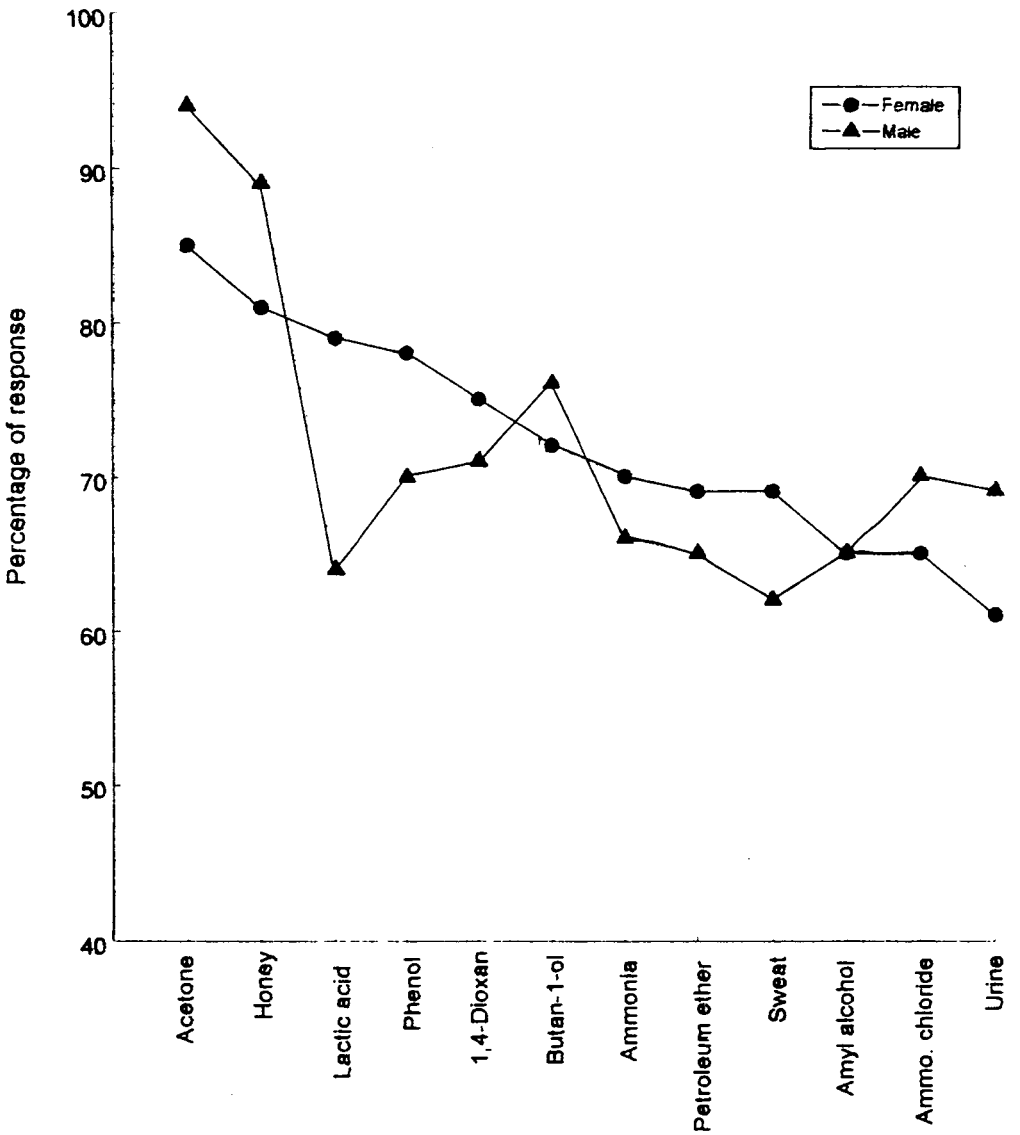

FIGURE- 7. Response of *A. subalbatus* (48-96h age) towards attractants. Female preferences in descending order and male's response to the same chemical.

In the case of 48-96h aged males, the order of preference as attractant is Acetone > Honey > Butan-1-ol > 1,4 Dioxan > Phenol > Ammonium Chloride > Urine > Ammonia > Petroleum ether > Amyl alcohol > Lactic acid > Sweat (Fig. 8).

In the case of >96h aged females, the order of preference as attractant is Lactic acid > Honey > Acetone > Phenol > Sweat > 1,4 Dioxan > Urine > Butan-1-ol> Ammonia > Petroleum ether > Ammonium chloride > Amyl alcohol (Fig. 9).

In the case of >96h aged males, the order of preference as attractant is Honey > Acetone > 1,4 Dioxan > Butan-1-ol > Ammonium Chloride > Phenol> Petroleum ether > Amyl alcohol > Ammonia > Lactic acid > Urine > Sweat (Fig. 10).

Age wise responses of both the sexes of A.subalbatus towards attractants are graphically represented and given in the figure 11-22. Salient features of the results obtained are as follows.

1. Among the attractants tested, maximum number of *A. subalbatus* were attracted to acetone in the case of 0-48h and 48-96h aged *A. subalbatus* in both the sexes (Fig. 5,6,7 and 8).

2. In the case of >96h aged mosquitoes, females showed greatest attraction towards lactic acid (Fig. 9) and males showed greatest attraction towards honey (Fig. 10).

3. When lactic acid was used as stimulant, 0-48h aged females showed almost least preference (11th preference among the twelve attractants) to this chemical (Fig. 5). But, as age increases to 48-96h, females showed greater preference (3rd preference among the twelve attractants) towards lactic acid (Fig. 7). Likewise, in >96h aged females, among the twelve attractants, first preference was lactic acid (Fig. 9). This observation indicates that in females, as age increases the attraction towards lactic acid also increases. However, in the case of males, all

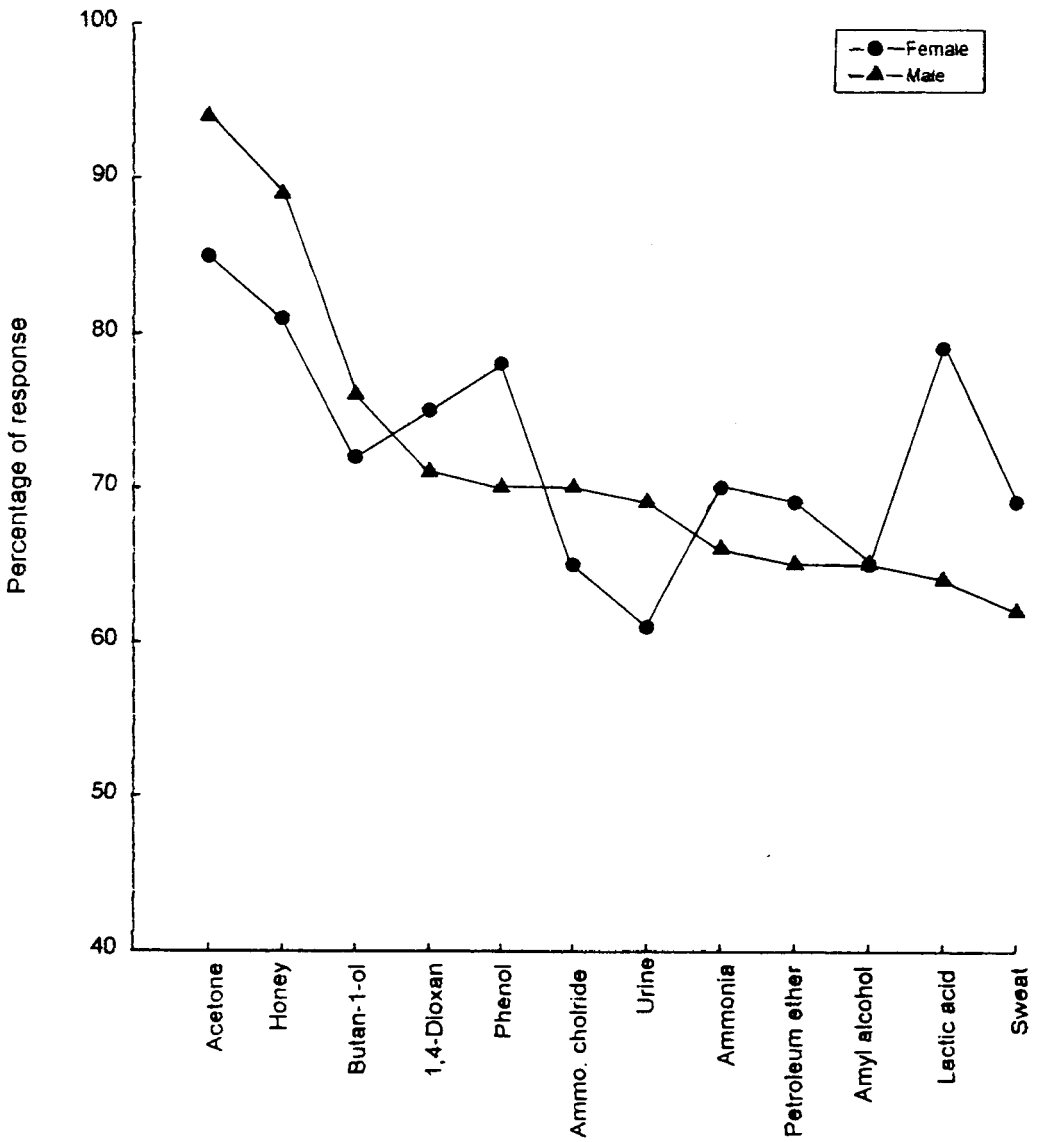

FIGURE-8. Response of *A.subalbatus* (48-96h age) towards attractants. Male preferences in descending order and female's response to the same chemical.

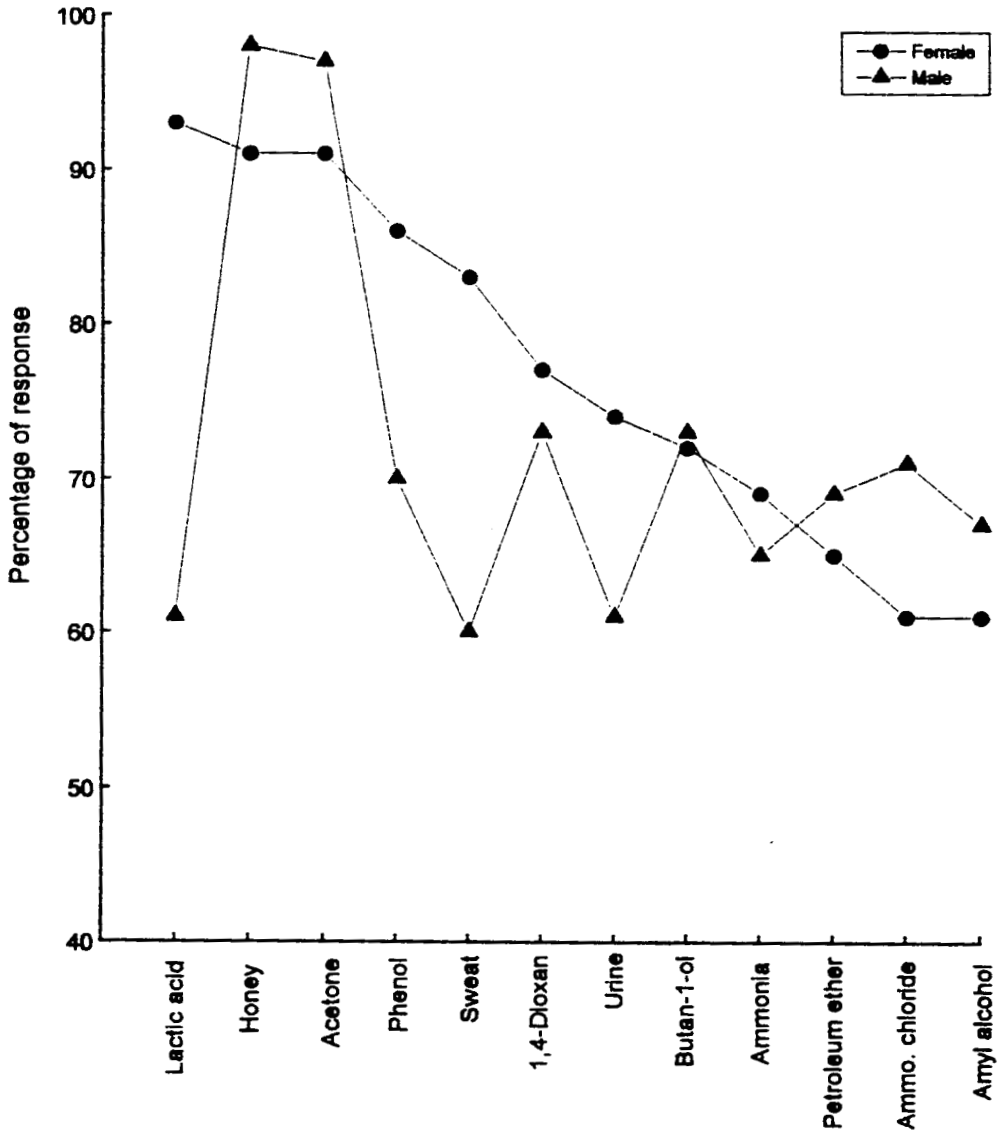

FIGURE-9. Response of *A .subalbatus* (>96h age) towards attractants. Female preferences in descending order and male's response to the same chemical.

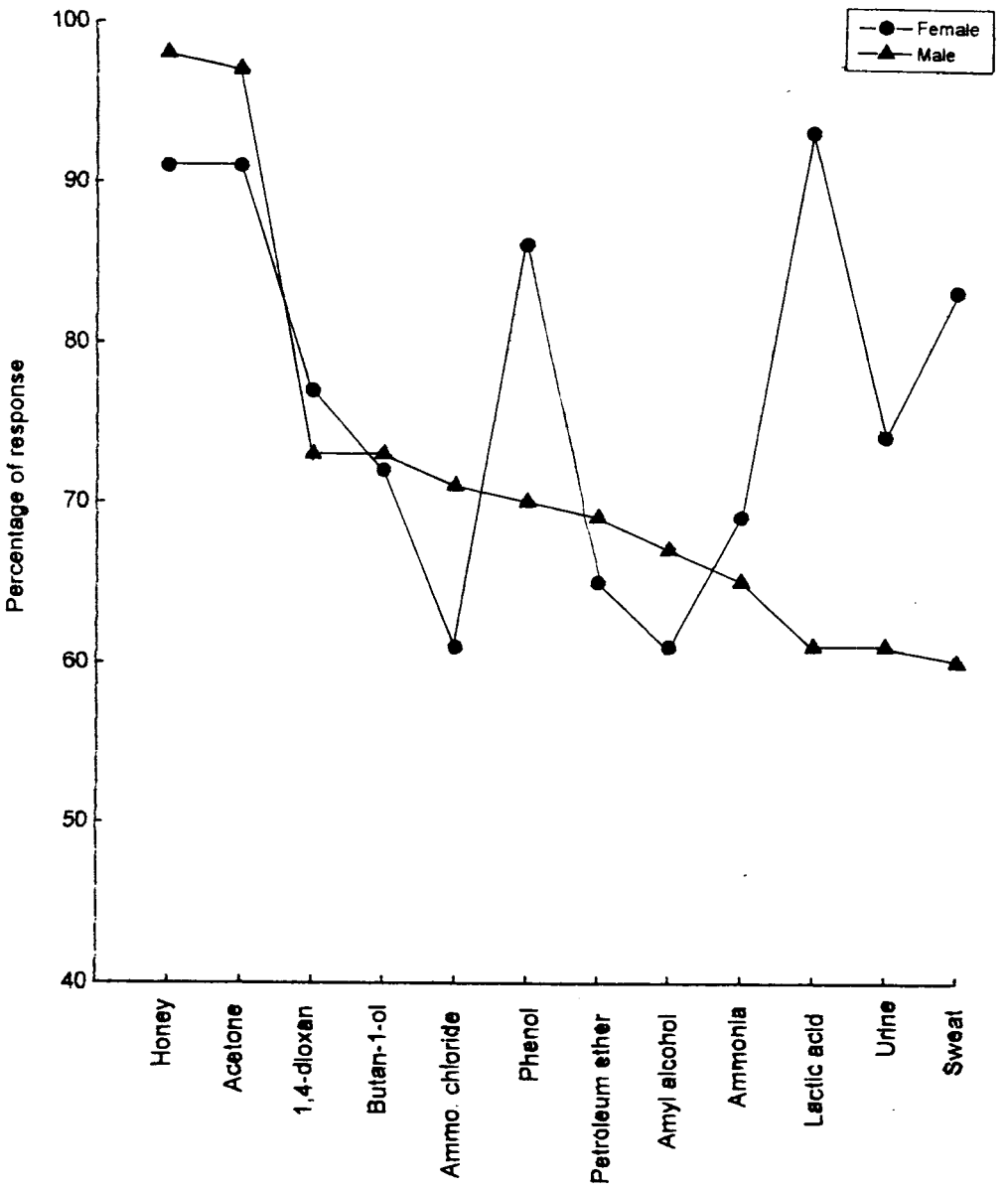

FIGURE- 10. Response of *A. subalbatus* (>96h age) towards attractants. Male preferences in descending order and female's response to the same chemical.

the age groups showed almost same preference towards lactic acid (Fig. 6, 8 and 10).

4. As in the case of lactic acid, females also showed same pattern of response towards sweat. However, the preference was somewhat lesser (to sweat) when compared to lactic acid (Fig. 5,7 and 9). But, males of all the three age groups showed least preference to sweat (Fig. 6, 8 and 10).

5. When acetone (Fig. 11) 1,4 dioxan (Fig. 12) and honey (Fig. 13) were used as a stimulants, significant age wise increase in response was observed in both the sexes.

6. In the case of phenol (Fig. 14), lactic acid (Fig. 15), sweat (Fig.16) and urine (Fig. 17), significant age wise increase in response was observed only in females. In the case of petroleum ether (Fig. 18) amyl alcohol (Fig. 19), and ammonium chloride (Fig. 20) only males showed significant age wise increase in response.

7. In the case of petroleum ether (Fig. 18), amyl alcohol (Fig. 19), and ammonium chloride (Fig. 20), 48-96h aged females showed greater attraction than the other two age groups. Like wise, 48-96h aged males showed greater attraction towards lactic acid (Fig. 15) and butan-1-ol (Fig. 21). Both the sexes (48-96h age) showed maximum attraction towards ammonia than the other two age groups (Fig. 22).

8. In the case of 1,4 dioxan (Fig. 12), phenol (Fig. 14) and females of all age groups were more sensitive than males of the corresponding age group. In contrast to this, males were more sensitive to acetone (Fig. 11), honey (Fig. 13), amyl alcohol (Fig. 19), ammonium chloride (Fig. 20), and butan 1-o1(Fig.21) than females.

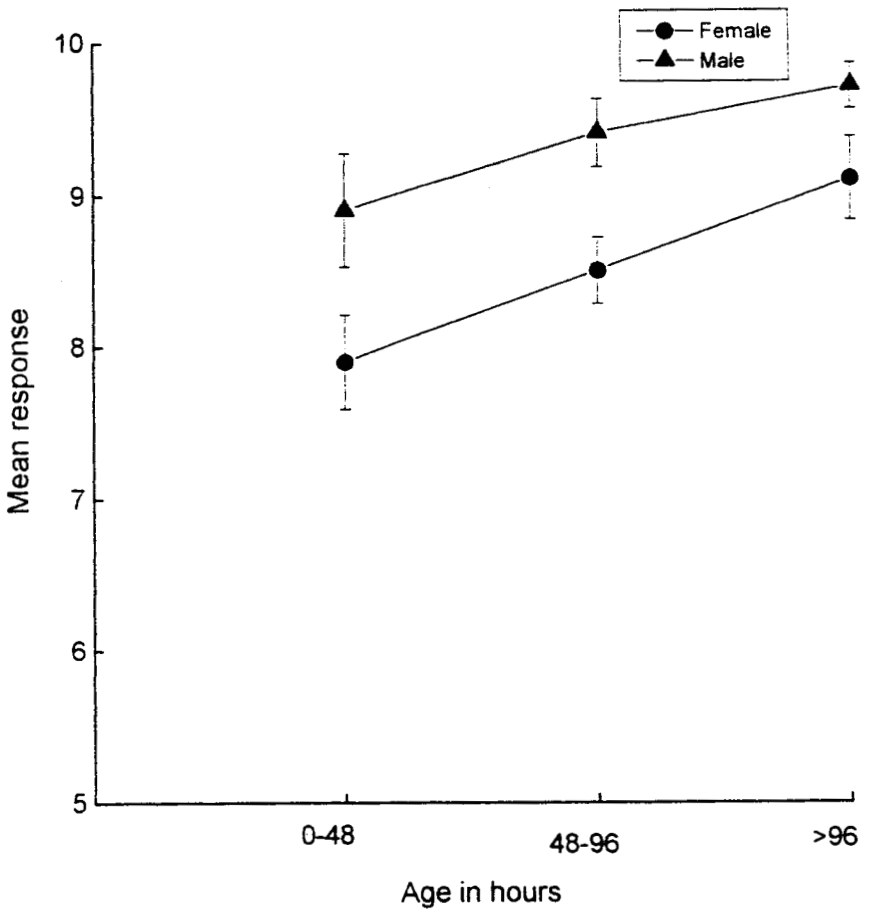

FIGURE-11. Response (Mean ±SEM) to acetone.

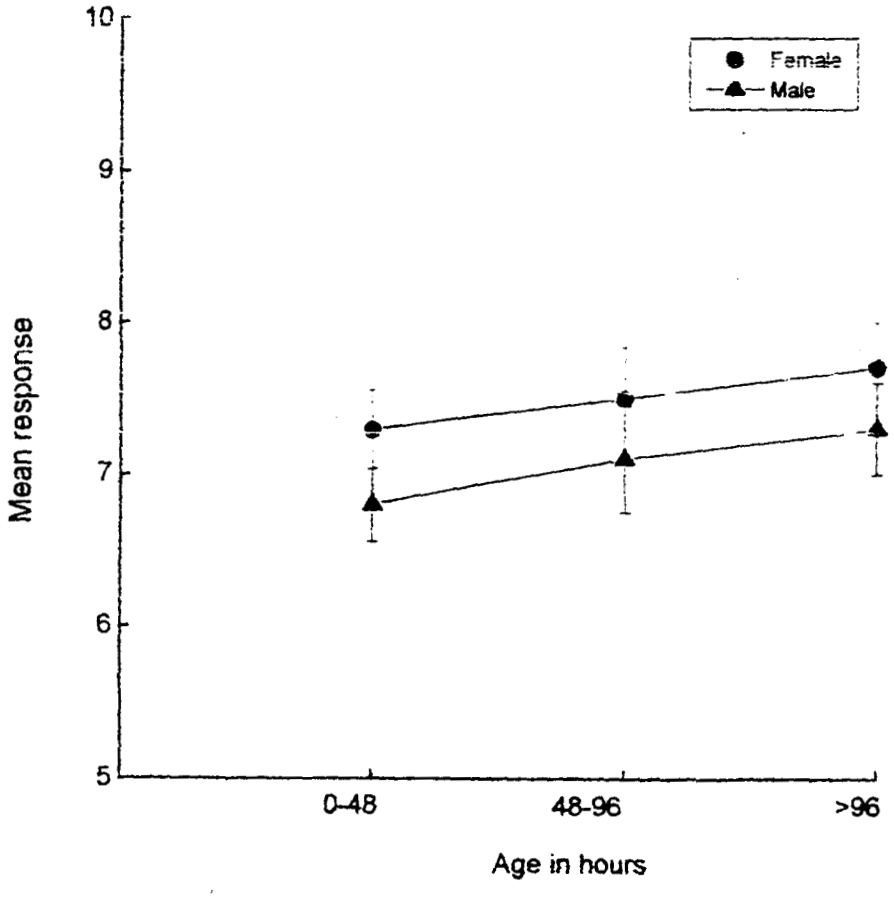

FIGURE-12. Response (Mean ± SEM) to 1,4-dioxan.

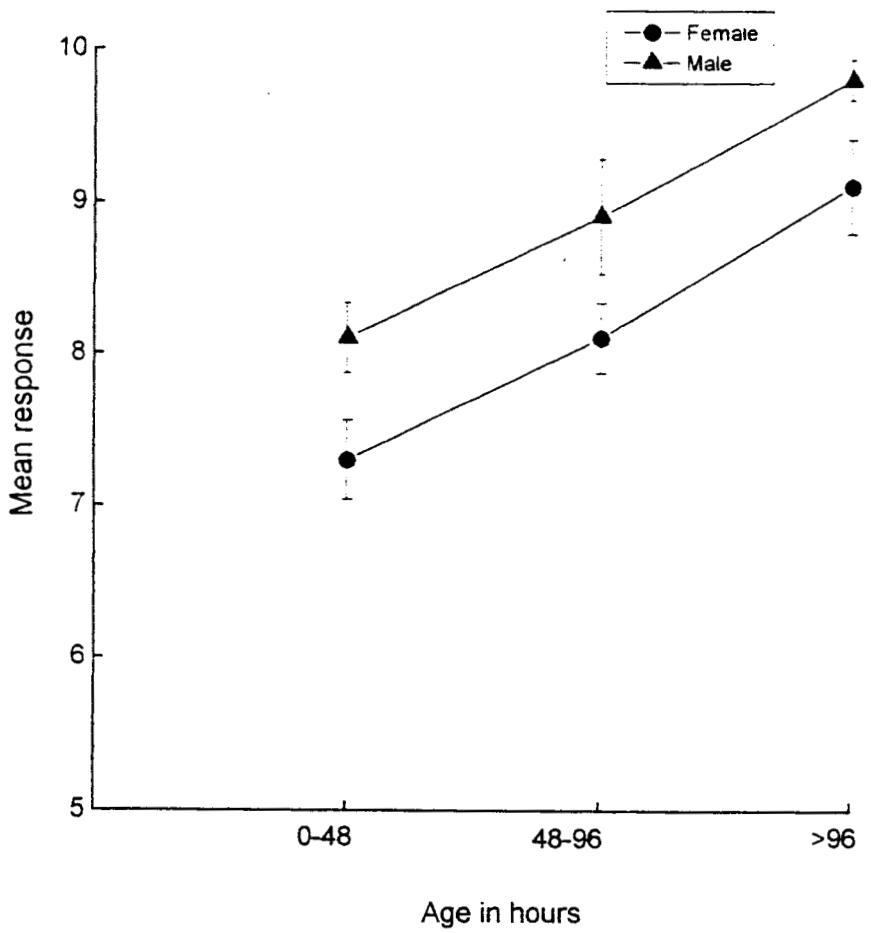

FIGURE-13. Response (Mean±SEM) to honey.

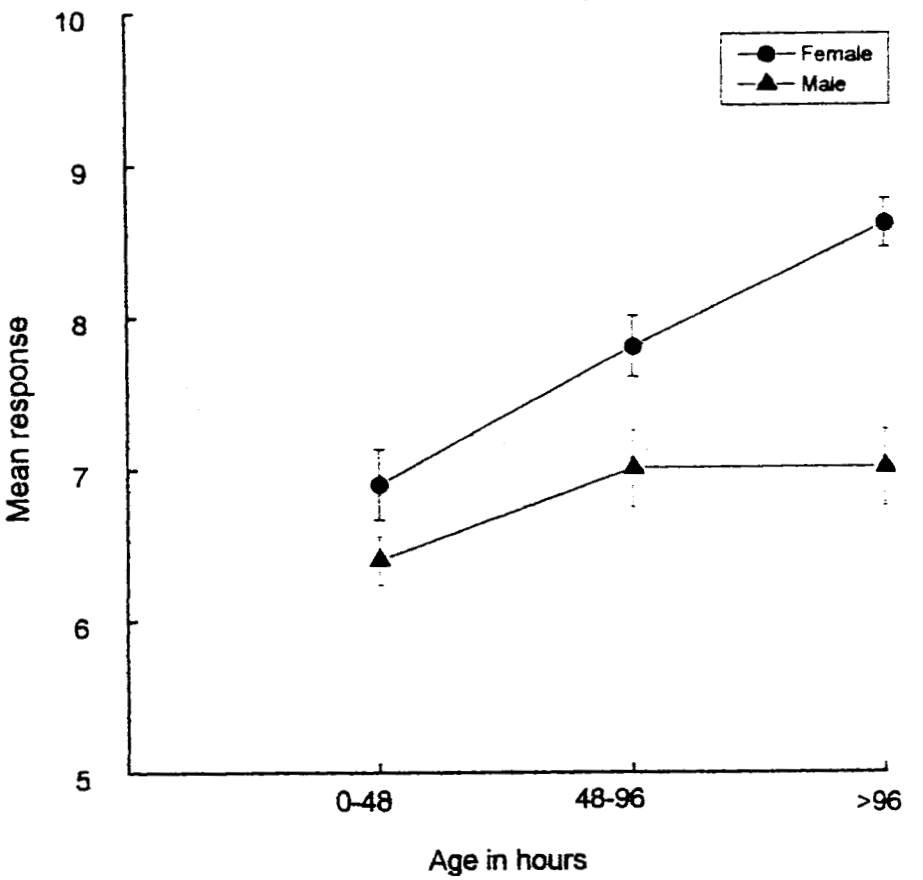

FIGURE-14. Response (Mean ±SEM) to phenol.

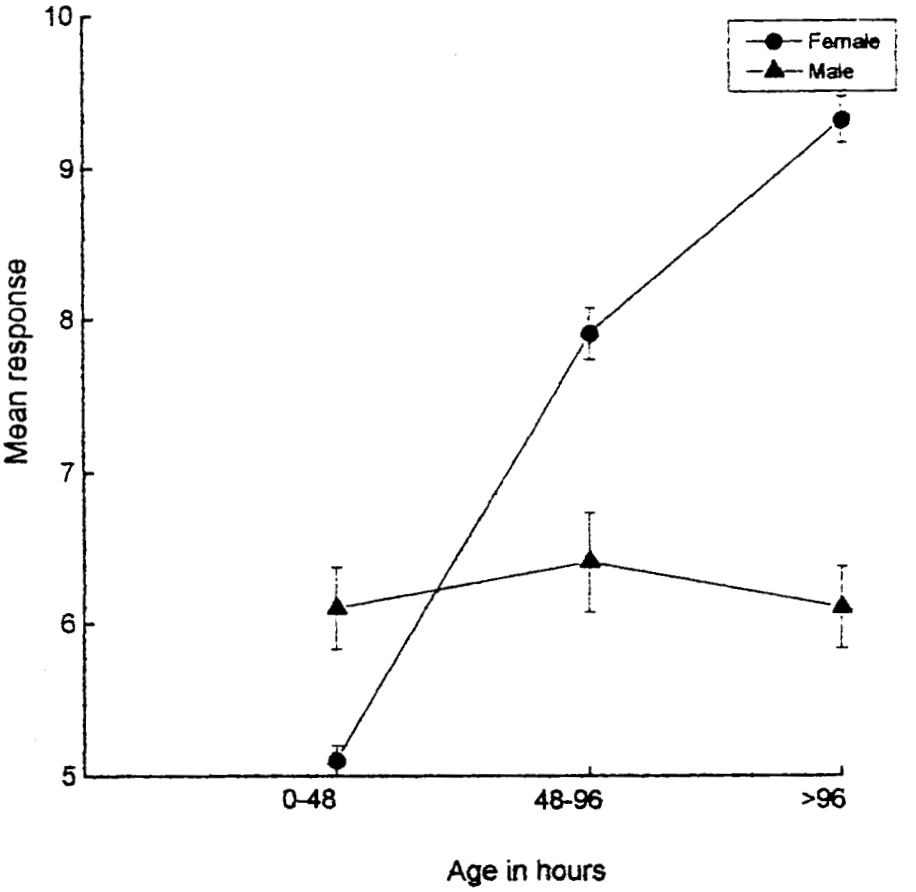

FIGURE-15. Response (Mean ± SEM) to lactic acid.

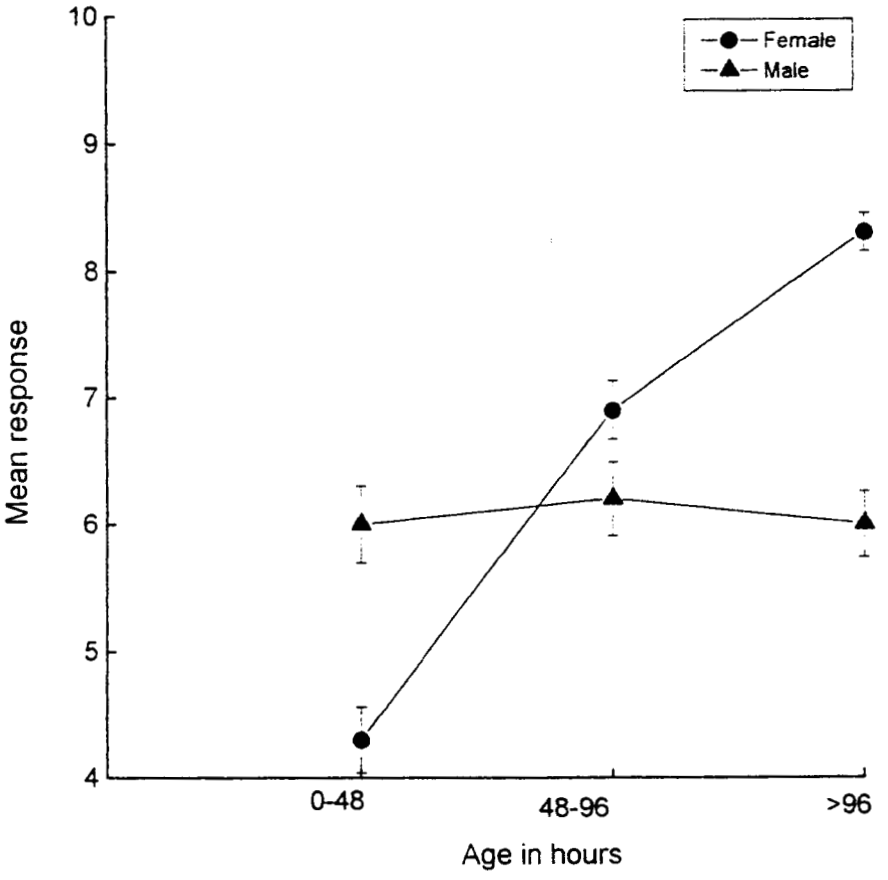

FIGURE-16. Response (Mean ± SEM) to sweat.

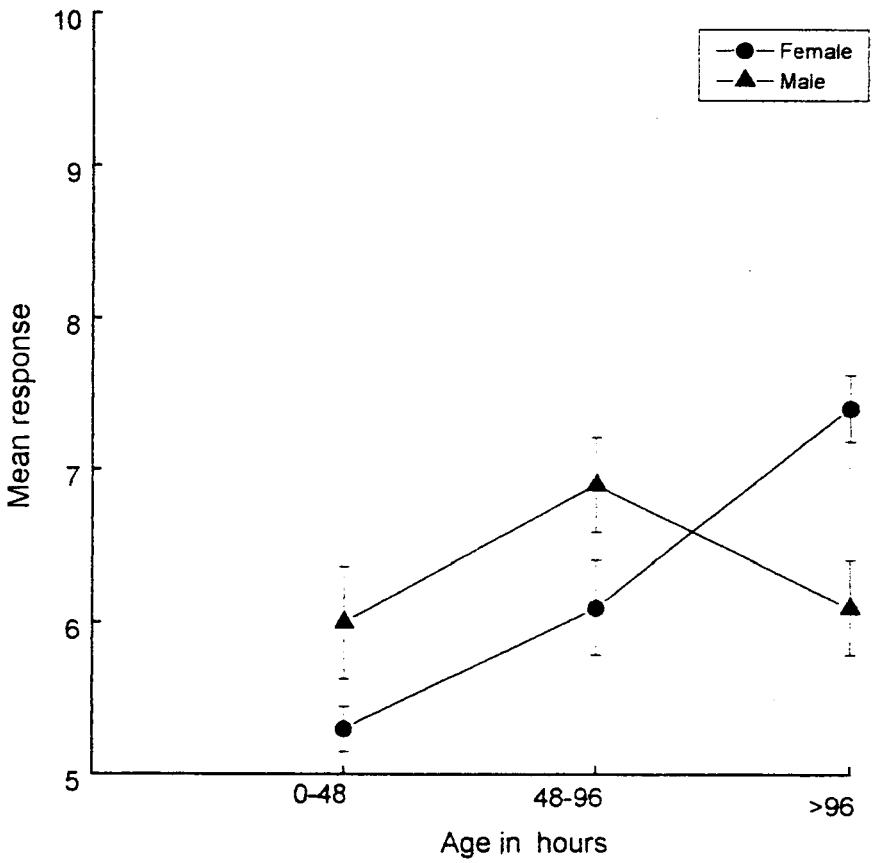

FIGURE-17. Response (Mean ± SEM) to urine.

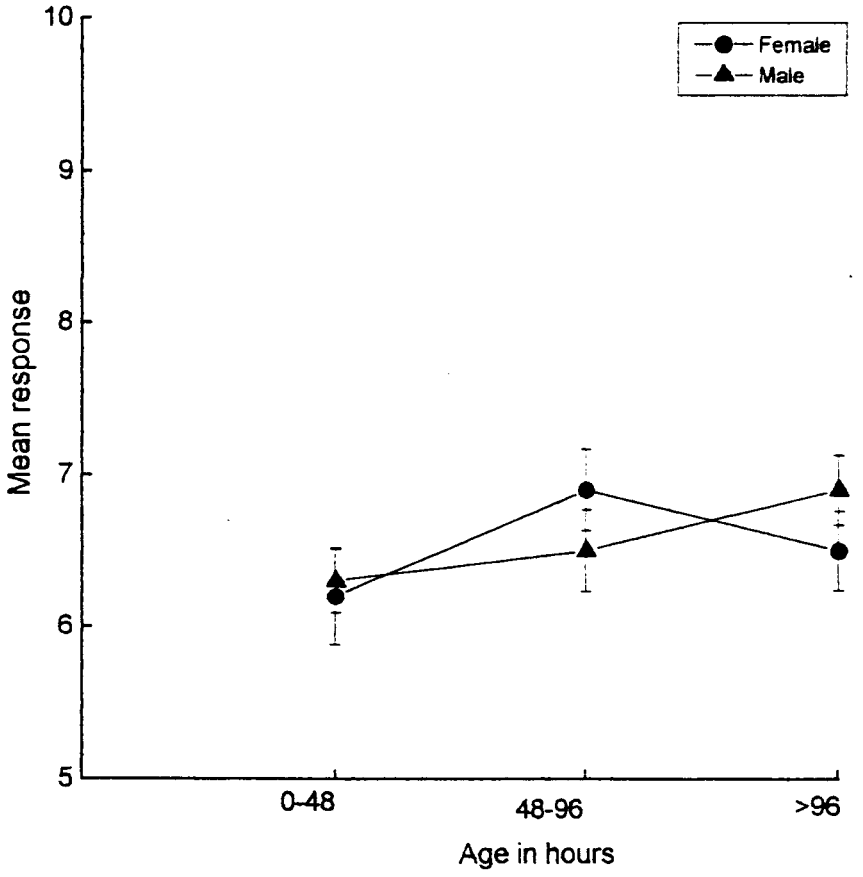

FIGURE-18. Response (Mean ± SEM) to petroleum ether.

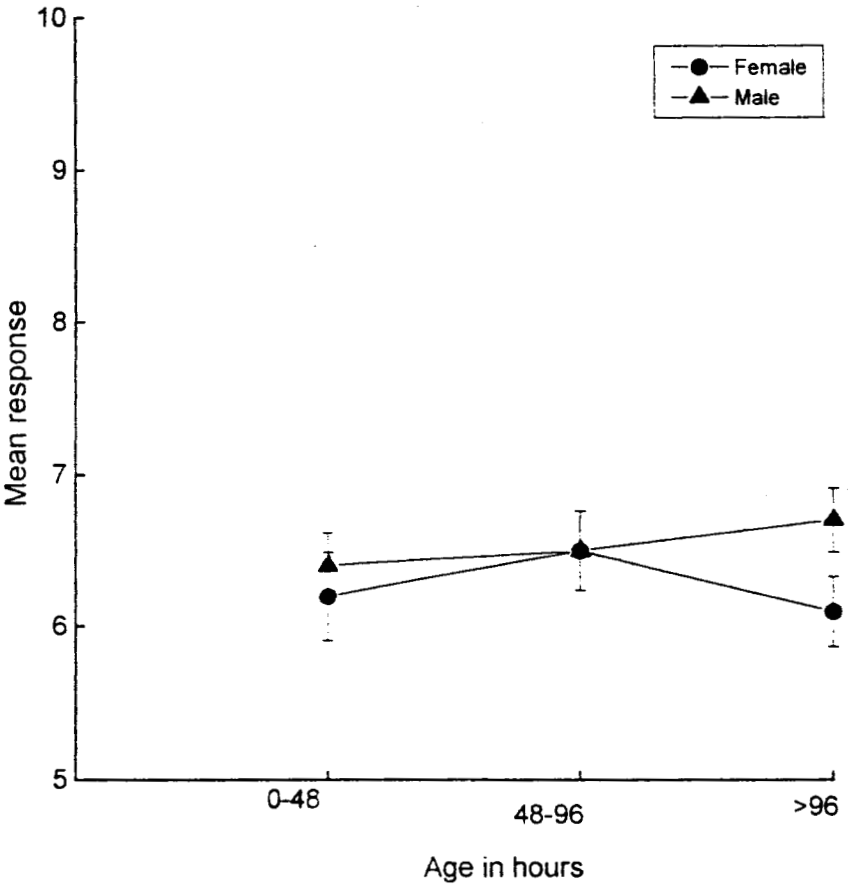

FIGURE-19. Response (Mean ±SEM) to amyl alcohol.

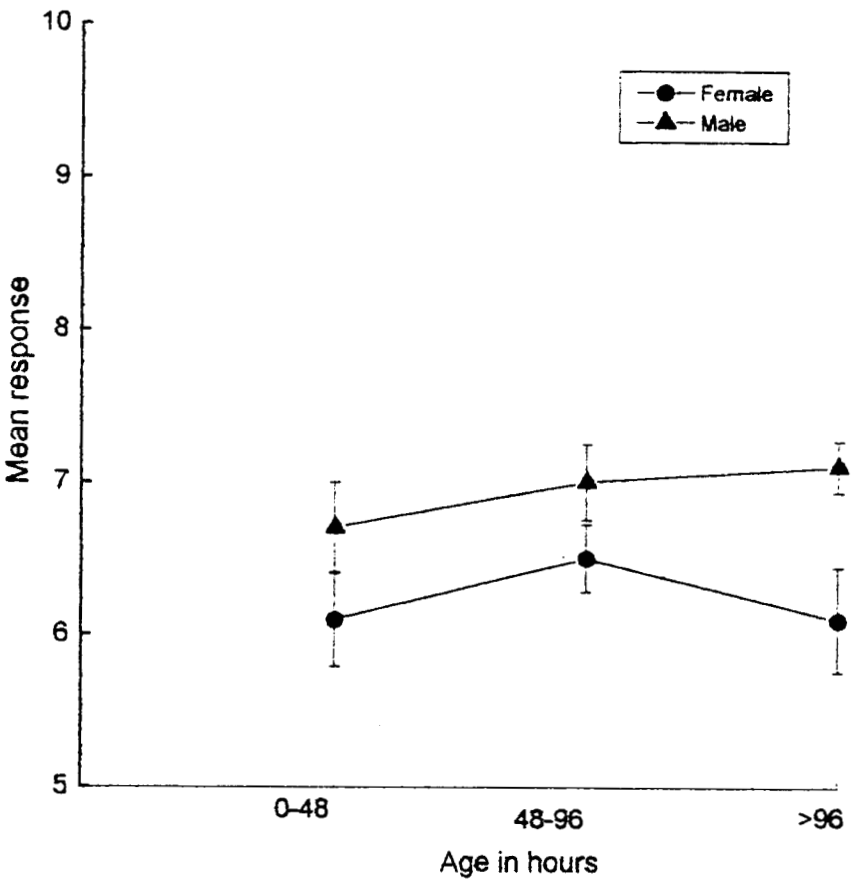

FIGURE -20. Response (Mean±SEM) to Ammonium chloride.

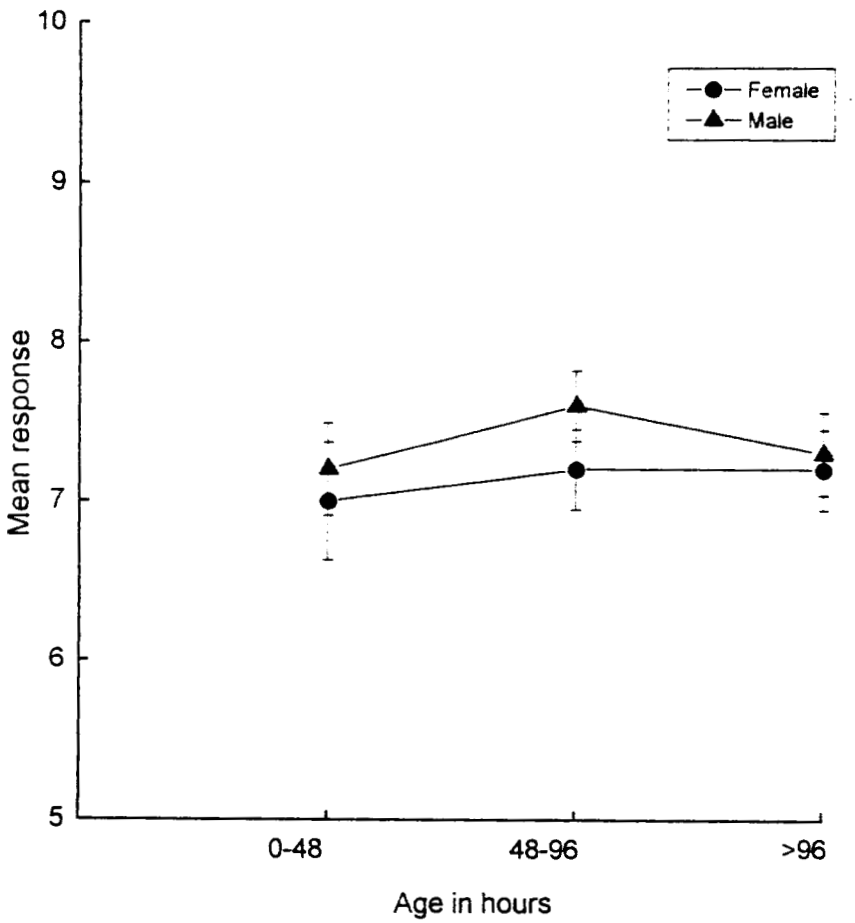

FIGURE- 21. Reponse (Mean±SEM) to butan-1-ol.

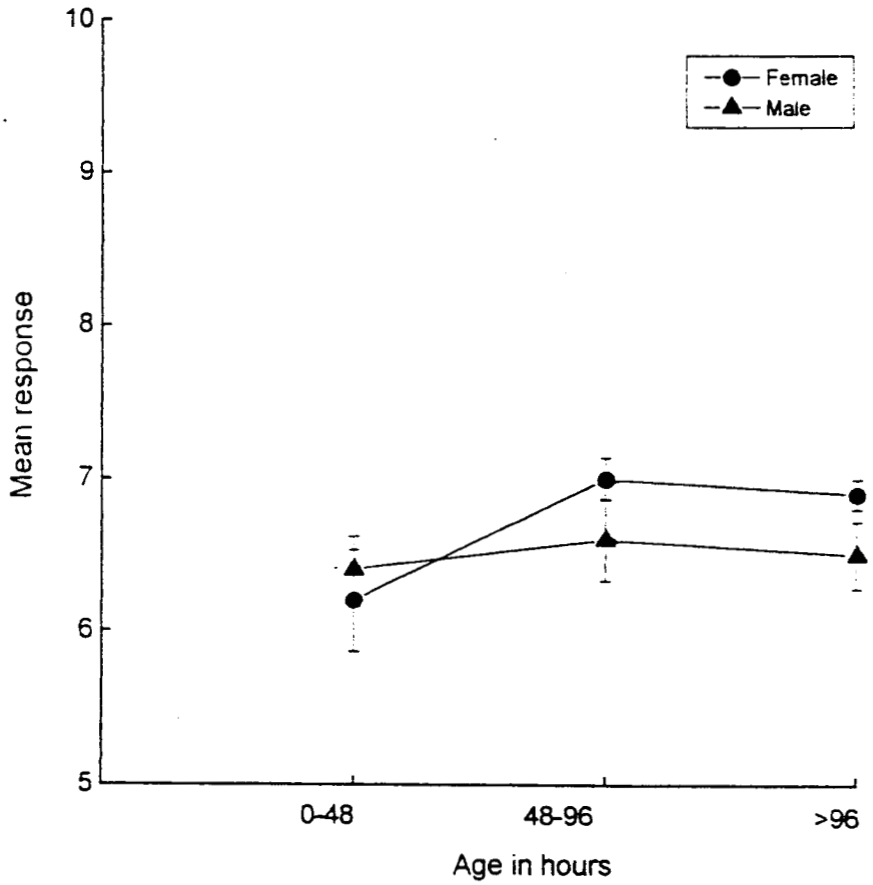

FIGURE-22. Response (Mean ± SEM) to ammonia.

9. In all the attractants tested, significant sex wise difference in response was observed.

2.3.2. REPELLENTS OF *A.SUBALBATUS*

Among the 60 plants tested for the behavioural experiment, only seven plants were found as repellents for *A. subalbatus* (Table - 3). Among the organic chemicals, only acetic acid was observed as a repellent (Table 6). Among the inorganic chemicals and natural products, none was found to be a repellent. The percentage of diffrent age groups (0-48h, 48-96h and >96h) of both the sexes of *A. subalbatus* showing repellent behaviour are shown in Table-10.

In the case of 0-48h aged females, the repellents are effective in this order : Acetic acid > *Azadirachta indica* > *Vitex negundo* > *Leucas aspera* > *Premna latifolia* > *Oscimum sanctum* > *Cymbopogon citratus*. (Fig. 23).

In the case of 0-48h aged males the repellents are effective in this order: *Acetic acid* > *A. indica* > *L. aspera* > *V. negundo* > *P. latifolia* > *C. citratus* > *O. sanctum* (Fig. 24).

As far as 48-96h aged females are concerned, the efficacy as repellent is in the order Acetic acid > *A. indica* > *V. negundo* > *L. aspera* > *P. latifolia* > *C. citratus* > *O. sanctum* (Fig. 25).

In the case of males (48-96h age) the order of efficacy as repellent is Acetic acid > *A. indica* > *L. aspera* > *V. negundo* > *P. latifolia* > *C. citratus* > *O. sanctum* (Fig. 26).

In the case of >96h aged females, the order of efficacy as repellent is Acetic acid > *P. latifolia* > *L. aspera* > *V. negundo* > *A. indica* > *C. citratus* > *O. sanctum* (Fig. 27).

TABLE - 10

Percentage of mosquitoes showing repellent behaviour

Sl. No	Name of the repellent	Age of Females (in hours)			Age of Males (in hours)		
		0-48	48-96	>96	0-48	48-96	>96
	Plants						
1	*Leucas aspera*	68	73	75	69	71	78
2	*Vitex negundo*	69	75	73	65	70	71
3	*Cymbopogon citratus*	60	66	68	61	66	68
4	*Ocimum sanctum*	61	63	60	60	64	65
5	*Premna latifolia*	67	70	79	63	67	70
6	*Azadirachta indica*	79	81	71	74	76	68
	Organic Chemical						
1	Acetic acid	80	86	87	87	90	91

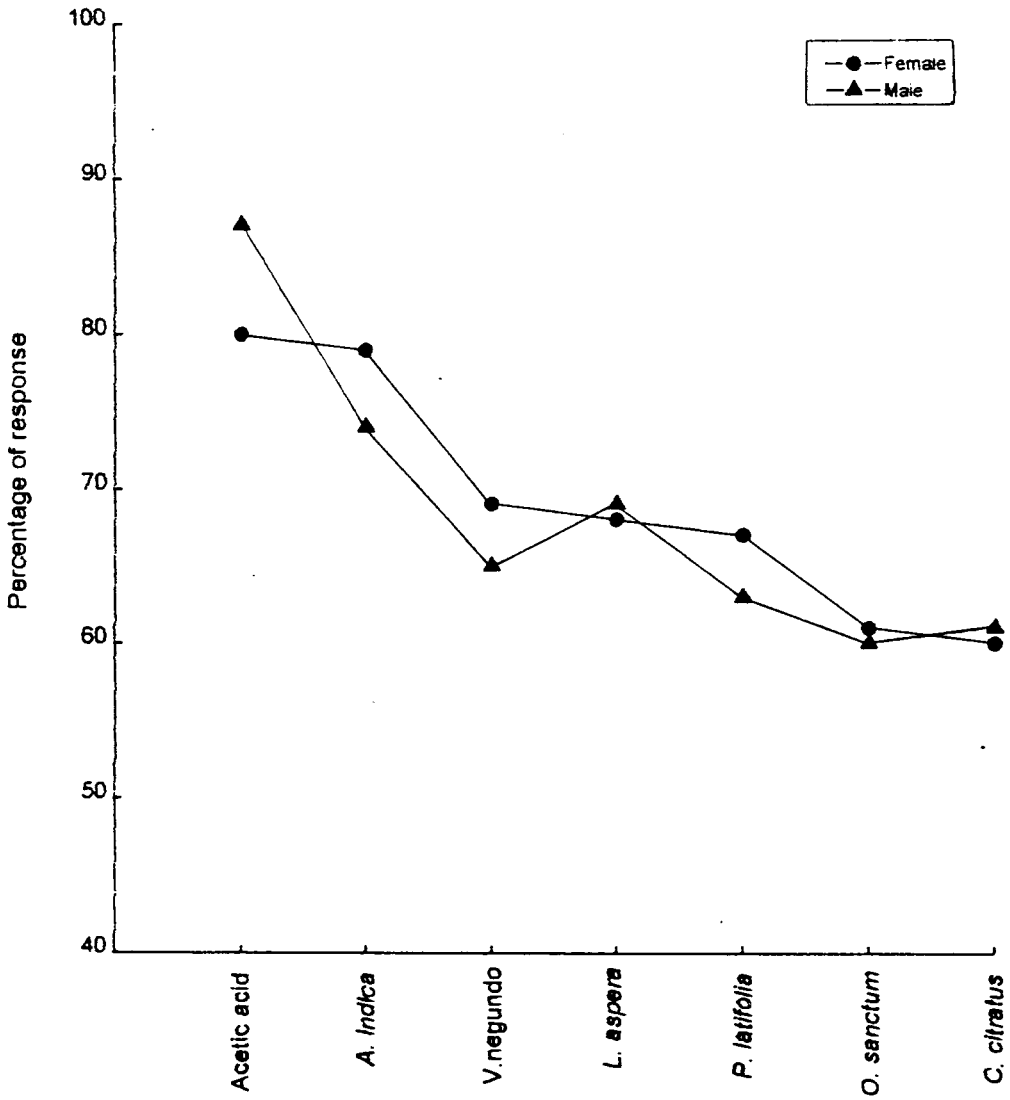

FIGURE-23. Response of *A. subalbatus* (0-48h age) towards repellents. Female responses in descending order and male's response to the same chemical.

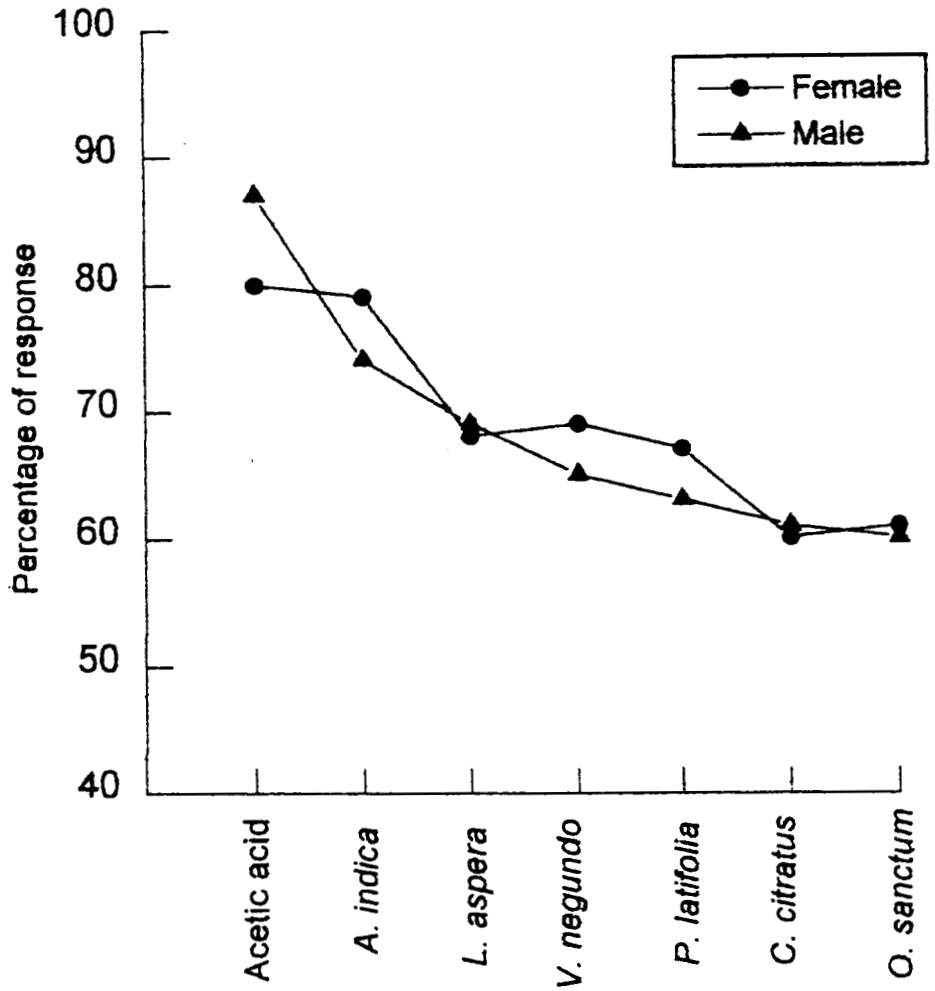

FIGURE- 24. Response of *A.subalbatus*(0-48h age) towards repellents. Male responses in descending order and female's response to the same chemical.

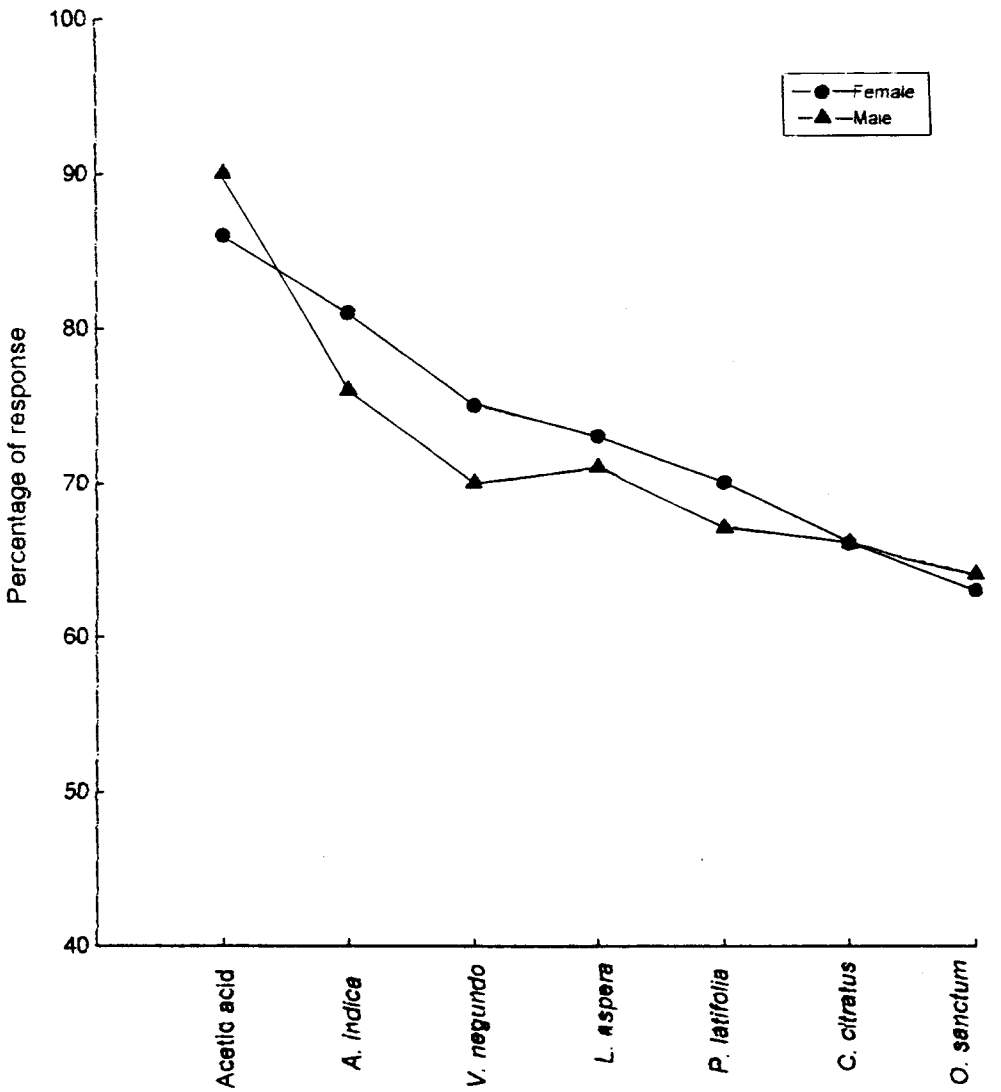

FIGURE -25. Response of *A. subalbatus* (48-96h age) towards repellents. Female responses in descending order and male's response to the same chemical.

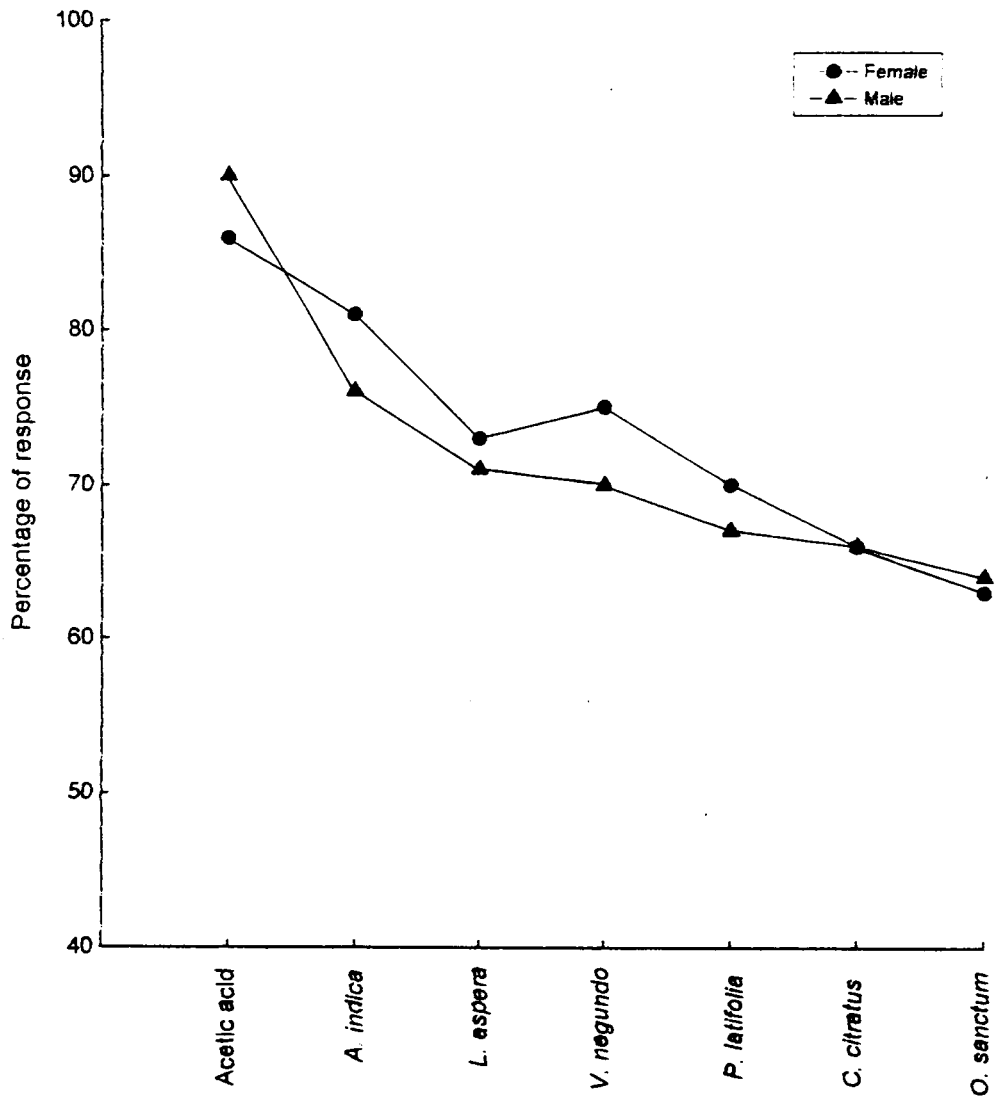

FIGURE -26. Response of *A. subalbatus* (48-96h age) towards repellents. Male responses in descending order and female's response to the same chemical.

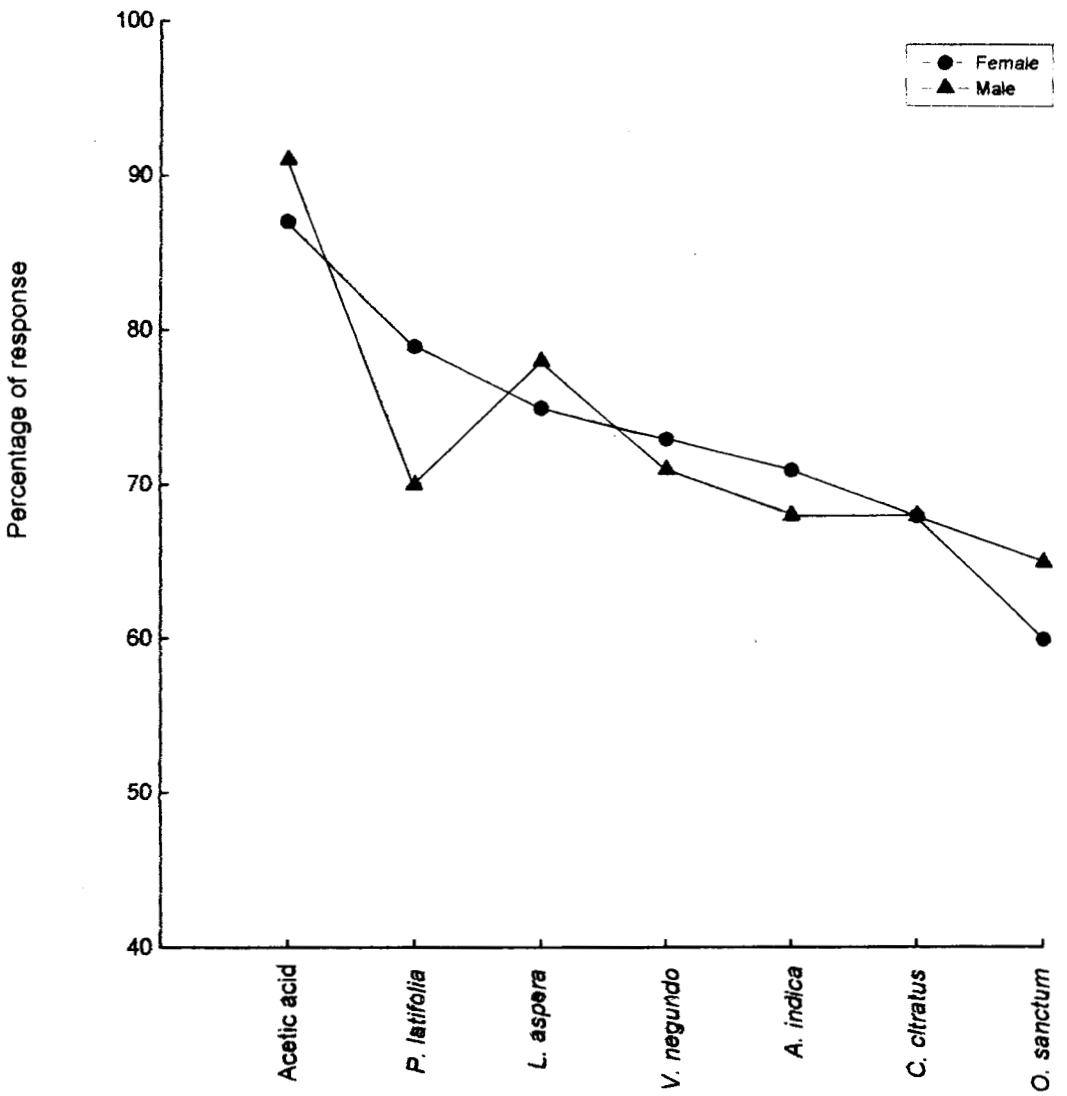

FIGURE -27. Response of *A. subalbatus* (>96h age) towards repellents. Female responses in descending order and male's response to the same chemical.

The order has changed in males as Acetic acid > *L. aspera* > *V. negundo* > *P. latifolia* > *A. indica* > *C.citratus* > *O. sanctum* (Fig. 28).

Age wise responses of males and females towards each repellent is graphically represented and shown in the figures 29-35. Salient features are as follows:

1. Unlike attractants, all the age groups (0-48h, 48-96h and >96h) of both the sexes showed maximum repellence towards the same chemical i.e. acetic acid (Fig. 23, 24, 25, 26, 27 and 28).

2. Among the plants tested, except >96h aged mosquitoes, all other age groups of both the sexes showed greater repellence towards *A. indica* (Fig. 23, 24, 25 and 26).

3. In the case of >96h aged females, the best herbal repellent was *P. latifolia* (Fig. 27) and for males of the same age, the best repellent was *L. aspera* (Fig. 28).

4. Except 0-48h aged females, other two age groups of both the sexes showed least repellence towards *O. sanctum* (Figs. 24, 25, 26, 27 and 28) whereas, 0-48h aged females showed least repellence to *C. citratus* (Fig. 23).

5. In the case of Acetic acid, males showed greater sensitivity than females (Fig. 29). But in the case of *A. indica* (Fig. 30), *P. latifolia* (Fig. 31) and *V. negundo* (Fig. 32) females showed greater sensitivity than males. Towards *C. citratus* (Fig. 33) both the sexes showed almost similar response.

6. In the case of Acetic acid (fig. 29), *P. latifolia* (Fig. 31) and *L. aspera* (Fig. 34) significant age wise increase in response was observed only in females. When *V. negundo* (Fig. 32) and *O. sanctum* (Fig. 35) were used as

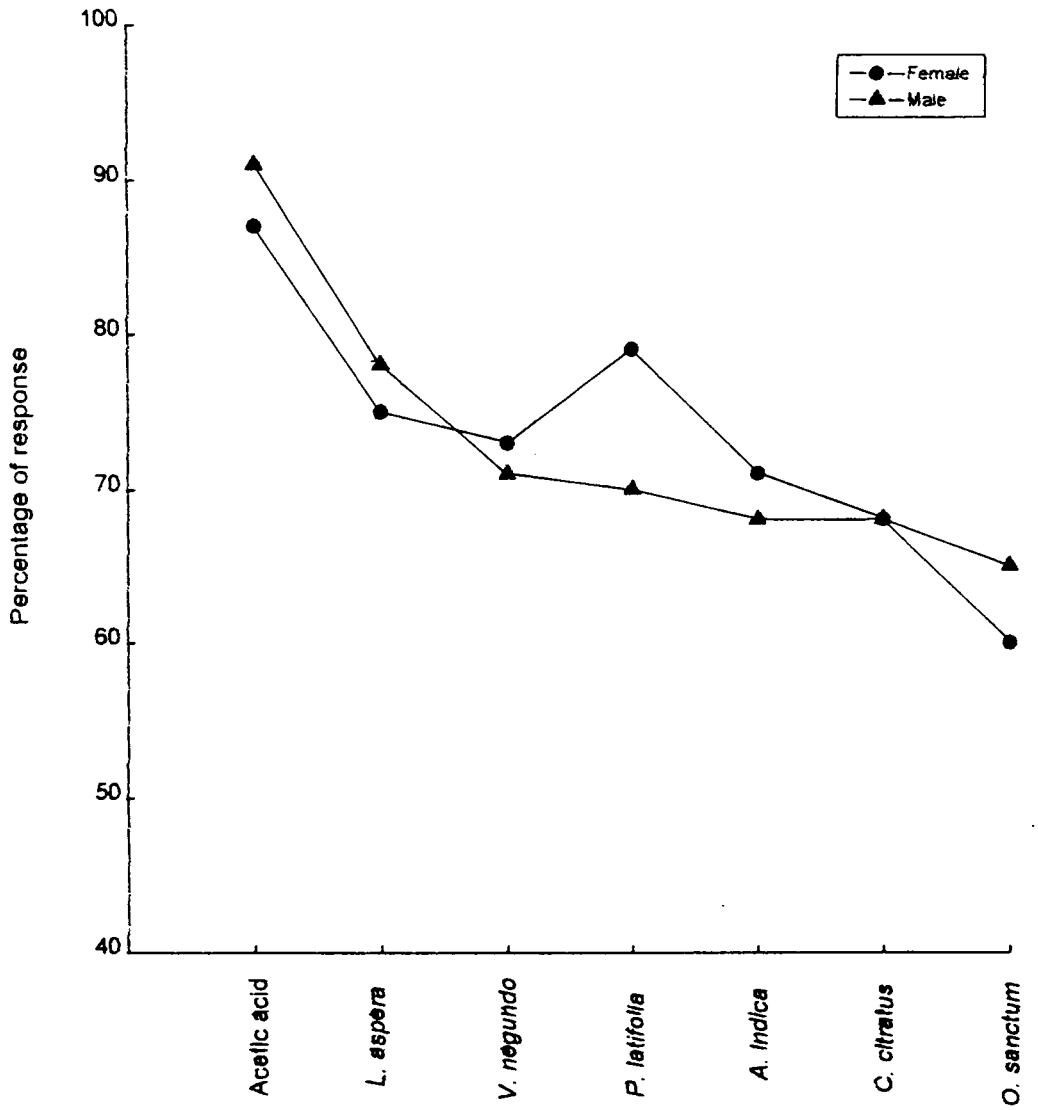

FIGURE -28. Response of *A. subalbatus* (>96h age) towards repellents. Male responses in descending order and female's response to the same chemical.

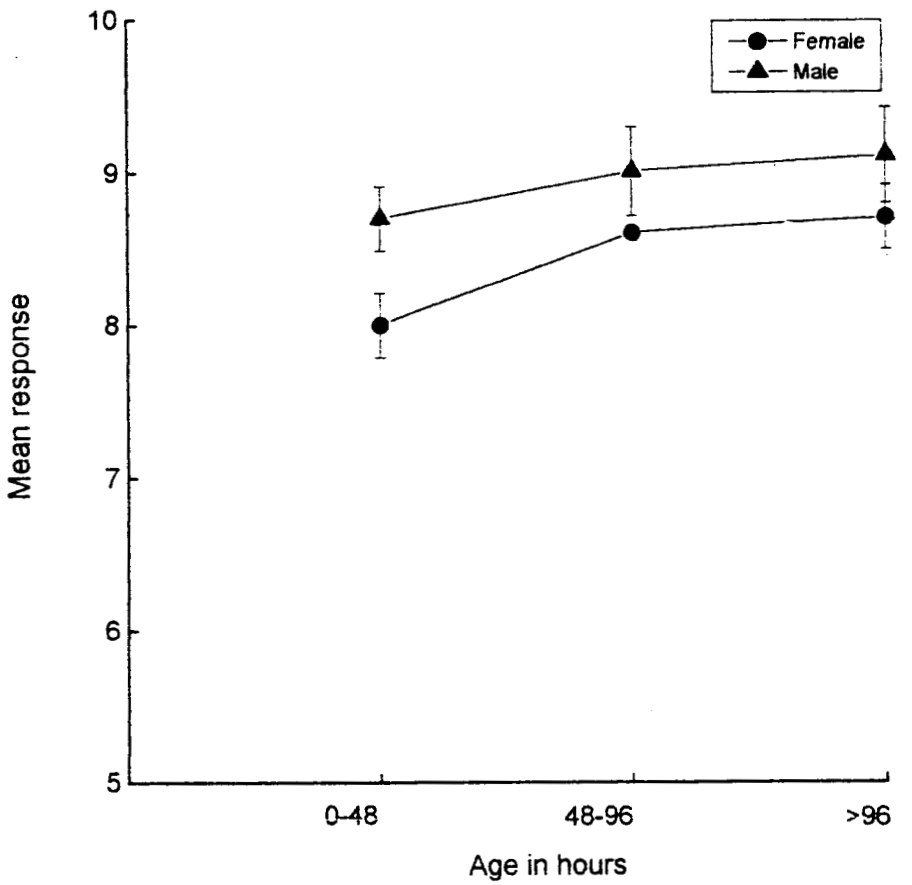

FIGURE-29. Response (Mean ± SEM) to acetic acid.

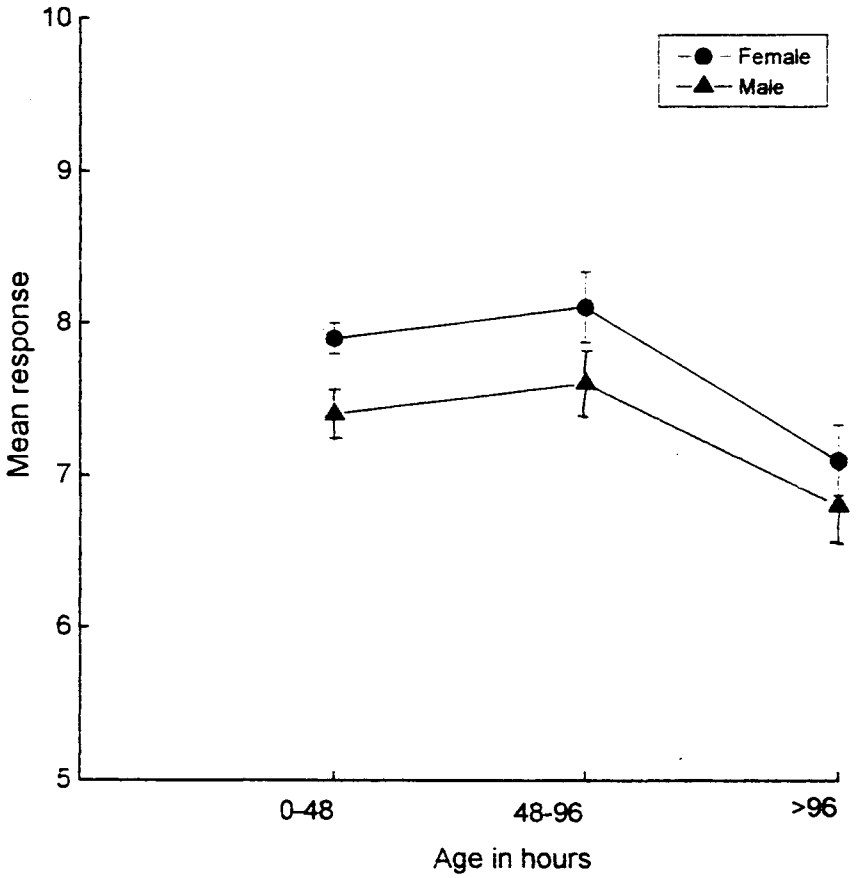

FIGURE-30. Response (Mean ± SEM) to *A. indica.*

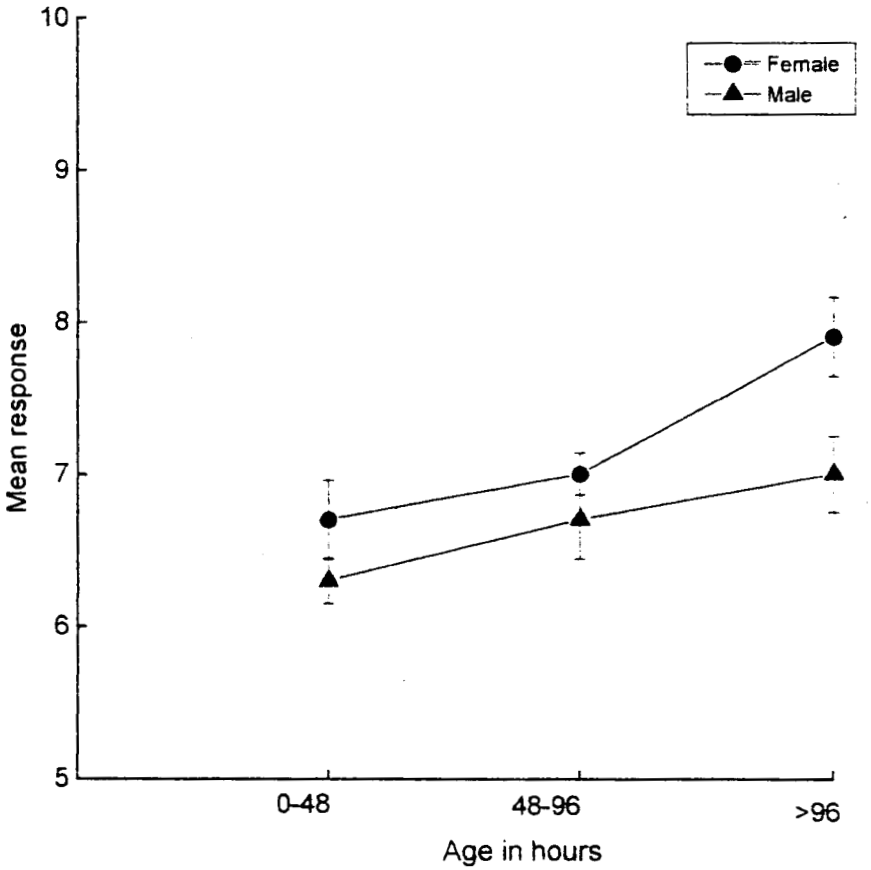

FIGURE-31. Response (Mean ± SEM) to *P. latifolia*.

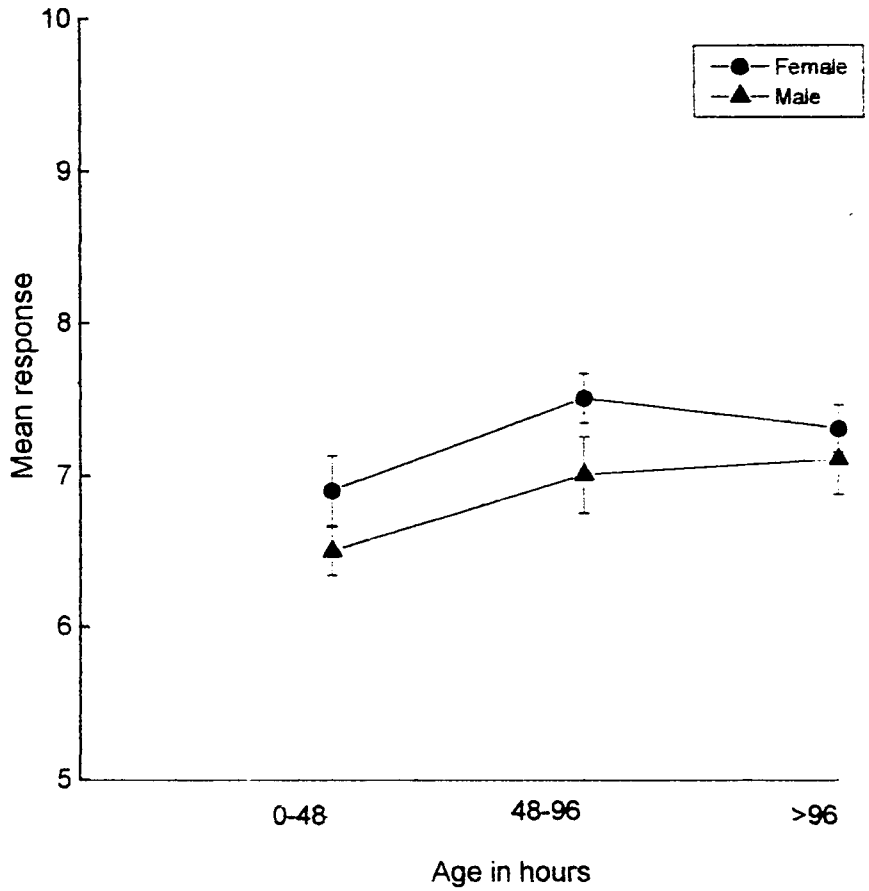

FIGURE-32. Response (Mean ± SEM) to *V. negundo.*

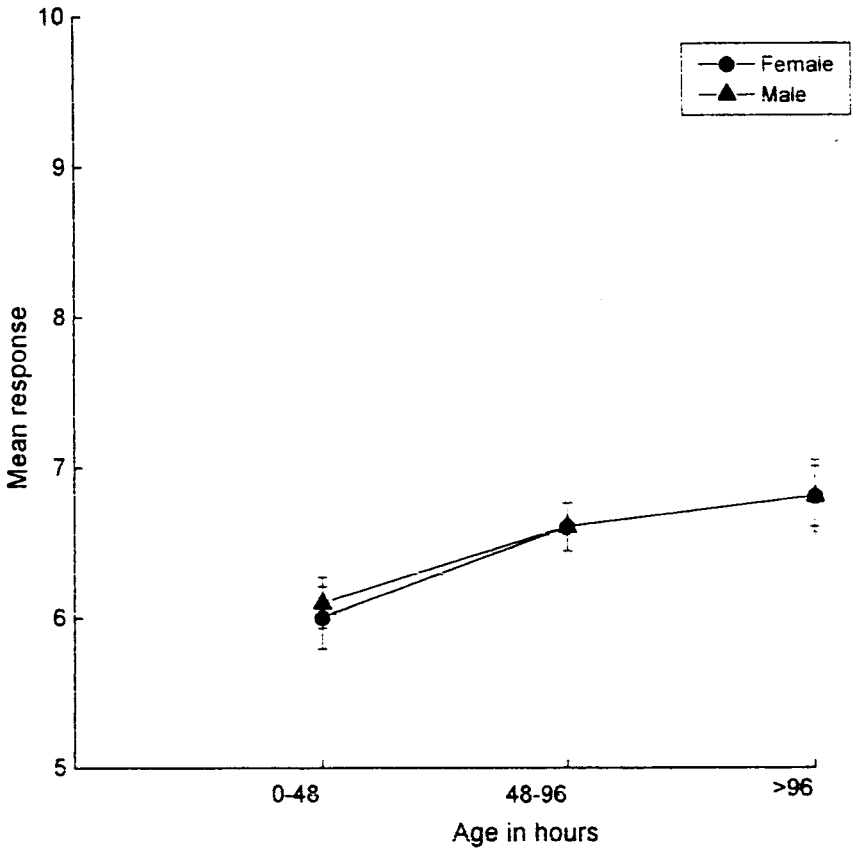

FIGURE- 33. Response (Mean ± SEM) to *C. citratus*

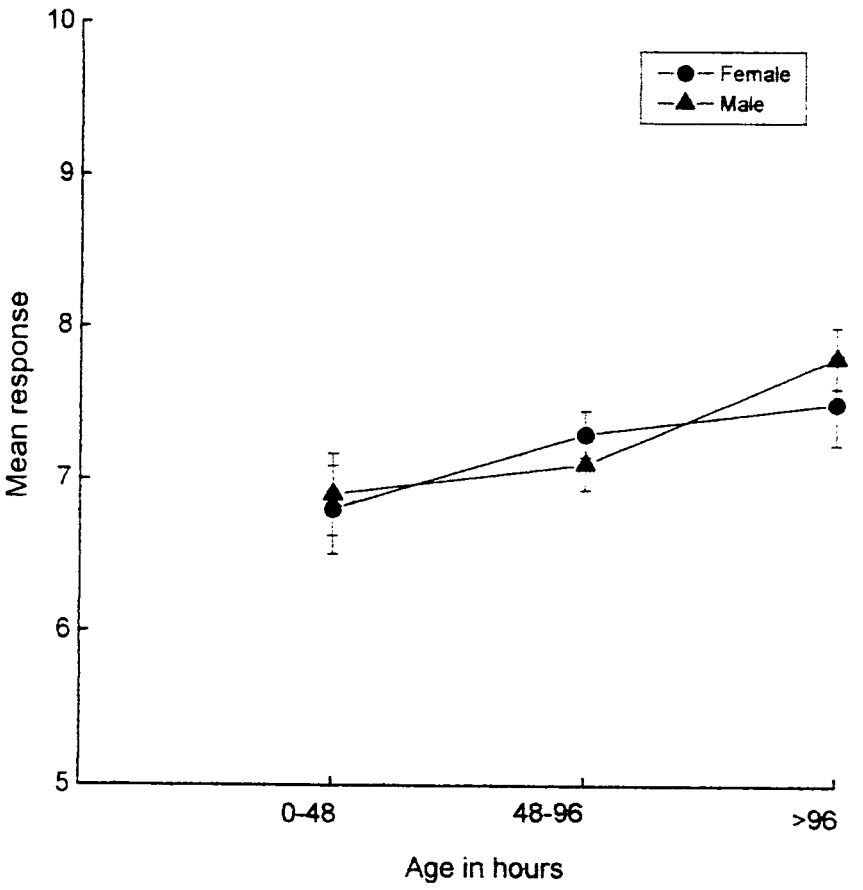

FIGURE -34. Response (Mean ±SEM) to *L. aspera*.

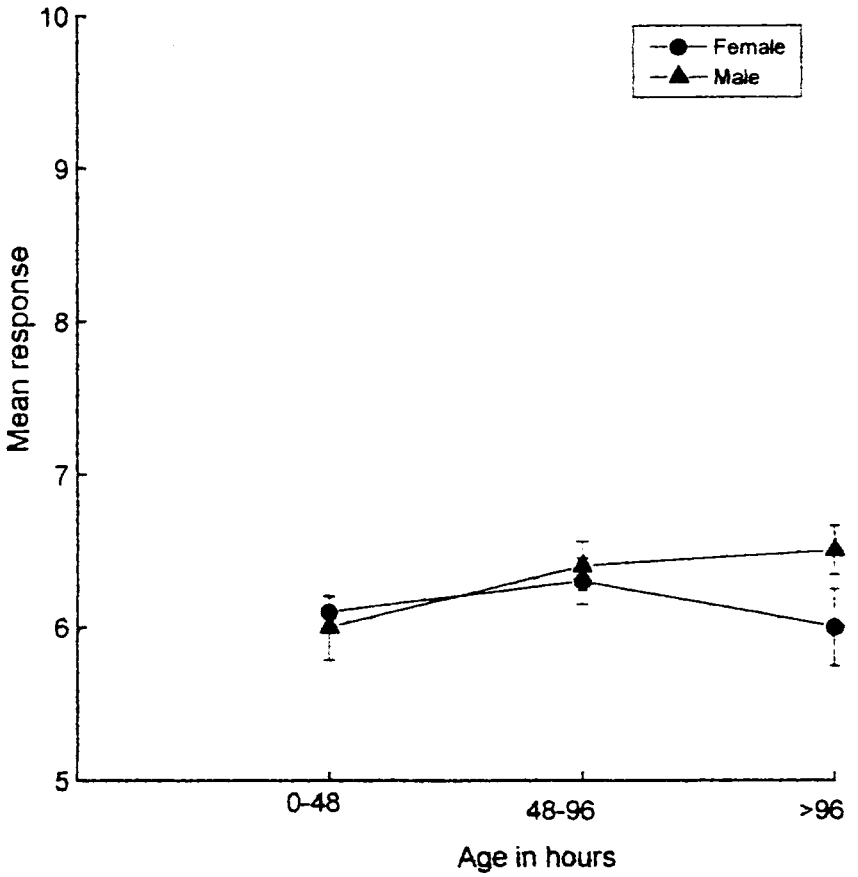

FIGURE- 35. Response (Mean ±SEM) to *O.sanctum*.

stimulants, significant age wise increase in response was observed only in males.

7. When *V. negundo* (Fig. 32) and *O. sanctum* (Fig. 35) were used as stimulants, 48-96h aged females showed greater repellence than the other two age groups. In the case of *A. indica* (Fig. 30) 48-96h aged males and females showed greater repellence than the other two age groups.

8. In all the repellents tested, there was significant sex wise difference in response to each one of the test-stimulant (Figs. 29-35).

2.3.3. OVIPOSITION ATTRACTANT OF *A. SUBALBATUS*

Eggs collected from different oviposition pools were tabulated in the table 11. It is also represented graphically in the Figure 36. It was found that maximum number of mosquitoes preferred to lay their eggs in the trough containing arecanut husk, yeast, acetone and tap water mixture (arecanut mixture), from which a mean of 532 eggs were collected. Next preference for oviposition was the trough containing arecanut husk and tap water from which the mean number of eggs collected were 242. A mean of 188 eggs were obtained from the trough with humus and tap water. Troughs containing acetone-tap water and yeast tap water possess 156 and 150 eggs respectively. Least option for the mosquitoes to lay their eggs was the trough containing tap water alone. The mean number of eggs from tap water was only 80. From this result, it is assumed that the arecanut mixture is an effective oviposition attractant for *A. subalbatus*.

Details of the statistical evaluation of the results are given in Appendix I.

TABLE - 11

Number of eggs obtained from different oviposition attractants

Sl. No	Attractants used	Mean of eggs
1	Arecanut Acetone Yeast mixture	532
2	Aracanut + Tapwater	242
3	Humus + Tapwater	188
4	Acetone + Tapwater	156
5	Yeast + Tapwater	150
6	Tapwater	80

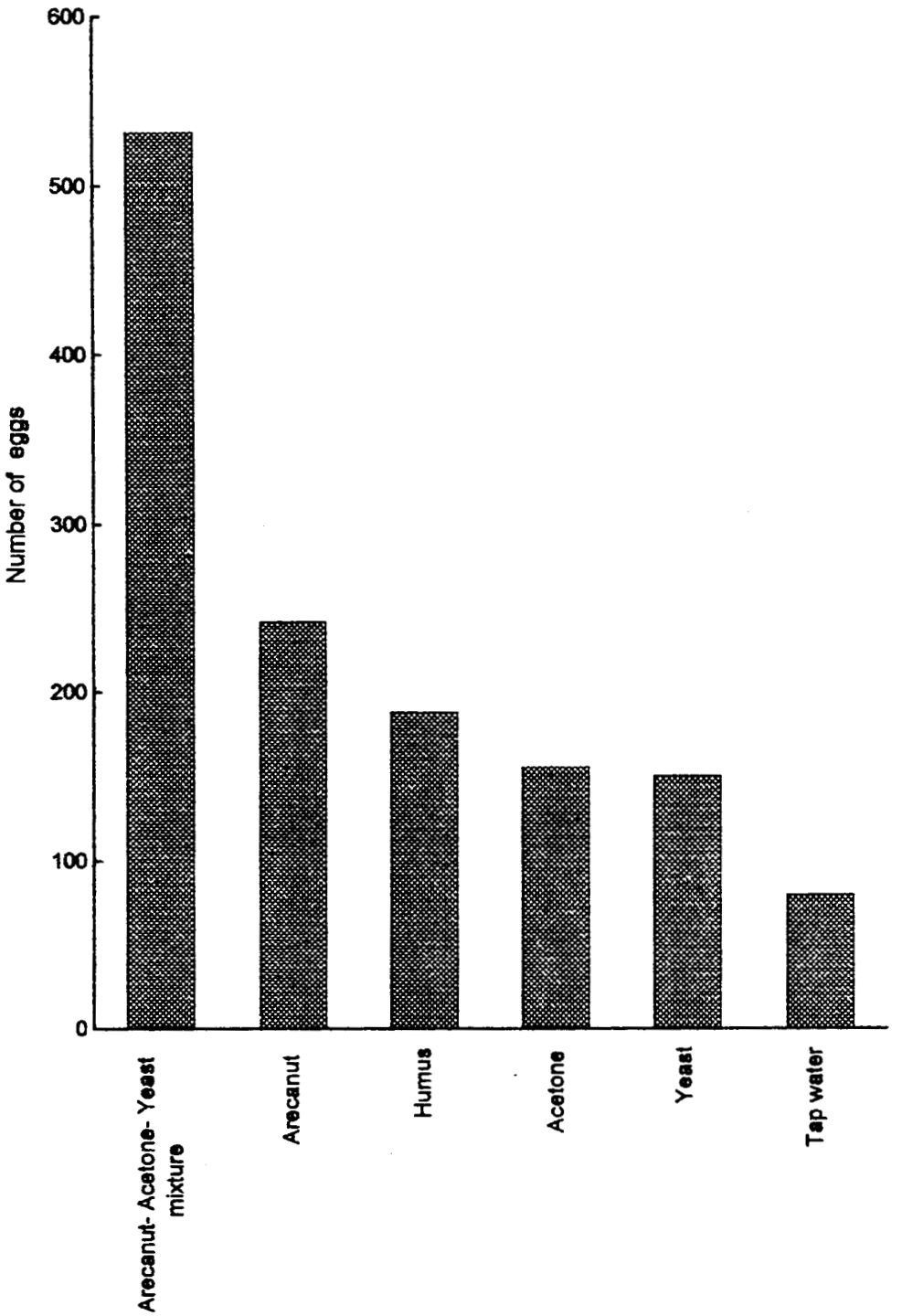

FIGURE-36. Mean number of eggs obtained from different oviposition attractants.

2.4. DISCUSSION

2.4.1. ATTRACTANTS

The present study has examined the effect of some plants, organic and inorganic chemicals and natural products as attractants or repellents of *A. subalbatus*. Among the plants tested it was found that no one acts as attractant. This may be because mosquitoes are attracted only towards the odour coming from the flowers, honey or fruits. This suggestion is based on the studies of Mc Crae *et al.* (1969) who observed that floral nectar is the best known source of sugars for mosquitoes. Joseph (1970) observed the preference of fruits as food in mosquitoes. Thorsteinson and Brust, 1962; Harada *et al.*, 1971, 1972, 1974 and Healy and Jepson, 1988 also noted the preference for honey, flowers and fruits in mosquitoes. The present study was conducted with the leaf extracts of plants.

Eventhough *A. subalbatus* were not attracted to the plant extracts, a good number of organic chemicals tested were found to attract mosquitoes. These chemicals were 1,4-dioxan, acetone, butanol, phenol, petroleum ether, lactic acid and amyl alcohol. Among these organic chemicals, acetone was found to be the best attractant for males and younger females. Acetone is known to be a common ingredient of many fruits. That may be the reason for the attractive behaviour of mosquitoes, towards acetone.

Kline *et al.* (1990) observed the potential of butanone, lactic acid and phenol as attractants for mosquitoes. In the present study, *A. subalbatus* also showed attractance towards these chemicals. It was found that 93% of the females (>96h age) were attracted to lactic acid where as it was only 61% in the case of males (>96h age). Lactic acid is a volatile by-product of metabolism of warm blooded

animals that is exploited by mosquitoes for use as host attractant for feeding (Bowen *et al.*, 1994a). This chemical is detected by specific olfactory receptors on the mosquito antennae that are acutely sensitive to physiological air-borne levels of latic acid (Bowen, 1991). It is well known that only female mosquitoes injest blood as their food. As lactic acid is a host related stimulant, female mosquitoes are more attracted towards lactic acids when compared to males. The present study also showed that males were also attracted to lactic acid to some extent. This is in agreement with Davis's (1977) findings, which suggest that like females, males of *Aedes aegypti* also possess lactic acid sensitive neurones. So in the case of *A. subalbatus* also, males seem to possess lactic acid sensitive neurones in their antenna. Davis (1977) explained that this sensory neurones serve to bring male mosquitoes into close proximity with females that have had or are about to have a blood meal. This would provide for the fertilization of female mosquitoes that were most likely to produce eggs. That means, lactic acid, a host related stimulus enhances the chances for mating by causing male mosquitoes also to be in a location with blood-fed females. Signals which are evoking response for mate finding is always lesser when compared to signals for feeding. For females, lactic acid is a host related stimulus for feeding but for males it is only a mate location signal.

Plant volatiles contain different kinds of alcoholic compounds like 2-butoxy-ethanol, 1-octen-3-ol, 1-hydroxy-2-butanone, which are reported to produce spontaneous neuronal activities (Bowen, 1992a). Hence attempt has been made to test the effect of butan-1-ol and amyl alcohol, which are related alcoholic compounds. When butan-1-ol and amyl alcohol were used as stimuli, 72% and 61% of female *A. subalbatus* (>96h age) were attracted towards these chemicals while the percentage of males (>96h age) attracted were 73 and 67 respectively. The positive results indicate that butan-1-ol and amyl alcohol may also have stimulatory effect on the sensory cells of both the sexes of *A. subalbatus*. Similarly, the

response of *A. subalbatus* to phenol was also analyzed, which showed that 86% of the female *A. subalbatus* (>96 h age) were attracted towards it while it was 70% in the case of males of the same age group. Phenol is known to be a plant volatile, which could act as an attractant of mosquitoes (Kline *et al.*, 1990). Beehler *et al.* (1993, 1994) found that phenol acts as an oviposition attractant for the mosquito, *C. quinquefasciatus*. The reason for the higher percentage of females being attracted towards phenol in the present study, could be explained in the light of above mentioned report. In this case, female *A. subalbatus* may be having more sensitivity than males as phenol may be an oviposition attractant for these mosquitoes also.

In the case of 1,4 dioxan, females were more sensitive whereas for petroleum ether both males and females were showing almost equal response.

Among the inorganic compounds tested, ammonia and ammonium chloride were found as attractants. Ammonia appears to be generally excitatory to all mosquito chemoreceptor neurons regardless of the type of neuron or the sex of the mosquito (Davis, 1977). The present results showed that 70% of the females (48-96h age) were attracted to ammonia while it was 66% in males (48-96h age). Likewise, when ammonium chloride was used as stimulant 65 and 70 percent of 48-96h aged females and males respectively were attracted. So the present study supports the earlier findings.

It has long been known that sugars derived from plant nectar provides energy source for flight and metabolic maintenance for mosquitoes (Thorsteinson and Brust, 1962; Mc Crae *et al.*, 1969; Schaefer and Washino, 1970; Magnarelli, 1978; Klowden, 1986). It is interesting to note that maximum number of males showed attraction (98%) towards honey, when compared to other natural products. Females also showed rather high response (91%) towards honey. However, greater percentage of males were attracted to honey than females. This response may be

because honey is only food resource for males, whereas females injest blood and honey as alternate sources of food.

In search of the role of sweat, as a possible attractant, experiments were conducted to observe the response of *A. subalbatus*. Males (>96h age) attracted towards this stimulant was only 60% whereas the percentage of females (>96h age) responded was 83%. Since sweat is the excretory product of warm blooded animals on which female mosquitoes feed, this result is not surprising. Male mosquitoes may be using sweat as a mate location signal to reach the location where the blood fed females may be present.

When urine was used as stimulant, 67% of the males (maximum response was observed in 48-96h age group) showed attractive response to it whereas the percentage of females responded was 74%. Since urine is an excretory product of host animals, female mosquito may use this as a host location signal for feeding whereas males might use the same for mate location only.

2.4.2. REPELLENTS

Among the leaf extracts of the 60 plants tested none was an attractant. However, some of these plant extracts were found to act as repellents against *A. subalbatus*. Among the repellents observed, male mosquitoes showed maximum repellent behaviour to *L. aspera* (78%) and females showed maximum repellent behaviour towards *A. indica* (81%) among all the age groups (Table 10). Studies of Pandian *et al.* (1994) explained that the smoke of the leaves of *L. aspera* are more toxic to *C. quinquefasciatus* than synthetic mosquito mats which contain 4% d-allethrin. Kalyansundaram and Das (1985) demonstrated the larvicidal efficacy of *L. aspera* against *C. quinquefasciatus*. The present results and these earlier reports together suggest that *L. aspera* is a very good herbal repellent against mosquitoes.

A. indica have long been used as repellent against many insects including mosquitoes. Azadirachtin is a potent disruptor of insect development and also an effective sterilant (Schmutterer, 1988). The repellent action of *A. indica* against other mosquito genus (*Culex, Anopheles*) was reported earlier (Deshmukh and Renapurkar, 1987; Sharma *et al.*, 1993; Sharma and Ansari, 1994). Deshmukh and Renapurkar (1987) explained the growth inhibitory properties of *A. indica* against *C. pipiens* fatigans. Sharma *et al.* (1993) investigated that 2% neem oil (*A. indica*) mixed in coconut oil when applied to the exposed body parts of human volunteers provided complete protection for 12 hours from the bites of all anopheline mosquitoes. Sharma and Ansari (1994) also evaluated the repellent action of neem (*A. indica*) oil against *Anopheles culicifacies* and *Culex* sp. However, no reports were found regarding the repellent action of *A. indica* against *A. subalbatus*. The present studies showed that *A. indica* is a best herbal repellent against *A. subalbatus*. Since only females were blood feeders, the action of *A. indica* towards females become more significant.

Another repellent obtained in this study was *V. negundo*, for which 75% females and 71% males were repelled (maximum response among all the age group) (Table 10). This result is in agreement with the earlier studies that the smoke of the leaves of *V. negundo* is toxic to *C. quinquefasciatus* (Pandian *et al.*, 1994) which is also effective against the larvae of *C. quinquefasciatus* (Kalayansundaram and Babu, 1992). Some plants known to contain toxic principles, can play a useful role in the control of vectors (Sujatha *et al.*, 1988). So, the present study confirms the repellent action of *V. negundo* against *A. subalbatus*.

Repellent property of *O. sanctum* against *Callosobruchus chinensis* (Coleoptera: Bruchidae) has been shown earlier by Rajini *et al.* (1993). Pupicidal effect of *O. sanctum* on *A. aegypti* was studied by Kumari *et al.* (1994). In the

present study, repellent action of *O. sanctum* against *A. subalbatus* was observed. It is found that 63% of the female (maximum percentage, from all the age groups) *A. subalbatus* showed repellence while it was 65% in the case of males. *O. sanctum* of family Labiatae is a medicinal plant grown in the houses throughout the country. Both males and females showed same percentage (68) of repellence towards *Cymbopogon citratus*. Kumar and Dutta (1987) studied the larvicidal activity of plant oils extracted from *Cymbopogon nardus*.

Among the organic chemicals tested, 87% of the females (>96h age) and 91% of the males (>96h age) showed repellence towards acetic acid. Davis (1976) reported that acetic acid is inhibitory to most antennal chemoreceptor neurons.

The present study has shown that these plant extracts could be effectively utilised as mosquito repellents. The efficacy of plant extracts was already reported (Kalansundaram and Babu, 1982; Sujatha *et al.*, 1988; Curtis *et al.*, 1990; Schreck and Leonhardt, 1991). Pandian *et al.* (1989) found that smoke of herbal leaves seems to be an effective mosquito repellent and this has distinct advantages over chemical repellents because it does not leave poisonous residues and does not pollute the air. Biologically active plant extracts are therefore, being studied for their potential efficacy to minimize the extent of pollution and to reduce the cost.

On the basis the above mentioned tests it may be inferred that *A. indica* is the best repellent against *A. subalbatus*. Considerable repellence is shown towards *P. latifolia* also. All the repellents tested have some medicinal value and are readily available in Kerala, so these plants can be used as an effective repellent against *A. subalbatus*, inexpensively. The wide distribution of these plants with its high repellence shows that they are promising agents against *A. subalbatus*.

Lehane (1991) stated that blood sucking mosquitoes usually have a delay period between their emergence from the egg and their first blood meal. Blood feeding has been reported to be initiated between 24 and 72 hours after a female mosquito emerges (Seaton and Lumsden, 1941; Bishop and Gilchrist, 1946; Laarman, 1955). In the present study, host related body fluids/ components (for blood feeding) plant volatiles as well as chemicals were tested to see whether any difference in response exists in different age groups. Interestingly, mosquitoes showed some increase in response as age increases. The mosquitoes tested were grouped into 3 different categories ie. 0-48h, 48-96h and >96h after emergence. In most cases, an age wise increase in response (either attraction or repulsion) was observed. However, in some cases, the response decreased in the >96h age group. But in all cases, an increase in response was observed in 48-96h age group mosquitoes (both males and females) when compared to 0-48h age groups. Newly emerged (0-24h) females showed almost no preference towards host-related stimuli (Lactic acid sweat and urine), when compared to the other stimuli. But as the age increases, host related stimuli were the most preferred ones when compared to the others. According to Lehan's (1991) arguement, after adult emergence, the female reproductive system of many blood-sucking insects undergoes a maturation period lasting several days. Blood meals taken before maturation do not add to the reproductive output of the insect, but visiting the host, during this period, on the other hand will greatly increase the insect's chances of being damaged or killed.

Apart from this, the response of male and female towards attractants looks almost similar upto 0-48h age. But at 48-96h age, females showed greater attraction towards host related stimuli. At >96h age, maximum number of males showed greater attraction towards honey and females showed attraction towards lactic acid. That means, after 4 days of emergence both the sexes showd first preference towards their normal source of food. This observation supports Davis's

(1984) contention, that within the first 24 hours following emergence of the adult mosquitoes, the chemosensory afferents of the grooved peg sensilla typically exhibit no spontaneous spike activity or no detectable response to a variety of chemical stimuli.

Present results may indicate that feeding behaviour is not expressed immediately following adult emergence. The reason for the delay may be that, at this age cuticle of the mouth parts of the mosquitoes may be insufficiently hard or thick. Hardening and theckening takes place during the teneral period approximately the first 24 hours after emergence (Lehane, 1991). As the time since feeding lengthens, the mosquito becomes increasingly hungry and more likely to begin feeding. So 48-96h aged mosquitoes may be more hungry than 0-48h aged ones. So naturally they may respond in a better way than the newly emerged ones.

2.4.3. OVIPOSITION ATTRACTANT

The location and selection of an oviposition site by a mosquito has been shown to be influenced, in part by airborne chemical stimuli (Christophers, 1960a; Gillet, 1971). Oviposition attractants are sufficiently volatile to be detected by the olfactory receptors of the mosquito at a distance from the source (Davis, 1976). The fowl smell is known to attract female mosquitoes for oviposition. Among the various oviposition attractants tested, arecanut-acetone-yeast mixture is shown to be best oviposition attractant for *A. subalbatus*. The decaying arecanut which contains many organic substances may be supporting the growth of yeast cells as well as other bacteria. The decomposition of these substances results in the production of fowl smell. Moreover, decomposition results in the release of various gases, which may stimulate the olfactory receptors of the gravid female moquitoes for oviposition. However, further studies are needed to specify which component of this fowl smelling gas is responsible for the attraction of mosquitoes. Present study

suggests that this mixture can be used as a good oviposition attractant for *A. subalbatus* which can be ultimately used for mosquito control (by destroying the collected eggs). Moreover, it can be utilised in the laboratory for effective rearing of *A. subalbatus* for research purposes.

CHAPTER 3

Electrophysiological Aspects of Chemoreception

3.1. CHEMORECEPTION: SOME GENERAL ASPECTS

Receptor cells sensitive to chemicals are among the most important components of an insect sensory system. Such cells are designated as chemoreceptors and the physiological processes which occur in these cells upon chemical stimulation are termed chemoreception (Hodgson, 1974). In recent years, chemosensory physiology has become a field of active research ranging from ciliates to mammals.

Insect chemoreception is generally divided into three categories viz. olfaction, contact chemoreception and common chemical sense or general chemical sense (Hodgson, 1974). The olfactory sense is mediated by chemical stimuli in a gaseous state at relatively low concentration while contact chemoreception is mediated by chemical stimuli acting as liquids or solutions at relatively high concentration on contact. Common chemical sense is involved in reactions to high concentrations or irritating compounds which evoke avoidance reactions (Hodgson, 1974).

Olfactory or gustatory systems have to react to a considerable number of different molecules. One central question concerns the number of chemoreceptors in a given organ or organism and their range of specificity. There are specific receptors for each class of molecules or even for each molecule, so that, so many different receptors are seen in an insect according to their way of life i.e., whether they are free living, temporary ectoparasite, permanent ectoparasite or endoparasite. These differences in life-style are reflected in the number of receptors which different blood-sucking insects posses (Chapman, 1982). The more independent host seeking insects posses the most receptors. Moreover, adaptation of the sensory organs occurs in response to the blood-sucking habit. If we closely observe the chemoreceptors of mosquitoes we can see another pattern to the blood-sucking

habit, where the female mosquitoes bear between two to four times the number of chemoreceptors as the non-blood feeding male (Chapman, 1982; Lehane, 1991).

The significance of chemoreception in insects has long been recognised by entomologists may be because their sensory process triggers a wide variety of most important behavioural patterns. Physiologists have focussed attention upon these chemoreceptors because insect chemoreceptors are well suited for the study of the basic mechanisms of chemoreception at cellular level.

Studies on chemoreception stemmed from observations on the behaviour of animals in the field or under stimulated conditions in the laboratory (Hodgson, 1974). The pioneering studies of Von Frisch (1934) are outstanding examples of this approach. Behavioural experiments reached a new level of physiological precision during 1940s when Dethier and Chadwick (1948) carried out experiments on the blow fly, *Phormia regina*. Such studies on mosquitoes also commenced in the same period which mainly include feeding behaviour, host seeking behaviour, oviposition and mating behaviour.

3.1.1. HOST SEEKING

Laarman (1955, 1958) observed the host seeking behaviour of anophiline mosquitoes and used the term 'feeding drive' to describe the condition of the mosquito in which it responds to host stimuli by orientating to and attacking the host. The blood feeding behaviour of mosquitoes is fundamental to their importance as vectors (Klowden and Lea, 1979a). Exogenous factors which activate and orient mosquitoes to their host have been well studied (Hosoi, 1958; Brown, 1958; Kellog and Wright, 1962; Clements, 1963; Daykin *et al.*, 1965). Ovarian development is one of the endogenous factors which may influence the mosquito's

host seeking behaviour (Clements, 1963). Biting was inhibited much less by the presence of mature eggs in the ovary in virgin females of *Aedes aegypti* than it was in inseminated females (Clements, 1963).

Laviopierre (1958a, b) found that inseminated *Aedes aegypti* showed a reduced biting activity which coincided with egg development; he ruled out distension from mature oocytes or an enlarged fat body as a source of inhibition, and suggested that 'Blood avidity' was under hormonal control, closely associated with events in the gonotrophic cycle. Judson (1967) examined the basis of differences in the biting behaviour between inseminated and uninseminated *A.aegypti.* The retention of eggs by mosquitoes significantly inhibited the biting response compared to females which had oviposited (Judson, 1968). Khan and Miabach (1970) and Edman *et al.*, (1975) also attributed the inhibition of biting to the presence of developing oocytes. Edman *et al.*, (1975) and Klowden and Lea (1978) proved that large meals inhibit the mosquito's host seeking behaviour. There is thus evidence that an endogenous rhythm with a period of several days exists, distinct from the gonotrophic cycle, affecting the intensity of biting .

Hosoi (1954) and Armstrong (1968) suggested that nutritional status controls the host seeking behaviour in mosquitoes. Klowden and Lea (1978) demonstrated that short term inhibition of host seeking does not occur in *Aedes aegypti* when less than 2.5µl blood is ingested. In 1979, they also examined the long term host seeking behaviour of *A.aegypti* after they received 1µl blood enemas. Gravid uninseminated mosquitoes were also inhibited but not to the same degree as inseminated mosquitoes. They also reported that host seeking is inhibited by a haemolymph-borne factor which is present during and after ovarian development. Klowden (1981) observed the initiation and termination of host seeking behaviour

during oocyte maturation, the mechanism involving a signal from the maturing ovaries that activates the release of a behavioural inhibitor from another site.

At least two endogenous mechanisms inhibit host seeking behaviour (Klowden, 1986). Abdominal distension resulting from blood meals above a certain threshold volume causes an immediate behavioural inhibition that is mediated by the nervous system (Klowden and Lea, 1978, 1979a, 1979b). Both these endogenous mechanisms can be modified by such factors as the age of mosquitoes (Klowden and Lea 1980, 1984) and whether the mosquito is inseminated (Judson, 1967; Klowden and Lea, 1979a). It is also reported (Klowden, 1986) that starvation or ingestion of carbohydrates can significantly modify the effects of blood meal on reproduction as well as on the expression of humoral host seeking inhibition in mosquitoes that do develop eggs.

Other factors which affect host seeking behaviour have been well investigated which includes, the regulation of sensitivity in the peripheral chemoreceptor system for host seeking behaviour by a haemolymph - borne factor in *Aedes aegypti* (Davis, 1984); behavioural and sensory analysis of host seeking behaviour in *Culex pipiens* (Bowen *et al.*, 1988); the role of olfaction in host seeking (Takken, 1991); sensory physiology of host seeking behaviour (Bowen, 1991); lack of correlation between peripheral receptor sensitivity and daily pattern of host seeking behaviour (Bowen, 1992); host seeking behaviour in the autogenous mosquito, *Aedes atropalpus* (Bowen *et al.* ,1994) and endogenous regulation of host seeking behaviour by a neuropeptide (Brown *et al.*, 1994)in mosquitoes.

3.1.2. HOST LOCATION

For mosquitoes, the location of the host is an integrated, but flexible behavioural package which gathers momentum as the host is tracked down (Lehane, 1991). From the observations of blood sucking insects both in the laboratory and field, there is clear evidence that a variety of host signals are used in host finding. In general, olfactory and visual stimuli are the most important signals when the mosquito is still at some distance from the host. Nearer to the host different stimuli become important, particularly heat.

Chemoreception is important in activating and orientating mosquitoes to a potential host animal. The chemical stimuli may directly cause the insect to switch over from endogenously driven appetitive searching to oriented host location behaviour. From the literature it is inferred that heat, moisture, carbon dioxide and various odours are the major factors which activate, orient and attract the mosquitoes towards their hosts.

3.1.2.1. Response to heat and moisture

The manner in which mosquitoes are attracted to objects heated above air temperature in laboratory and field experiments leaves no doubt that body temperature is an important factor in host finding (Howlett, 1910; Brown, 1951; Peterson and Brown, 1951). Howlett (1910) showed that convection currents were responsible for this attraction and not radiation. To find whether mosquitoes respond to high temperature as such or to temperature gradients, Laarman (1955) passed two streams of dry air into a cage containing *Anopheles labranchiae atroparvus* which had been activated by carbondioxide; one air stream was at

room temperature and the other was cooled by 1-2°C. The mosquitoes showed a clear preference for the cooler air, leading Laarman to conclude that the mosquitoes which orientate to heat sources are reacting to temperature gradients. Temperature may play a part in the selection of an individual host from others in close proximity to it. Electrophysiological studies of the temperature response in *Aedes aegypti* shows maximal spike frequency changes in the cold and hot receptors of their antennal sensilla coeloconica, in response to a temperature change of 0.2°C (Davis and Sokolove, 1975). Grossman and Pappas (1991) observed the relation between human skin temperature and mosquito blood feeding rate. In *Armigeres subalbatus*, such studies has been carried out by Srinivas *et al.* (1994), who showed a higher attractancy to relatively warmer skin of human hands.

Water vapour also clearly affects the behaviour of mosquitoes. In a large cage olfactometer *Aedes aegypti* approached a stream of warm air of 80-90% R.H than one of 15-20% R.H (Brown, 1951). However, water vapour is not always attractive. Laarman (1955), working with a small cage olfactometer, found that the response of *Anopheles labranchiae atroparvus* to rabbit odour was greatly reduced after the relative humidity was raised to 90%. On the basis of the studies conducted by Brown. (1951), Laarman (1955), Smart and Brown (1956) Gouck and Bowman (1959), Clements (1963) suggested that mosquitoes orientate towards currents of moist air and show a strong tendency to alight at the source, provided the humidity is not raised to near saturation, so that moisture is probably an important part of the emanations of a host, acting over a short distance.

3.1.2.2. Response to carbondioxide

It is generally accepted that carbondioxide is involved in activation and orientation of virtually all blood sucking insects (Omer and Gillies, 1971; Bursell,

1984, 1987; Warnes and Finlayson, 1985). In mosquitoes, it is the change in concentration of carbondioxide rather than the level of carbondioxide encountered which is the important factor in eliciting behavioural responses (Wright and Kellog, 1962; Lahane,1991). Mosquitoes are sensitive to very small changes in carbondioxide levels (Brown, 1951; Brouwer, 1960; Laarman, 1955; Mayer and James, 1969). Electrophysiological recordings from the carbondioxide receptors on mosquito palps showed responses to changes as small as 0.01% (Kellog, 1970). He also conducted a detailed study on carbondioxide receptors in *Aedes aegypti*. In 1971, Omer and Gillies found that palpectomized female mosquitoes lost their ability to respond to carbondioxide.

As well as acting as an activating agent carbondioxide, also acts as a stimulus, orienting blood sucking insects to hosts (Omer and Gillies, 1971; Warnes and Finlayson, 1985). This can be neatly shown in mosquitoes by filtering carbondioxide from the breath of a host (Laarman, 1958). Edman (1979), in his field experiments clearly demonstrated that carbondioxide orientate mosquitoes towards small vertebrates. Other studies include relative attractiveness of carbondioxide to parous and nulliparous mosquitoes (Feldlaufer and Crans, 1979), role of carbondioxide in host finding (Gillies, 1980), potential of carbondioxide as an attractant (Kline *et al.*, 1990), inter active effects of 1-octan-3-ol and carbondioxide (Kline *et al.*, 1991), responses of mosquitoes to carbondioxide and 1-octan-3-ol (Kemme *et al.*, 1993), differential responses of Aedes and Culex mosquitoes to octenol or light combination with carbondioxide (Van Essen *et al.*, 1994), and electrophysiological responses of receptor neurones of maxillary palp sensilla to carbondioxide (Grant *et al.*, 1995) in mosquitoes.

3.1.2.3. Response to odours

Several investigators have isolated and identified various chemical substances that are to some extent act either as attractant or as repellent. Olfactory stimuli implicated in host location include lactic acid, acetone, butanone, octanol and phenolic components of urine (Lehane, 1991). Odour is important in activation, orientation and is of significance in the attraction phase of host location in mosquitoes (Lehane, 1991).

Lactic acid is a volatile by-product of anaerobic metabolism that is used by mosquitoes as a host attractant. Acree *et al.* (1968) isolated L-lactic acid from human hosts, which was proved as a mosquito attractant. Smith *et al.* (1970) found L-lactic acid as a factor in the attraction of *Aedes aegypti* to human hosts. Lactic acid has been demonstrated to attract female mosquitoes to the source of an air flow that contains lactic acid and a small amount of carbondioxide (Acree *et al.*, 1968; Smith *et al.*, 1970). Davis and Sokolove (1976) reported two types of lactic acid-sensitive peripheral receptor cells associated with the antennal grooved peg sensilla of yellow fever mosquito. One type of receptor neurone showed an increase in spike discharge rate when exposed to lactic acid, where as the second type showed a decrease in spike frequency in the presence of lactic acid. Further more, Smith *et al.* (1970) found a behavioural synergism of lactic acid plus carbondioxide. In addition, there is evidence to support the notion of a causal relationship between the changes in sensitivity of neurones to lactic acid and the presence of host seeking behaviour (Davis, 1984, 1984a). But the responsiveness of the lactic acid-excitable receptor is variable, being about ten times less sensitive in the fed non-active host-seeking mosquito compared to a hungry one and this change in

responsiveness is induced by a hormone released from the mosquitoes fat body during oogenesis (Klowden and Lea, 1979b; Davis, 1984; Klowden *et al.*, 1987).

It has been argued that variable responsiveness of the lactic acid receptors is sufficient to account for changes in the behaviour patterns of mosquitoes without the need to evoke central nervous system involvement (Davis *et al.*, 1987). The theory is that in the unfed mosquito the summed output of the excited and inhibited receptors in the presence of lactic acid is positive (i.e. above the spontaneous level produced by the resting receptors) and drives host-seeking behaviour. Conversely, in the fed mosquito, because of the lowered responsiveness of the lactic acid excited receptors (brought about by the fat body hormone), the summed output of the receptors in the presence of lactic acid is negative, leading to inhibition of host-seeking behaviour (Davis *et al.*, 1987). Davis (1988) also studied the structure-response relationship of lactic acid-excited neurones in the antennal grooved peg sensilla of *Aedes aegypti*. His investigations determined the specificity and sensitivity of lactic acid-excited neurones to the host attractant, lactic acid. The absence of host-responsiveness during adult diapause in *Culex pipiens* is similarly correlated with the absence of highly sensitive receptors (Bowen *et al.*, 1988). In this species, diapause apparently causes a delay in maturation of the receptors responsible for detecting host attractants (Bowen *et al.*, 1988). Diapause termination is accompanied by the development of high receptor sensitivity and the appearance of host seeking behaviour (Bowen, 1990). The rate of maturation of the lactic acid-sensitive neurones is relatively rapid in *Aedes atropalpus* (Bowen *et al.*, 1994). They also reported that lactic acid receptors of high sensitivity are present on the antennae of females that are gravid in the early stages of egg development.

Sweat, blood and urine have been investigated as likely sources of odour (Clements, 1963). Some workers have obtained negative results with sweat (Howlett, 1910; Rudolfs, 1922; Reuter, 1936) but others have obtained clear responses to it (Brown, 1951; Thompson and Brown, 1955). Dummies whose clothing was soaked with human sweat from the armpit and general body surface was significantly more attractive to Aedes in the field than controls with equally moist clothing (Brown, 1951, 1958). Van Thiel and Weurman (1947) clearly demonstrated that free blood attracts mosquitoes and showed that traps containing heated defibrinated pig's blood caught 7-9 times more *Anopheles labranchiae atroparvus* released into a very large cage surrounding the traps in the open air than did traps producing warmth and moisture alone. Burgess and Brown (1957) found that *Aedes aegypti* approached whole bovine blood exposed in a fairly large cage. Other studies include blood feeding requirements of the mosquito (O'Meera and Evans, 1973); blood feeding behaviour of adult *Aedes aegypti* (Jones and Pillit, 1973); frequency of blood feeding in *Aedes aegypti* (McClelland and Conway, 1974); blood feeding activity of partially engorged *Culex nigripalpus* (Edman *et al.*, 1975) and engorgement response of Anopheles mosquitoes to blood fractions (Galun *et al.*, 1985 b). Human urine in certain dilutions was shown by Roessler (1961) to attract female *Aedes aegypti.*

Rudolfs (1922) investigated the responses of female *Aedes sollicitans* and *Aedes contator* to a number of pure compounds and found that different compounds had attractive, repellent or activating effects. In 1958, Hosoi, found Adenosine 5'-phosphates to be a stimulating agent in blood for inducing gorging in the mosquito. Galun *et al.*, (1963) traced the stimulation by ATP in *Aedes aegypti.* Culicines respond maximally to ADP while aedine mosquitoes prefer ATP (Galun, 1987; Galun *et al.*, 1988). Jones and Madhukar (1976) investigated

the effect of sucrose in blood avidity in mosquitoes. Friend (1981) studied the response to ATP and sucrose in *Culiseta inornata*. Gorging response of *Aedes aegypti* (Galun *et al.*, 1985 a) and *Culex univittatus* (Galun and Fried man, 1992) to adenine nucleotides were also well studied.

Roessler (1960,1961) found that phenol strongly acts as a repellent to *Aedes aegypti* but that the position isomers of diphenol showed varying degrees of attractiveness. He also observed that mosquito showed attraction to an air stream which had passed over hydroquinone (para-diphenol), resorcinol (meta-diphenol) and catechol (ortho-diphenol).

3.1.3. MORPHOLOGICAL ASPECTS

Chemoreceptor cells of insects are modified epithelial cells and that the central axon of the receptor is formed by ingrowth of an extension process towards the central nervous system i.e., the receptor cells are true primary sense cells (Wigglesworth, 1953). Chemoreceptors in insects have been reviewed by Snodgrass (1926). Frings and Frings (1949), Dethier and Chadwick (1948) and Wigglesworth (1953). The advent of electron microscope and superior histological techniques have made available more detailed information about the minute anatomy of insect chemoreceptors since 1950s. The scanning electron microscopy has revealed much about the cuticular modifications associated with chemosensory structures During nineteen fifties, several electrophysiological techniques made possible a more precise measurement of the responses of chemoreceptor cells to analyse the function of single chemoreceptor cell (Hodgson *et al.*, 1955). These techniques soon proved useful to analyse insect chemoreception including that of mosquitoes.

In insects, feeding and distribution of food are controlled by integrated responses of the peripheral sensilla, central nervous system and stomatogastric system. Olfactory sensilla are found on the antennae (Slifer and Sekhon, 1962; Steward and Atwood, 1963; McIver,1970, 1974, 1978) and in some species upon palps (McIver and Charlton, 1970; Lewis, 1972; McIver, 1972; McIver and Siemicki, 1975a, b). Contact chemoreceptors (Owen 1963, 1965, 1968; Salama, 1966; Sinitsyna, 1971) are found grouped upon tarsi (Fier *et al.*, 1961; McIver and Siemicki, 1978), labella (Fier *et al.*, 1961; Owen *et al.*, 1974; Pappas and Larsen, 1976 b), labrum (Lee, 1974; Lee and Craig, 1983 b; Anna Liscia *et al.*., 1993) and within the cibarium (Lewis 1972; Lee, 1974; Lee and Davies 1978; McIver and Siemicki, 1981; Lee and Craig, 1983a).

In mosquitoes of many species three morphological types of olfactory sensilla were recognised (Steward and Atwood, 1963) and described as sharp trichoid (type A1), shorter blunt trichoid (A2) and smaller thorn like basiconic sensilla (A3). But in *Aedes aegypti* five types of olfactory sensilla have been described on the antennae namely, grooved pegs, long pointed sensilla trichodea, short pointed sensilla trichodea, long blunt sensilla trichodea and short blunt sensilla trichodea (Linda and Bronwen, 1994).

Mosquitoes use chemosensilla on the tarsi, labella and labrum to identify food substances (Clements 1992). Labella bear a large number of sensilla, mostly contact chemoreceptors and mechanoreceptors but including two proprioceptors (Muller, 1968; Owen, 1971; Larsen and Owen, 1971; Owen *et al.*, 1974; Puchkova, 1976). Labrum bears three pairs of sensilla (Lee, 1974; Lee and Craig, 1983 b; Clements, 1992). A pair of apical sensilla located at the tip of the stylet, a pair of subapical sensilla located below the apical sensilla (Clements, 1992) and a pair of campaniform sensilla located near the opening of the labral food canal are contact chemoreceptors.

Two types of receptors seem to be involved in the feeding of *Aedes aegypti* (Salama, 1966) and the chemoreceptors on the tarsi, labella, labrum and cibarium co-operate to accomplish feeding. In males and females of *Culiseta inornata*, the two labellar lobes at the tip of the proboscis bear hairs averaging 70μ in length which contain two lumina and the experiments have proved that they are chemosensory in function (Feir *et al.*, 1961). Hairs containing two lumina occur on the tarsi of various species of Anopheles, Culex and Aedes (Grabowski and Dethier, 1954) and it is known that tarsi mediate responses to sugar and salt solutions (Frings and Hamrum, 1950; Wallis, 1954a; Feir *et al.*, 1961). Observation of mosquito behaviour suggests that the females use their tarsal sense organs in selecting an oviposition site (Clements, 1963). These organs are sensitive to a number of inorganic ions (Wallis, 1954a, b; Hudson, 1956). The only organ on the stylet is a pair of peg organs near the tip of the labrum, described in the female *Anopheles maculipennis* (Robinson, 1939) and in both sexes of *Aedes aegypti* (Christophers, 1960). The inner surface of the cibarial pump of both males and females bears a number of sense organs, some of which are contact chemoreceptors. In *Aedes aegypti*, they comprise a number of short spines and fine hairs and two campaniform sensilla, all innervated from the frontal ganglion (Day, 1954). The inner surface of the cibarial pump bears three types of sensillum: uniporous peg sensilla, aporous articulated setae and campaniform sensilla (Uchida 1979; Lee and Craig, 1983a) which are respectively chemoreceptors, mechanoreceptors and proprioceptors. The number and distribution of the sensilla are broadly similar in male and female mosquitoes (Clements 1992).

Maxillary palp bear three types of sense organs, mechanosensory setae, multiporous peg sensilla and single campaniform sensillum (McIver and Charlton, 1970; McIver 1972; McIver and Siemicki, 1975a, b).

3.1.4. ELECTROPHYSIOLOGY

Various electrophysiological techniques were used to study the physiology of chemoreceptors. Insect chemoreceptor cells, like other receptor cells exhibit two main types of electrical changes during their activity. Graded potentials (receptor potential or generator potential) which spread electronically along dendrites and all-or-none impulses (Spike potential or action potential) which are conducted from the vicinity of cell bodies along afferent axons to the central nervous system (Hodgson, 1974).

The first recordings of afferent impulses from individual chemoreceptor cells were obtained from the labellar chemoreceptors of the blow fly, *Phormia regina* (Hodgson *et al*, 1955; Hodgson and Roeder, 1965). Electrophysiological experiments in mosquitoes proved that receptor cells are very sensitive to carbondioxide, giving phasic response of 2-4 times the spontaneous impulse rate on exposure to 0.01% change in concentration (Kellog, 1970). He also observed that A3 basiconic sensilla of Aedes antennae respond to water vapour. Lacher (1967) showed that in *Aedes aegypti*, cells of A1 trichoid sensilla are generally excited by fatty acids while their resting activity is depressed by essential oils; those of A2 trichoid sensilla are excited by higher fatty acids, depressed by lower fatty acids and variable in response to essential oils. Thus the A2 cells are considered by Lacher (1967) to behave as odour generalists towards essential oils and as specialists towards fatty acids. Lacher (1967) also found that in *A. aegypti*, cells associated with the A1 type antennal sensilla trichodea (McIver, 1978) respond to essential plant oils (terpenes) by decreasing their firing rate. Receptors associated with A2 type sensilla trichodea (McIver, 1978) in *Aedes triseriatus* and *A. aegypti* respond to cresols and related compounds but the same study generally found no

response of any kind to other plant related stimuli (Davis, 1976; Bently *et al.*, 1982). Bowen (1992) described the olfactory receptors associated with A2 type sensilla trichodea in *Culex pipiens* females that are sensitive to specific terpenes.

Lactic acid is detected by specific olfactory receptors on the mosquito antennae that are acutely sensitive to physiological air-borne levels of lactic acid (Bowen, 1991). These peripheral receptors (olfactory receptors) play an important role in the control of mosquito-host responsiveness. Electrophysiological recordings from lactic acid sensitive receptors on the antennae of female *Aedes aegypti* have shown that host responsive females always have some highly sensitive lactic acid receptors (Bowen *et al.*, 1994). Likewise non-host responsive females do not have highly sensitive receptors (Davis, 1984, 1984a).

Physiological studies of the peripheral sensory system in mosquitoes have focussed on the response of olfactory receptor neurones in antennal sensilla to various synthetic and natural stimuli (Davis, 1977; Davis and Sokolove, 1976; Davis, 1985). It has been suggested that number and distribution of particular chemoreceptors on the antennae and maxillary palps in both sexes of various species of mosquito are related to difference in the insect's relative preferences for hosts (McIver, 1971; Braverman and Hulley, 1979). Bowen (1992) described the electrophysiological characteristics of antennal olfactory receptors of female *Culex pipiens*, that are sensitive to specific terpenes. Anna *et al.*, (1993) explained the electrophysiological responses of labral apical chemoreceptors to adenine nucleotides in *Culex pipiens*. A comparative physiological study of chemoreceptors in several species might reveal important clues about the way in which chemical stimuli initiate and modulate host seeking behaviour (Grant *et al.*, 1995).

Extensive literature is available in many aspects *viz.* olfaction, chemoreception, host finding using electrophysiological techniques in female mosquitoes. However, there have been very few reports regarding males. McIver (1971) compared the sense organs on the antennae and maxillary palps of selected male Culicine mosquitoes. In 1977, Davis made extracellular recordings of single chemoreceptor neurones on the antennae of male *Aedes aegypti* and compared with similar recordings from homologous sensilla on the antennae of female. In this study he found that in both male and female mosquitoes the grooved peg sensilla were associated with two types of neurones sensitive to lactic acid. Nijhont and Sheffield (1979) studied the antennal hair erection in male mosquitoes. McIver (1980) observed the sensory aspects of mate finding behaviour in male mosquitoes.

3.1.5. OBJECTIVES

From the earlier literature it was found that all the studies were mainly carried on the genus Culex, Anopheles or Aedes. Studies on chemoreceptive physiology of *A. subalbatus*, which is one of the most common mosquito of our country, remains to be elucidated. In the light of the earlier studies mentioned so far, further investigations are necessary to explain the neurophysiological basis of chemoreception in mosquitoes. The electrophysiological studies may help to demonstrate the contact chemoreceptive sensilla on the proboscis of *A. subalbatus*. These studies may also help to show whether *A. subalbatus* possess sensilla that respond to different stimulants tested. The present study attempts to gather experimental evidences to explain whether any difference exists between male and female chemoreception on the basis of electrical activity of labrum and labellum.

3.2. MATERIALS AND METHODS

3.2.1. PROCEDURE:

Electrophysiological recording procedure (Plate-3 and Fig-37) was modified after Anna *et al.* (1993). Both sexes of mosquitoes starved for 24 hours, to aggravate its feeding, were used for electrophysiological studies. These mosquitoes were removed from polythene bags with forceps. The mosquitoes were kept in a freezer for 2-3 minutes to make them quiescent. The legs and wings were cut off immediately after taking from the freezer. The mosquito was fixed to the metal mount using plasticine, with dorsal side up. The indifferent electrode is a golden wire of 3cm length with one end pointed and the other end connected to a miniature golden amphenol plug (No.220-PO2-100, Bunker Ramo USA) of the wire leading to the junction box. The pointed end of this electrode was inserted into the head under a microscope. The labrum or labellum of the mosquito was inserted into the recording electrode. The recording electrode is a glass micropipette containing Insect Ringer. This was made from a capillary tube by pulling it under a flame. The tip diameter of the electrode was kept as small as feasible to minimise evaporation of electrolyte solution from the electrode tip. The length of this electrode was 4cm and filled with Insect Ringer containing test solution which was kept in contact with the golden wire, and is connected to the input of the preamplifier through the junction box. Crocodile pin was clipped to the aluminium foil on the metal mount and connected to the junction box for proper earthing.

The recording of electrical activity was made using a four channel ink writing paper polygraph system with a monitor oscilloscope (Model RMP-6004, Nihon Kohden, Japan). The evoked neuronal activity picked up by the micro

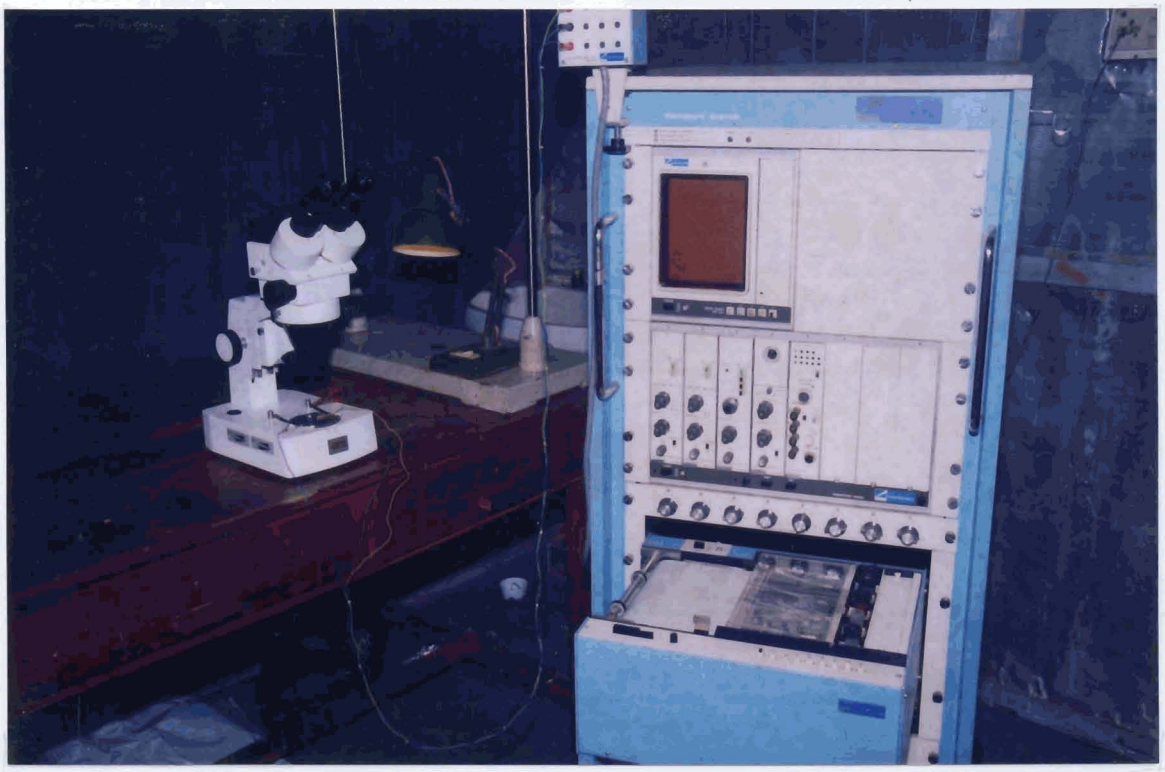

PLATE – 3. Electrophysiological recording setup.

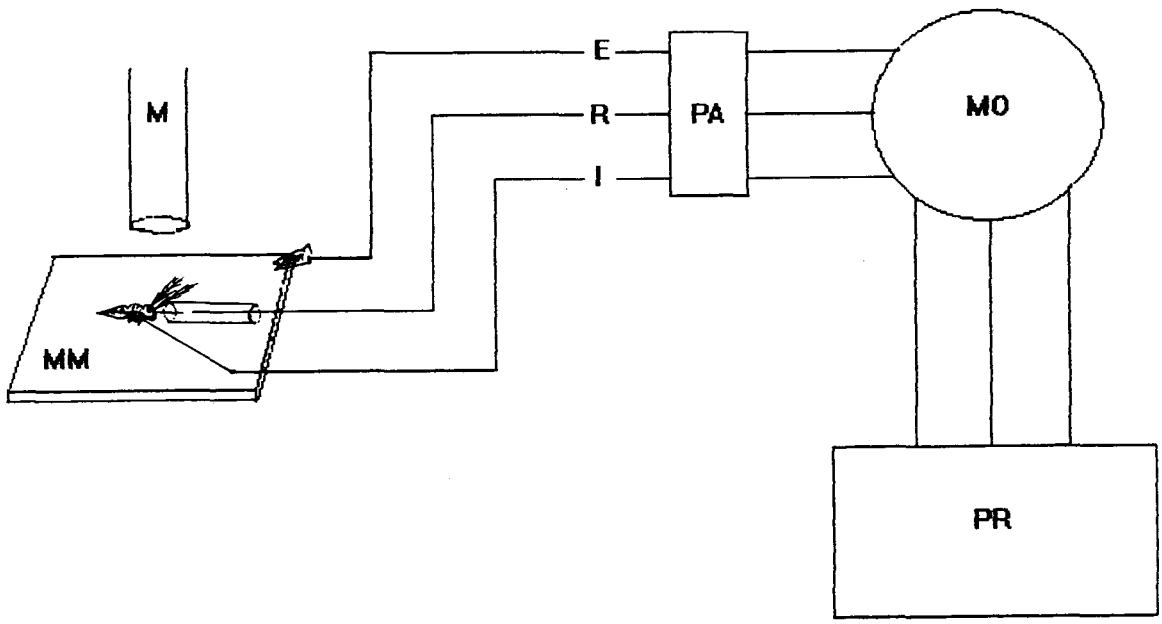

FIGURE-37. Schematic representation of recording electrical activity.

M-Microscope MM-Metal Mount E-Earth
R-Recording electrode I-Indifferent electrode
PA-Pre-amplifier MO-Monitor Oscilloscope PR-Pen recorder

electrode was amplified and displayed on oscilloscope as well as recorded on an ink writing polygraph. The experiments were carried out at room temperature during 14.00h. and 18.00h. The recordings were made at a sensitivity of 0.05mV/division, with time constant 0.3sec; amplifier high frequency was 100hz and chart speed 1mm/sec.

3.2.2. TEST SOLUTIONS

The different test solutions used were dextrose, lactic acid and acetic acid (0.5M and 1M) and Insect Ringer as control. The solutions were prepared as follows:

1) Insect Ringer: Sodium chloride 8gm.
Sodium carbonate 0.2gm.
Potassium chloride 0.2gm.
Calcium chloride 0.2gm.
Distilled water 1000ml

2) Dextrose: 0.5M and 1M dextrose solution was prepared using Insect Ringer.

3) Lactic acid: 0.5M and 1M lactic acid solution was prepared using Insect Ringer.

4) Acetic acid: 0.5M and 1M acetic acid was prepared using Insect Ringer.

3.2.3. RESPONSE TO STIMULI

Labellar response of males and labral and labellar response of females to dextrose, lactic acid and acetic acid were studied. Insect Ringer alone was taken as

control. Spike frequency difference between test and control and across sensilla pattern were analysed.

3.2.4. ANALYSIS OF ELECTRICAL ACTIVITY

Labral and labellar response of male and female mosquitoes were observed with the same stimulant to observe the time response, across sensilla pattern and total cell frequency. The electrical activity was recorded for a minimum of 10 seconds from each mosquito soon after introducing the test solution. The spikes were counted for each 2 second segment for a total period of 10 seconds. The frequency of spikes obtained was compared between test and control of each sex and between male and female. The spike amplitude obtained were categorised into three types. The highest amplitude spikes were taken as the responses of cell I, the medium amplitude spikes were considered as the response of cell II and the smaller amplitude spikes as that of cell III. Total number of spikes (CI + CII + CIII) were also counted for statistical analysis. Across sensilla pattern was analysed for a 10 second period. Prestimulus (response to Insect Ringer alone) as well as poststimulus (response to stimulant dissolved in Insect Ringer) response were compared for both the concentrations of test solutions. Intensity effect was studied from the data by comparing the difference between the spike frequency elicited by two different concentrations. Experiments were repeated six times in both control and tests.

3.3.5. STATISTICAL ANALYSIS

All the data were represented as mean $\pm$ S.D. The statistical evaluation of the results were carried out using ANOVA test.

3.3. RESULTS

3.3.1. MORPHOLOGY OF MOUTHPARTS OF *A. SUBALBATUS*

Mosquito mouthparts are adapted for the uptake of fluids, and those of the females are used both to probe flowers and to pierce skin. The terminology for the mouthparts provided by Harbach and Knight (1980) and Clements (1992) is generally followed in the present study.

As shown in Plate IV and Figure 38 proboscis arises from a snout-like projection of the cranium. Inside the proboscis are hidden the delicate mouthparts which will be described separately. On other side of the proboscis, there is the maxillary palpi, each made up of 5 segments. The maxillary palp is short and blunt in females and lengthwise it is only about one third of the proboscis. But in males, it is longer than proboscis with pointed tip.

Next to the maxillary palp on either side is the antenna, composed of 14 segments. In male mosquitoes the antennal hairs are of plumose type (long and bushy) and in female mosquitoes the antennal hairs are small and less bushy and are of pilose type. Males are easily distinguished by their conspicuous, plumose antennae which contrasts with the pilose antennae of the female.

In females, the labium which is the least modified of the mouthparts, is relatively a stout organ composed of three parts; a long trough like prementum, a pair of labella which articulate on the distal end of the prementum, and a terminal ligula. At its extreme base the prementum is flattish but elsewhere its walls curve upwards to form a trough, the premental gutter, which serves as a sheath for the stylets when they are not used in feeding. The labella are fleshy organs. Ligula is a short pointed lobe between the labella. The piercing stylets are the labrum, paired

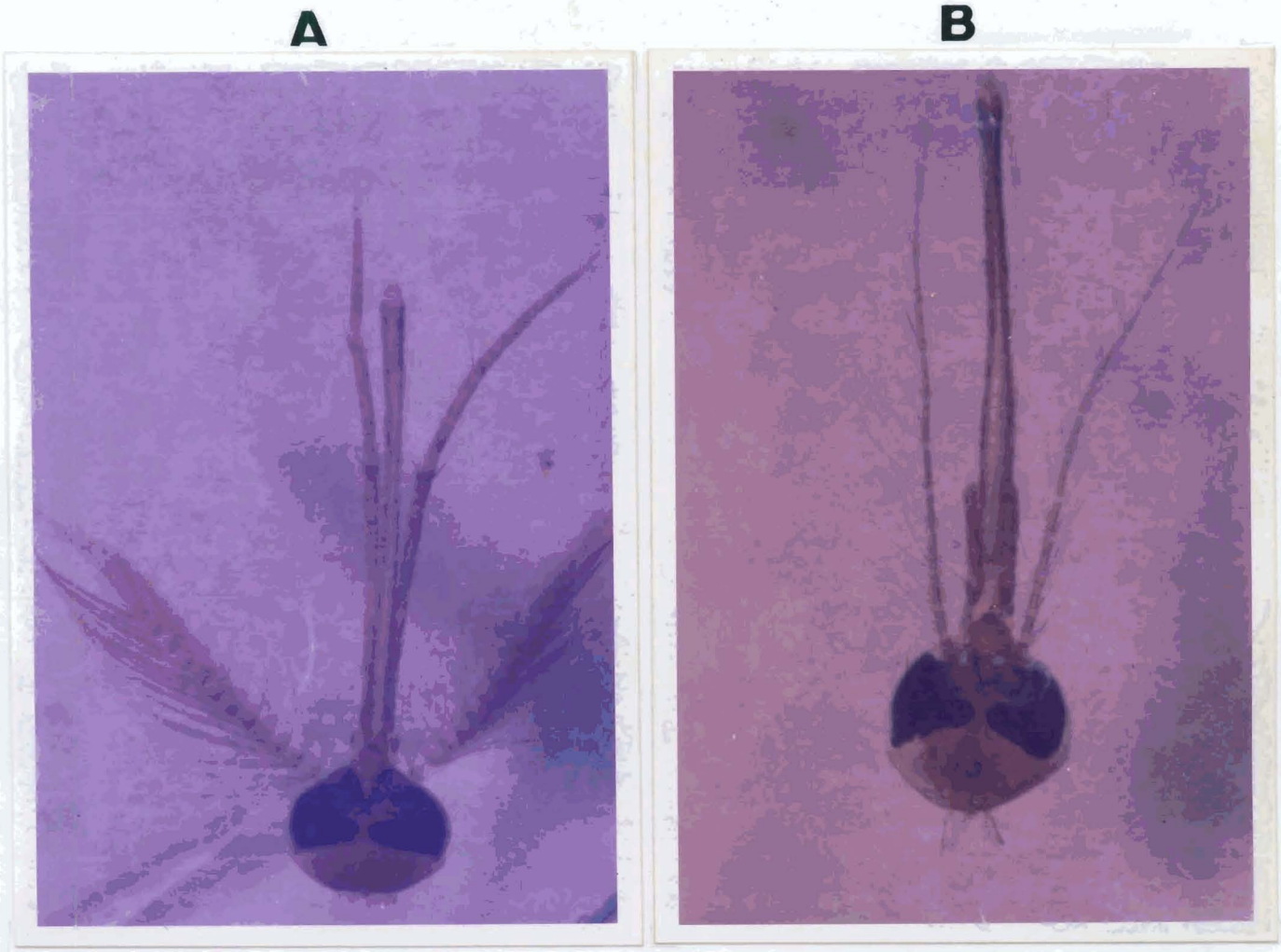

PLATE – 4. Head and mouth parts of *A. subalbatus*.

A - Male B - Female

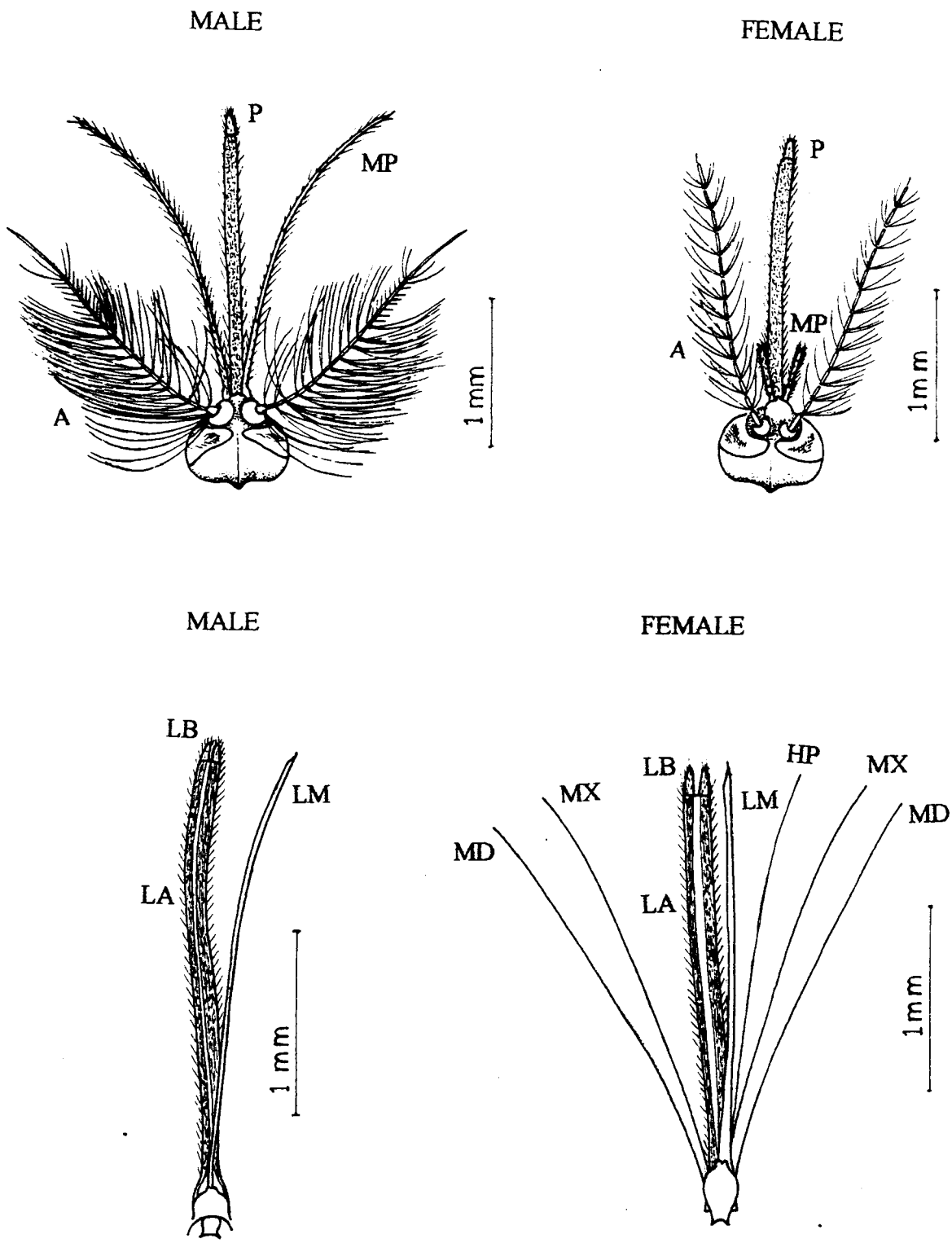

FIGURE-38. Morphology of mouth parts

A-Antenna MP-Maxillary palp P-Proboscis LA-Labium
LB-Labellum LM-Labrum MD-Mandible
MX-Maxilla HP-Hypopharynx.

mandibles and paired maxillae. In mosquitoes, the labrum is the largest, stiffest and most dorsal stylet in the fascicle and bears three pairs of sensilla. The mandibles are extremely thin, delicate stylets. At the base of the proboscis, the mandibles occupy a lateral position in the fascicle and lie above and below each other. The hypopharynx is a delicate flat unpaired stylet with a thickening along its midline which contains the salivary canal. The maxillae are the principal piercing organs of mosquitoes. The sclerotized tips of maxillary stylets are sharply tapered (Fig. 38).

The male mosquitoes probe flowers and other plant organs. Their mouthparts have the same general form as those of the female but some stylets are missing. The male labium is a strong flexible organ which resembles that of the female. The male labrum, which contains the food canal, has a forked tip. Mandibles and maxillae are absent in males. The hypopharynx which contains the salivary canal, is fused with the prementum and its tip with the ligula.

3.3.2. ELECTROPHYSIOLOGICAL RESPONSES

Electrophysiologycal responses were categorized into prestimulus and post- stimulus responses. Pre-stimulus response was the spike activity from the insect ringer and was treated as control. After recording the pre-stimulus activity, the glass micropipette with insect ringer was replaced with another pipette with insect ringer containing test solution (dextrose, lactic acid or acetic acid). The response to this was considered as post-stimulus response. The mean spike frequency per second during the 10 second period immediately preceding the application of test solution (pre stimulus period) and the mean spike frequency per second during the 10 second period following test-solution application (Post-stimulus period) were calculated for each height (cell I-100 $\pm$ 5 μv, cell II-75 $\pm$ 5 μv and cell III-50 $\pm$ 5 μv) as well as for all the heights together (total cell frequency). In the case of males only labellum showed

chemosensory activity whereas females showed both labral as well as labellar activity towards all the test-solutions. Hence, to obtain the chemosensory activity of females, cumulative response (labral response + labellar response) was also calculated and compared it to males chemosensory response (labellar response alone).

3.3.3. SALIENT FEATURES OF THE RESULTS

3.3.3.1. Male-Female differences

1. Labrum of males does not show any response towards the three test stimuli as well as control.

2. 0-24 h aged mosquitoes (both the sexes) do not show any response either to test or to control.

3.3.3.2. Effect of dextrose

Perusal of table 12 shows that

1. When dextrose was used as stimulant, three different spike heights were observed from labellum of male (Plate-5 and Fig. 39) which shows that three types of cells on the labellum were responding to dextrose (cell I, cell II and cell III). The two spike heights, i.e., two cell types of labellum (cell I and cell II) and two cell types of labrum (cell I and cell II) were responding in females towards dextrose (Plate-5 and 6 and Fig. 39).

2. Female's labrum is more sensitive to dextrose than labellum (Table 12).

3. When test (Dextrose, 0.5 M) and control (pre-stimulus) were compared, significant decrease in spike frequency was observed in cell I and cell II of male labellum. Similarly, significant decrease in spike frequency was

TABLE – 12.

Response of *A. subalbatus* towards dextrose

	Age of mosquito	Sensory organ	Stimulus	Response of **Male** (No. of spikes/second)					Response of **Female** (No. of spikes / second)				
				Cell I	Cell II	Cell III	Total cell frequency	Maximum response	Cell I	Cell II	Cell III	Total cell frequency	Maximum response
Pre-stimulus	> 24 h	Labrum	Insect ringer						1.04± 0.3	2.42± 0.67		3.46± 0.68	4th sec
		Labellum		1.26± 0.28	1.92± 0.73		3.18± 0.76	10th sec	2.28± 0.27			2.28± 0.27	6th sec
Post-stimulus	> 24 h	Labrum	0.5 M dextrose						2.18± 0.51	3.54± 0.29		5.72± 0.5	10th sec
		Labrum	1M dextrose						4.56± 1.1	6.04± 1.82		10.6± 2.38	6th sec
		Labellum	0.5 M dextrose	0.68± 0.33	1.1± 0.78	2.36± 0.95	4.14± 1.64	6th sec	1.28± 0.21	2.62± 1.94		3.9± 2	4th sec
		Labellum	1M dextrose	0.92± 0.31	1.56± 0.43	2.82± 1.01	5± 1.39	2nd sec	0.78± 0.22	3.18± 0.8		3.96± 0.6	4th sec

Blank space indicates no response

Values are mean ± S.D

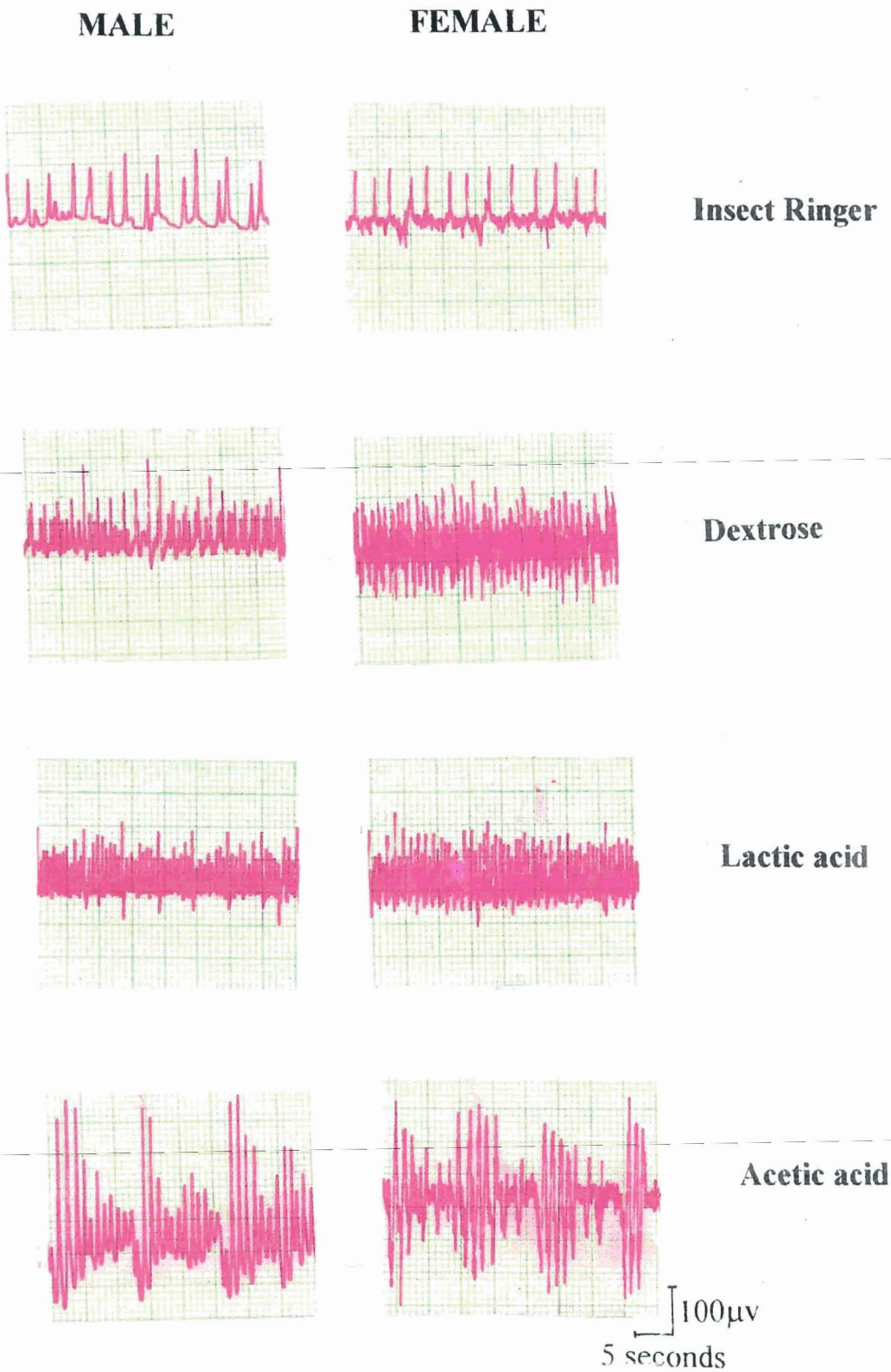

PLATE-5. Representative polygraph recordings from the labellum of *A.subalbatus* using test and control solutions.

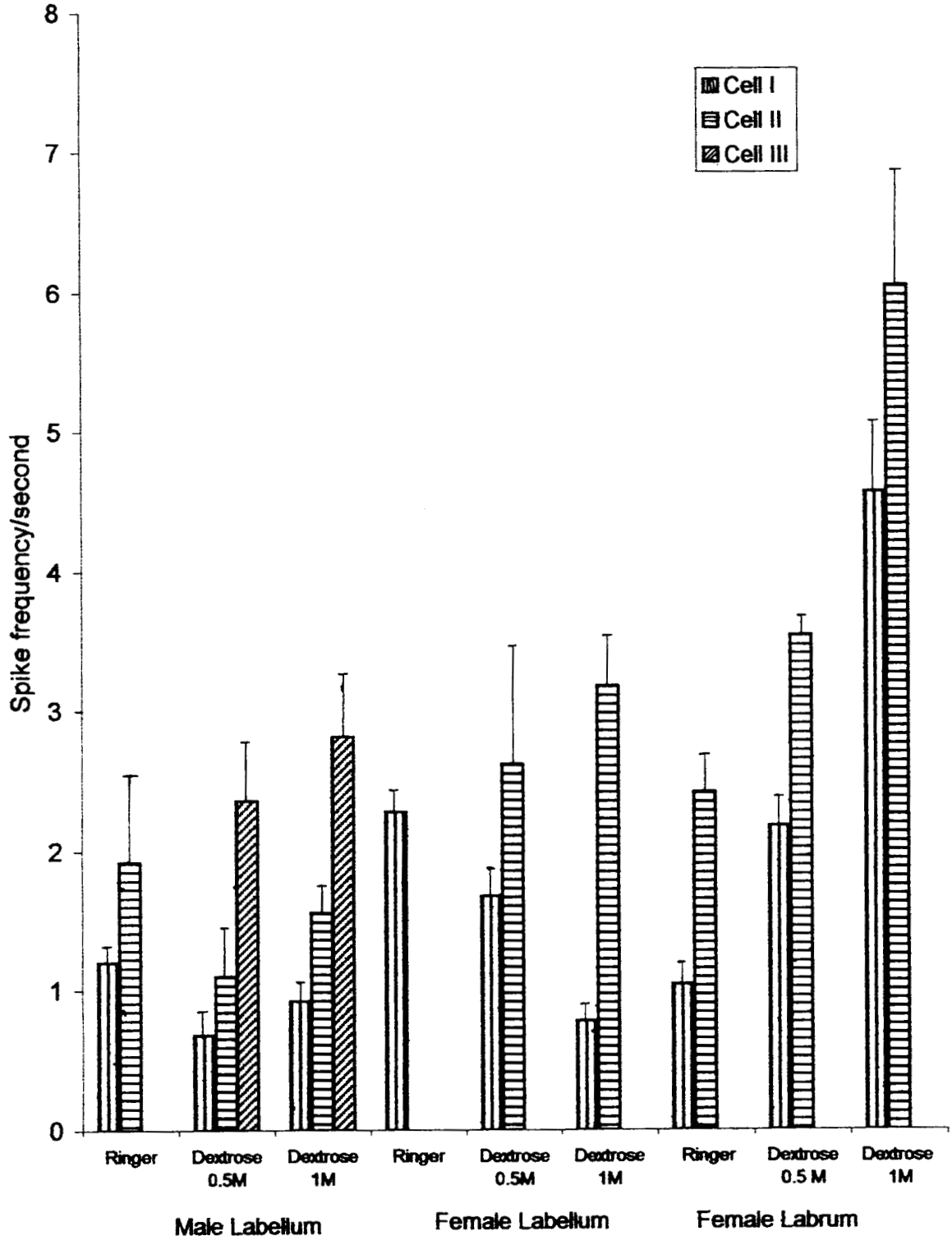

FIGURE - 39. Response of *A. subalbatus* towards Dextrose.

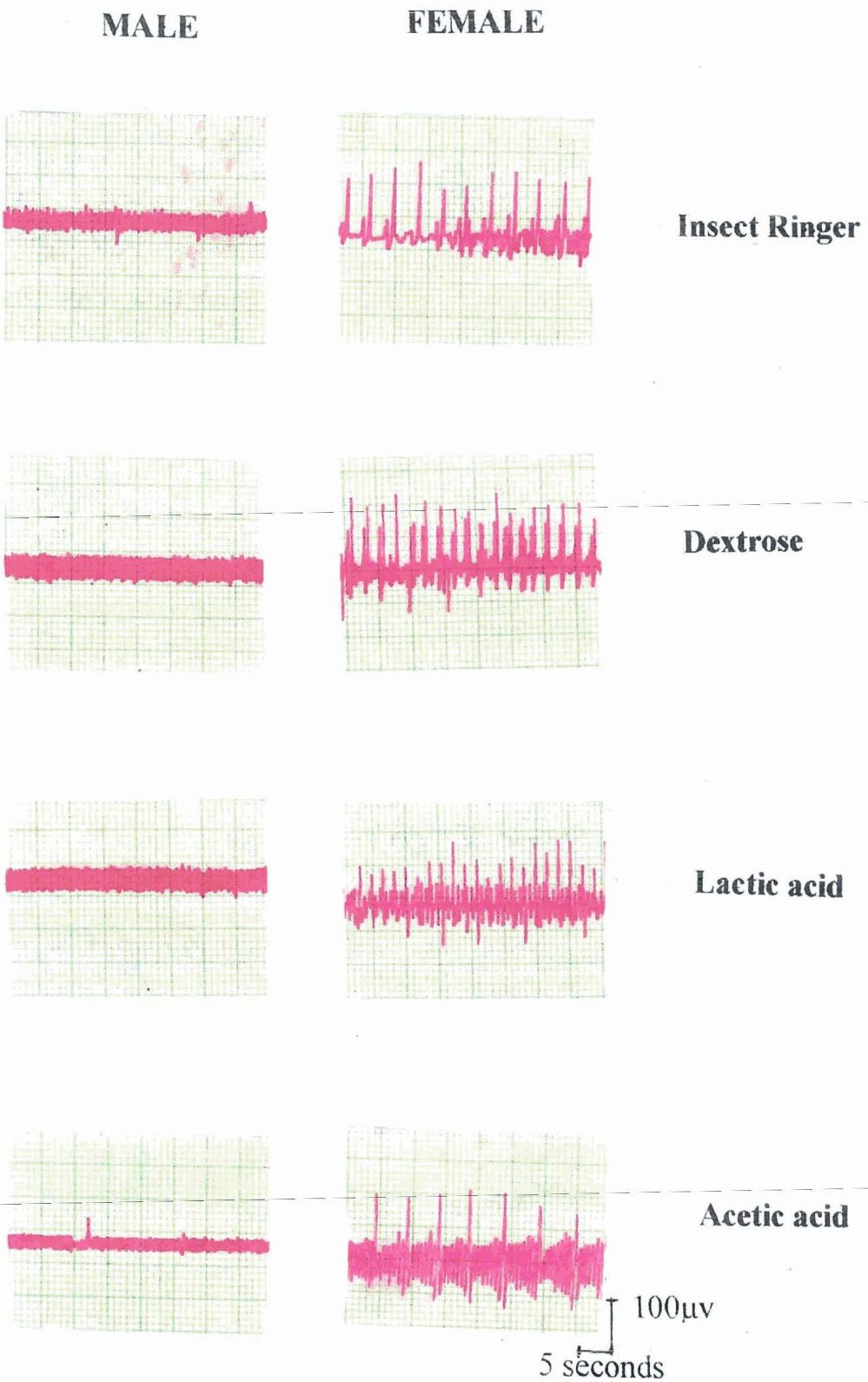

PLATE-6. Representative polygraph recordings from the labrum of *A.subalbatus* using test and control solutions.

observed in cell I of female labellum but cell II showed a significant increase in spike frequency. Significant increase in spike frequency was observed in the cell I and cell II of female labrum. Cell III does not show any response in females.

4. When males and females were compared, labrum of males does not show any response towards dextrose (Plate-6). Apart from this, in males, cell II of labellum showed significant decrease in response. But the same cell showed significant increase in respone in females.

5. When the concentration of the dextrose was raised from 0.5 M to 1 M, females and males showed significant increase in spike frequency in the cell I and cell II level. Moroever, the difference in total cell frequency was also statistically significant with increase in concentration in males and females. (Plate-7).

6. When total cell frequency was analyzed, significant increase in spike frequency was observed in the case of 0.5 M and 1 M dextrose when compared to control in male and female labellum (Fig. 40 & 41) and female labrum (Fig. 42).

7. When labellar responses between male and female were compared, males showed significant increase in response than females, for both the concentrations of dextrose. However, females showed significant increase in spike frequency when labral response of females were compared to labellar response of male (Plate-7, Fig. 40 and 42).

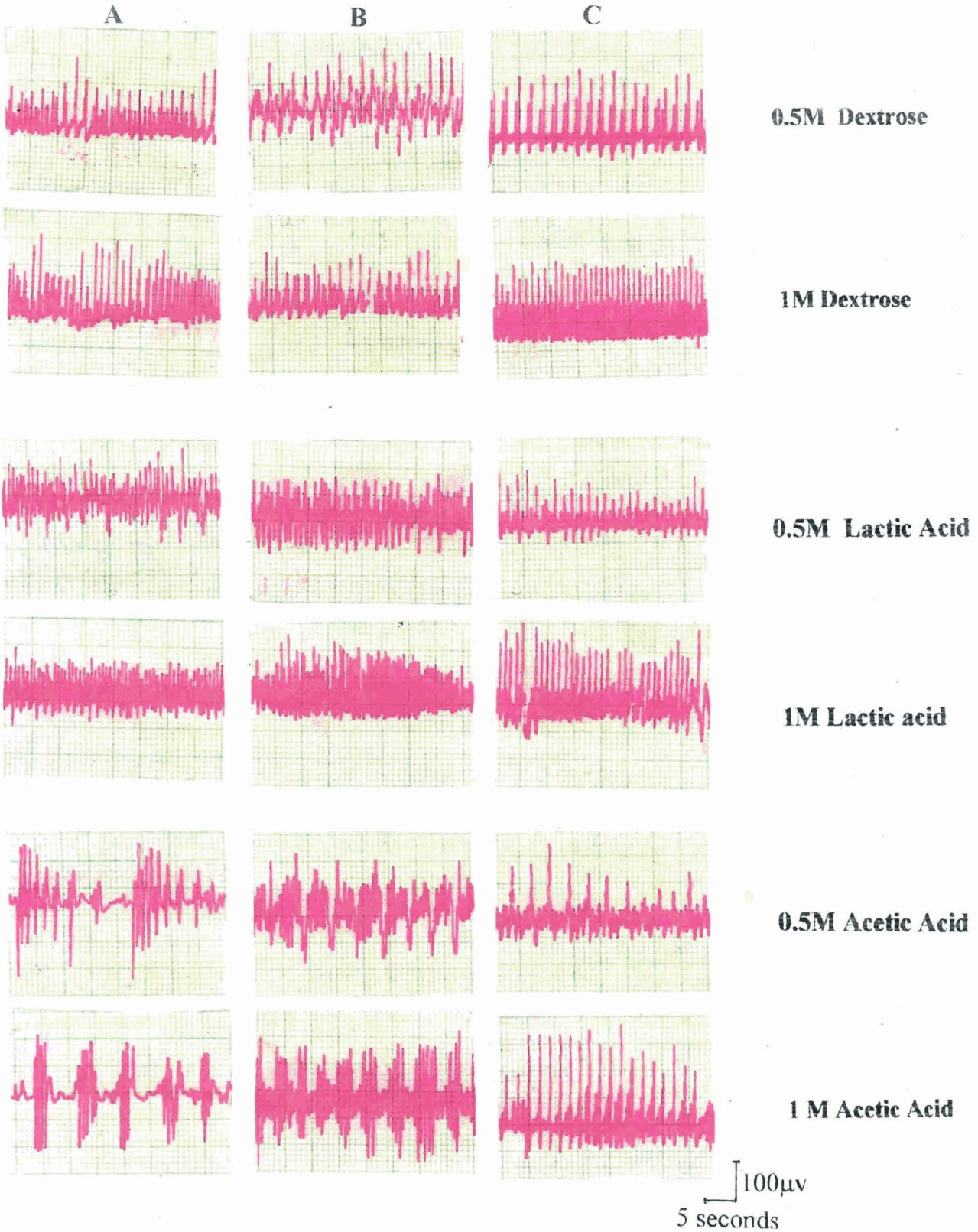

PLATE-7. Representative polygraph recordings showing concentration effect. A-Labellar response of male B-Labellar response of female C-Labral response of female

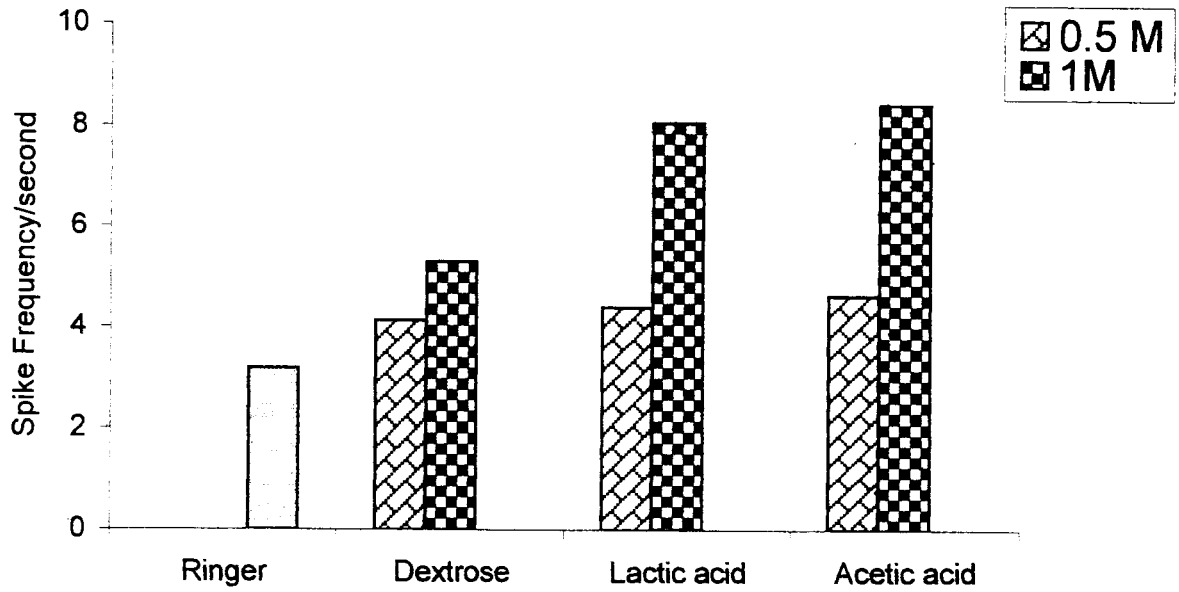

FIGURE - 40. Total cell frequency of male labellum with test (Dextrose, Lactic acid and Acetic acid) and control (Insect Ringer).

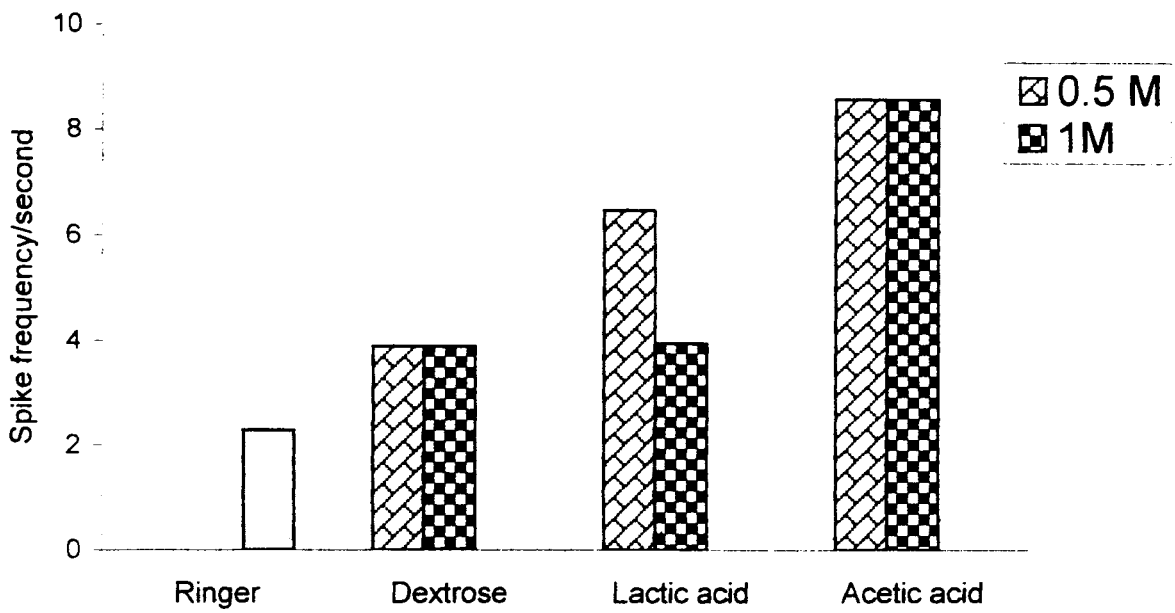

FIGURE - 41. Total cell frequency of female labellum with test (Dextrose, Lactic acid and Acetic acid) and control (Insect Ringer).

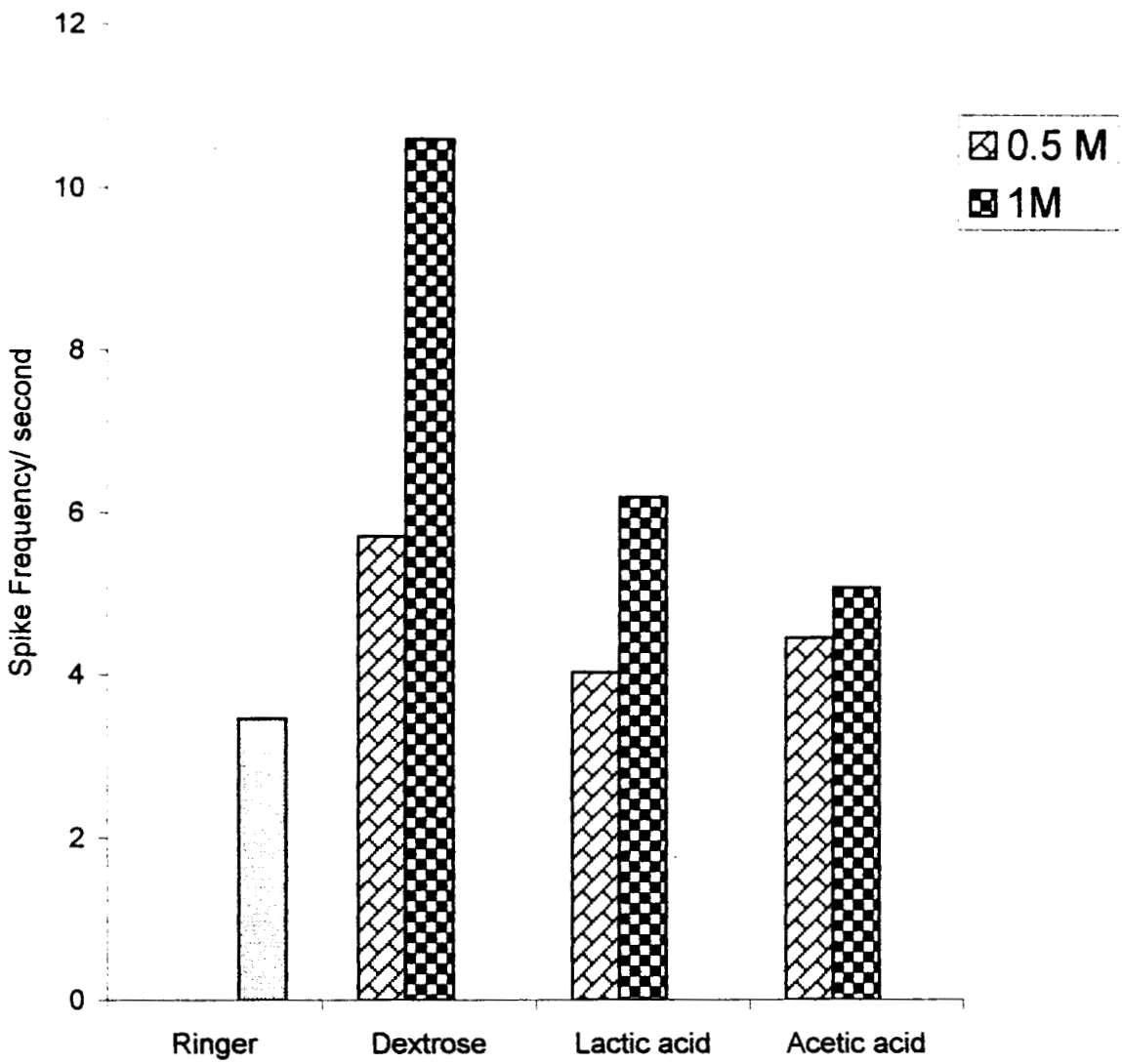

FIGURE - 42. Total cell frequency of female labrum with test (Dextrose, Lactic acid and Acetic acid) and control (Insect Ringer).

8. When labellar response of males were compared to cumulative response (labral + labellar response) of female, significant increase in spike activity was observed in females (Fig. 43).

3.3.3.3. Effect of Lactic Acid

Perusal of table 13 shows that

1. When lactic acid was used as stimulant, three different spike heights were observed from the labrum of females (Plate-6). But only two spike types were observed from the labellum of both the sexes (Plate-5). Females possess three lactic acid sensitive cell types on the labrum and two lactic acid sensitive cell types on the labellum (Plate-5 and 6). Males possess two cell types on the labellum (Fig. 44).

2. In females,labellum is more sensitive to lactic acid than labrum (Plate 7).

3. When test and control were compared, significant increase in spike frequency was observed from cell I and cell II of labellum in both the sexes. But significant decrease in spike frequency was observed from the cell I and cell II of female labrum whereas cell III showed significant increase in spike frequency (Plate 7).

4. When the concentration of lactic acid was raised from 0.5M to 1M, significant increase in spike frequency was observed in the cell I and cell II of female labrum and cell I and cell II of the labellum of both the sexes (Plate-7).

5. When total cell frequency was analysed significant increase in spike frequency was observed in the case of 0.5M and 1M lactic acid when compared to control in both the sexes (Fig. 40).

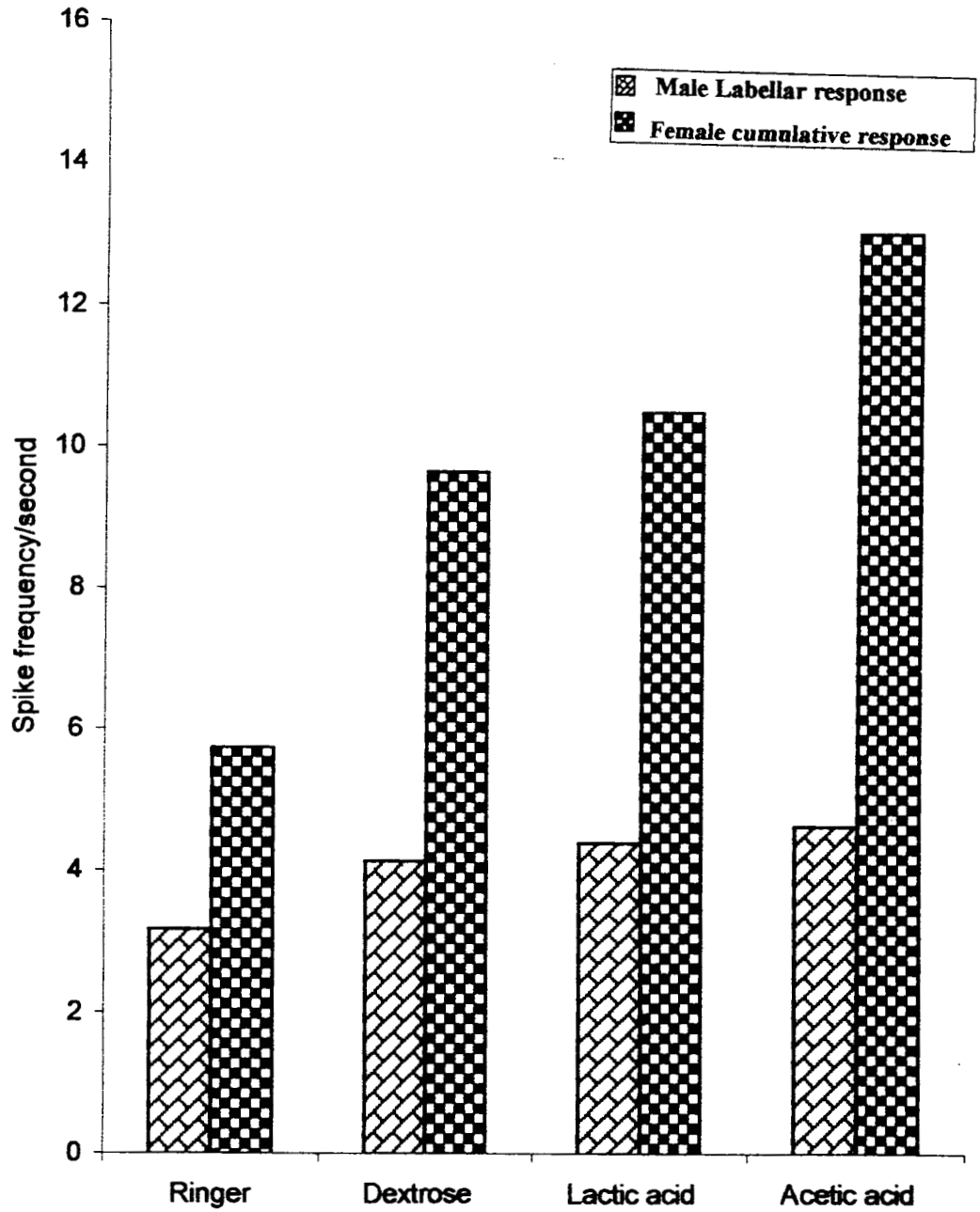

FIGURE - 43. Cumulative response(labrum + labellum) of female and labellar response of male towards test [Dextrose(0.5 M), Lactic acid (0.5 M) and Acetic acid (0.5 M)] and control (Insect Ringer).

TABLE – 13

Response of *A. subalbatus* towards lactic acid

	Age of mosquito	Sensory organ	Stimulus	Response of **Male** (No of spikes/second)					Response of **Female** (No of spikes / second)				
				Cell I	Cell II	Cell III	Total cell frequency	Maximum response	Cell I	Cell II	Cell III	Total cell frequency	Maximum response
Pre-stimulus	> 48 hr	Labrum	Insect ringer						1.04± 0.3	2.42± 0.67		3.46± 0.68	4th sec
		Labellum		1.26± 0.28	1.92± 0.73		3.18± 0.76	6th sec	2.28± 0.27			2.28± 0.27	6th sec
Post-stimulus	> 48 hr	Labrum	0.5M lactic acid						0.84± 0.28	1.42± 0.22	1.74± 0.73	4 ± 1.04	2nd sec
		Labrum	1M lactic acid						1.32± 0.16	1.94± 0.86	2.94± 0.7	6.2± 1.44	6th sec
		Labellum	0.5M lactic acid	2.12± 0.68	2.28± 0.72		4.4± 1.2	8th sec	2.44± 0.19	4.04± 0.49		6.48± 0.49	4th sec
		Labellum	1M lactic acid	4.04± 0.61	4.04± 0.72		8.08± 1.27	4th sec	3.18± 0.75	0.78± 0.22		3.96± 0.6	4th sec

Blank space indicates no response

Values are mean ± S.D

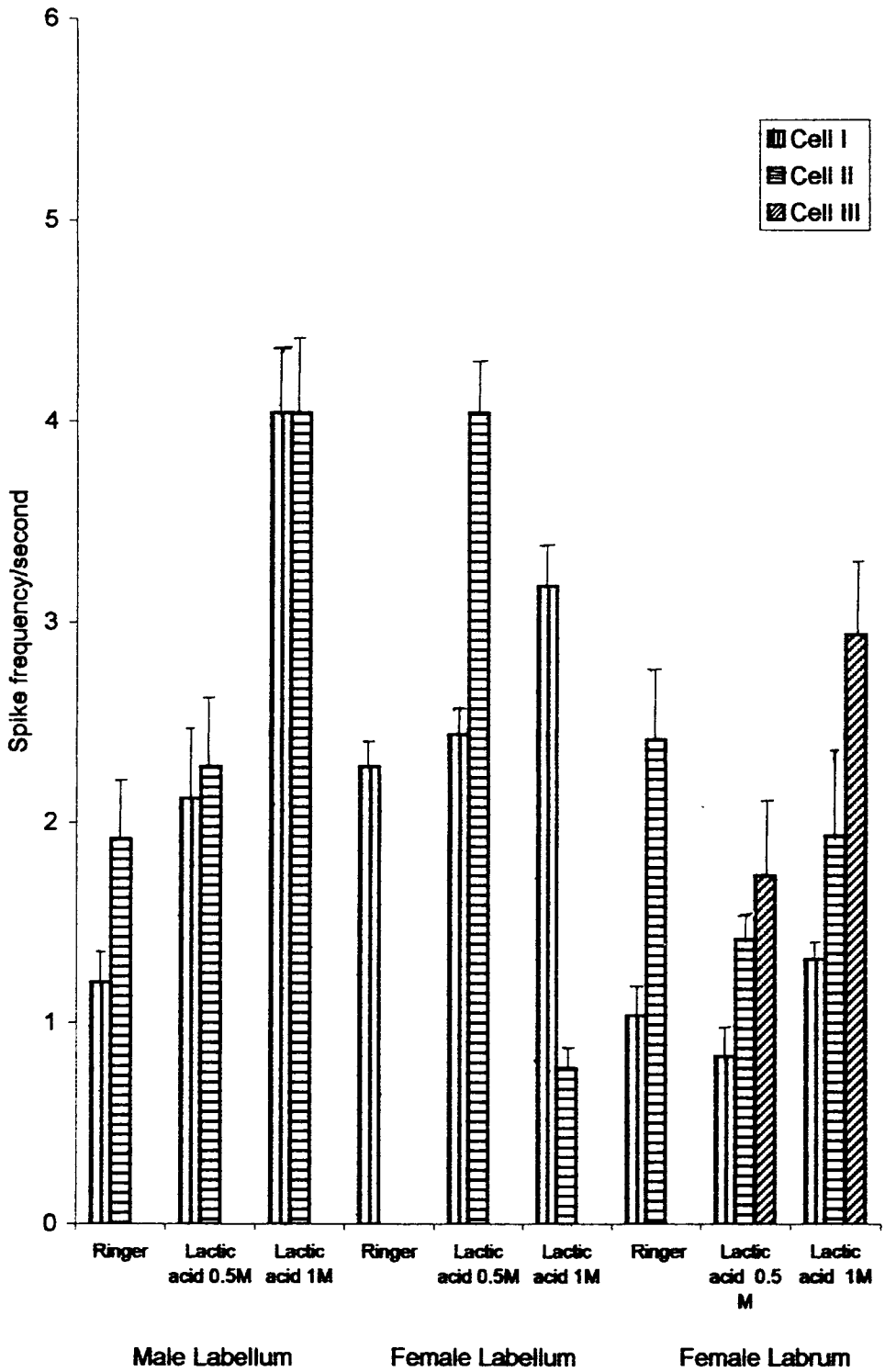

FIGURE - 44. Response of *A. subalbatus* towards Lactic acid.

6. When labellar response between male and female were compared, females showed significant increase in toal cell frequency, as far as 0.5M lactic acid was concerned. But in the case of 1M lactic acid males showed significant increase in spike frequency than females (Fig. 40 & 41).

7. When labellar response of males were compared to cumulative response (labral response + labellar response) of female, significant increase in spike activity was observed in females (Fig. 43).

8. When 0.5M and 1M lactic acid were compared, increase in response was observed in the case of cell II of male labellum, cell I, cell II and cell III of female labrum (Fig. 44).

3.3.3.4. Effect of Acetic Acid

Perusal of table 14 shows that

1. When the acetic acid was used as stimulant three different spike types were observed from the labellum of males (Plate-5) but only two spike types were observed from the labrum and labellum of females (Plate-5 and 6). This indicates that males possess three types of acetic acid sensitive cells on the labellum whereas females possess two types of acetic acid sensitive cells on the labrum as well as labellum (Fig. 45).

2. In females labellum is more sensitive to acetic acid than labrum (Plate 7).

3. When 0.5M acetic acid and control were compared, significant decrease in spike frequency was observed from cell I and cell II of male labellum. Significant increase in spike frequency was observed from cell I and cell II of female labrum and from the cell III of male labellum (Fig. 45). When 1M acetic acid and control were compared, significant increase in frequency was

TABLE – 14

Response of *A. subalbatus* towards acetic acid

	Age of mosquito	Sensory organ	Stimulus	Response of **Male** (No.of spikes/second)					Response of **Female** (No.of spikes / second)				
				Cell I	Cell II	Cell III	Total cell frequency	Maximum response	Cell I	Cell II	Cell III	Total cell frequency	Maximum response
Pre-stimulus	> 48 hr	Labrum	Insect ringer						1.04± 0.3	2.42± 0.67		3.46± 0.68	4th sec
		Labellum		1.26 ± 0.28	1.92± 0.73		3.18± 0.76	6th sec	2.28± 0.27			2.28± 0.27	6th sec
Post-stimulus	> 48 hr	Labrum	0.5 M acetic acid						1.5± 0.2	2.96± 1.11		4.46± 1.02	2nd sec
		Labrum	1 M acetic acid						1.12± 0.66	3.96± 1.46		5.08± 1.41	4th sec
		Labellum	0.5 M acetic acid	0.66± 0.11	1.32± 0.43	2.66± 0.58	4.64± 0.87	2nd sec	3.4± 0.54	5.2± 1.3		8.6± 1.67	2nd sec
		Labellum	1 M acetic acid	1.1± 0.41	2.08± 0.59	5.28± 1.03	8.46± 1.16	6th sec	2.8± 0.44	5.8± 2.48		8.6± 2.5	4th sec

Blank space indicates no response

Values are mean ± S.D

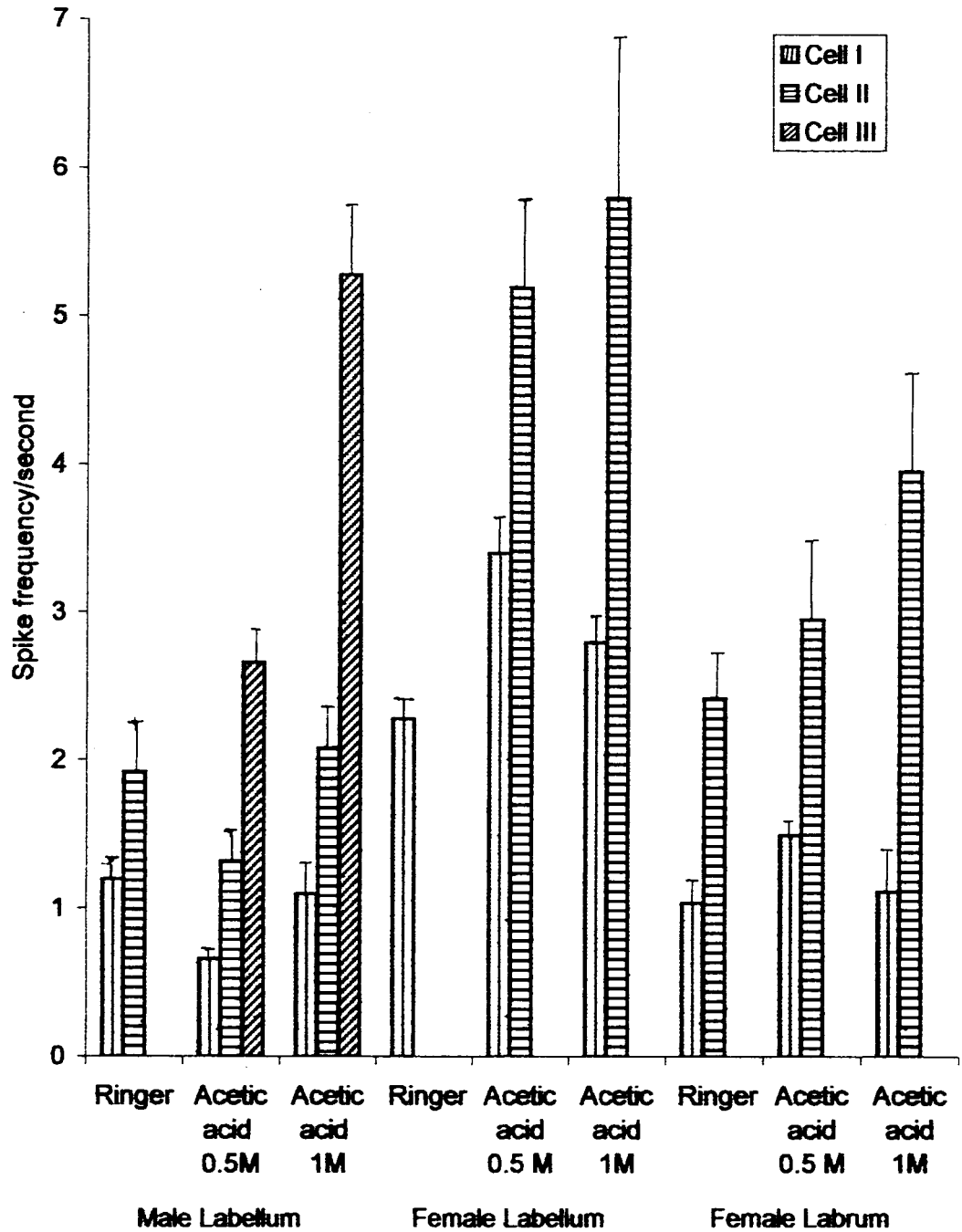

FIGURE - 45. Response of *A. subalbatus* towards Acetic acid.

observed from the cell II of female labrum and cell III of male labellum (Fig. 45).

4. When the concentration of acetic acid was raised from 0.5M to 1M, significant increase in spike frequency was observed from the cell I, cell II and cell III of males. In females significant increase in response was observed in the cell II (Plate-7 and Fig. 45).

5. When total cell frequency was analysed, significant increase in spike frequency was observed in the case of 0.5M and 1M acetic acid, when compared to control in both the sexes (Fig. 40, 41 and 42).

6. When labellar response between male and female were compared, females showed significant increase in spike frequency for both the concentrations of acetic acid.

7. When labellar response of males were compared to cumulative response of female (labrum + labellum), significant increase in spike activity was observed in females (Fig. 43).

8. When 0.5M and 1M acetic acid were compared, significant increase in response was observed only from the cell III of male labellum (Fig. 45).

3.3.4. ACROSS SENSILLA PATTERN

1. The pattern for each chemical used is different when the responses from cell I, II and III were plotted together over pre and post stimulus periods.

2. The across sensilla pattern seems to convey the quality message of each chemical.

3. More interestingly, between male and female mosquitoes the across sensilla pattern for the same chemical appears to be different.

4. In the case of dextrose, the pattern of cell III activity is dominant over the cell I and cell II of male labellum. In the case of female labellum and labrum, the across sensilla pattern shows that cell II is dominent over cell I and nil activity of cell III (Fig. 46 and 47).

5. In the case of lactic acid, cell II is dominant over cell I in male and female labellum and in the case of female labrum cell III is dominant over cell I and cell II (Fig. 48 and 49).

6. In the case of acetic acid, the pattern of cell III activity is dominant over the cell I and cell II in the case of male labellum. However, in the case of female labellum and labrum, the across sensilla pattern shows that cell II is dominant over the cell I and nil acivity in cell III (Fig. 50 and 51).

Details of the statistical evaluation of the results are given in Appendix- II.

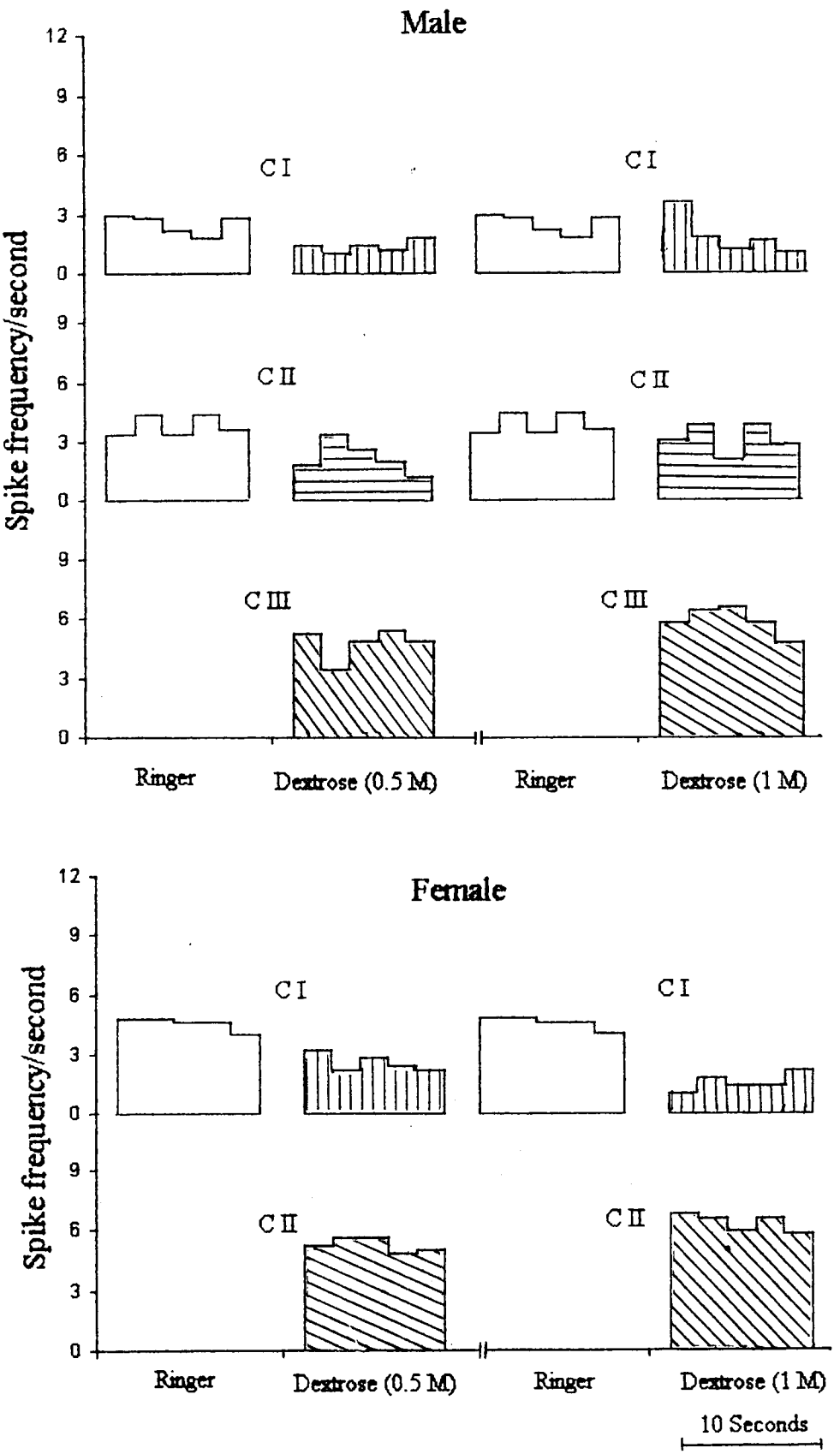

FIGURE- 46. Across sensilla pattern for dextrose from male and female labellum.

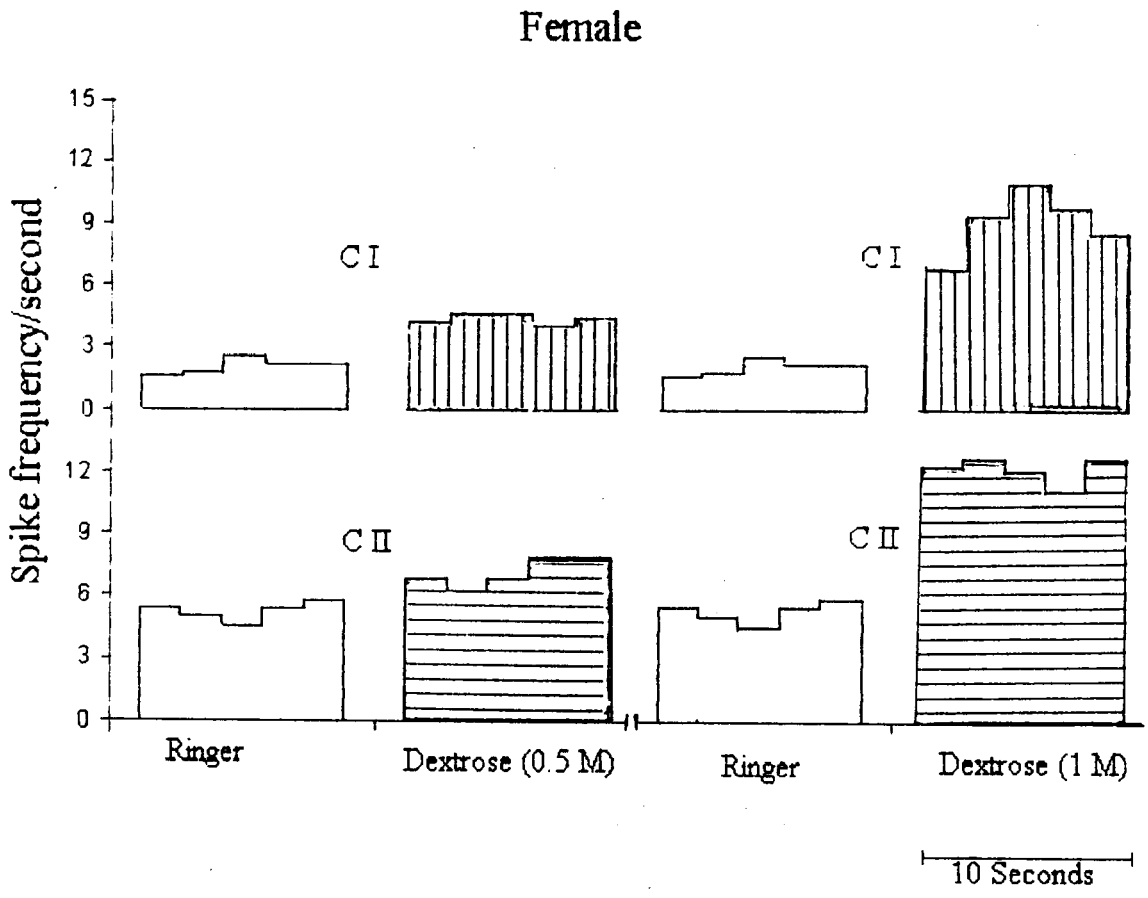

FIGURE- 47. Across sensilla pattern for dextrose from female labrum.

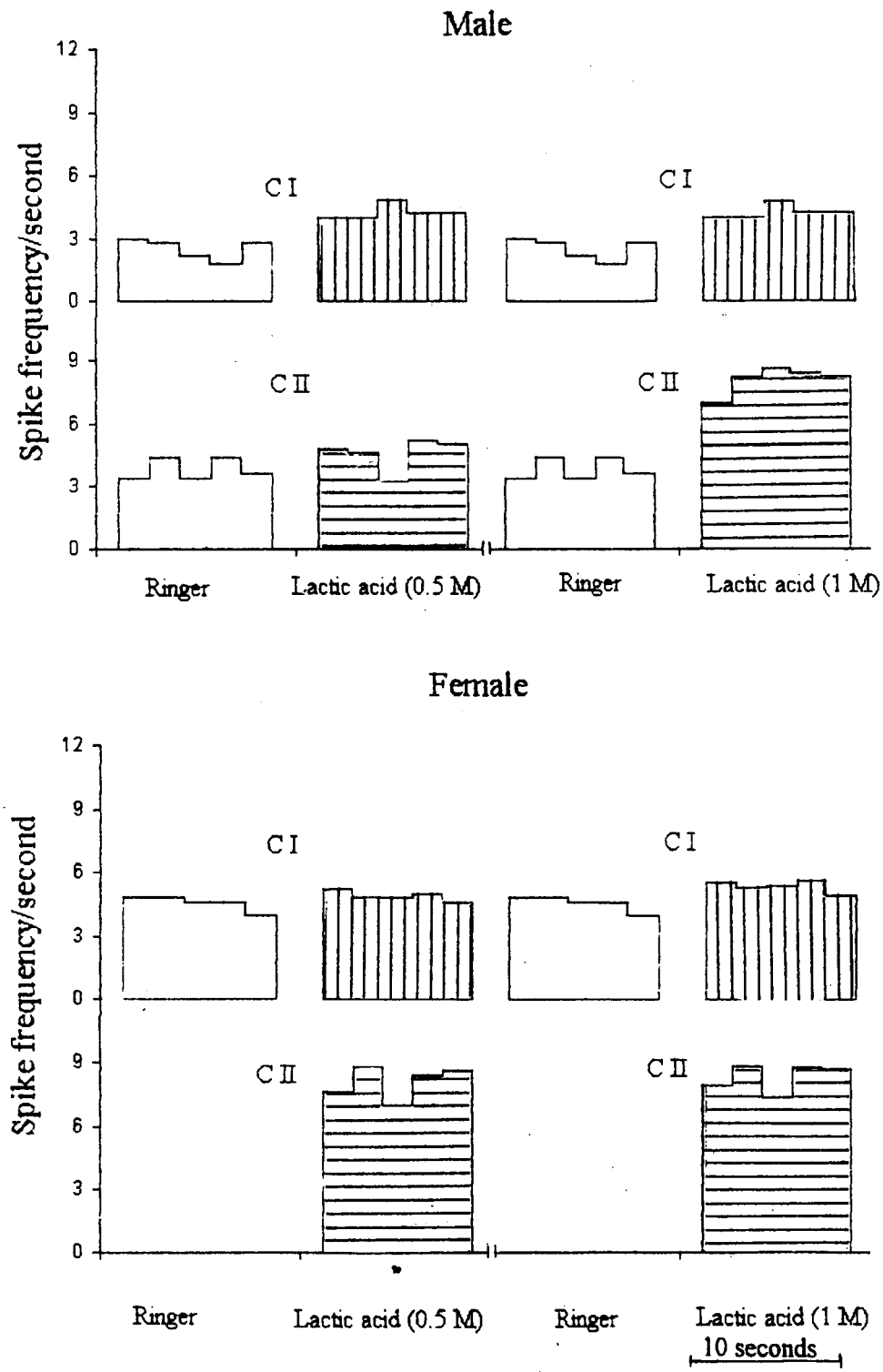

FIGURE- 48. Across sensilla pattern for lactic acid from male and female labellum.

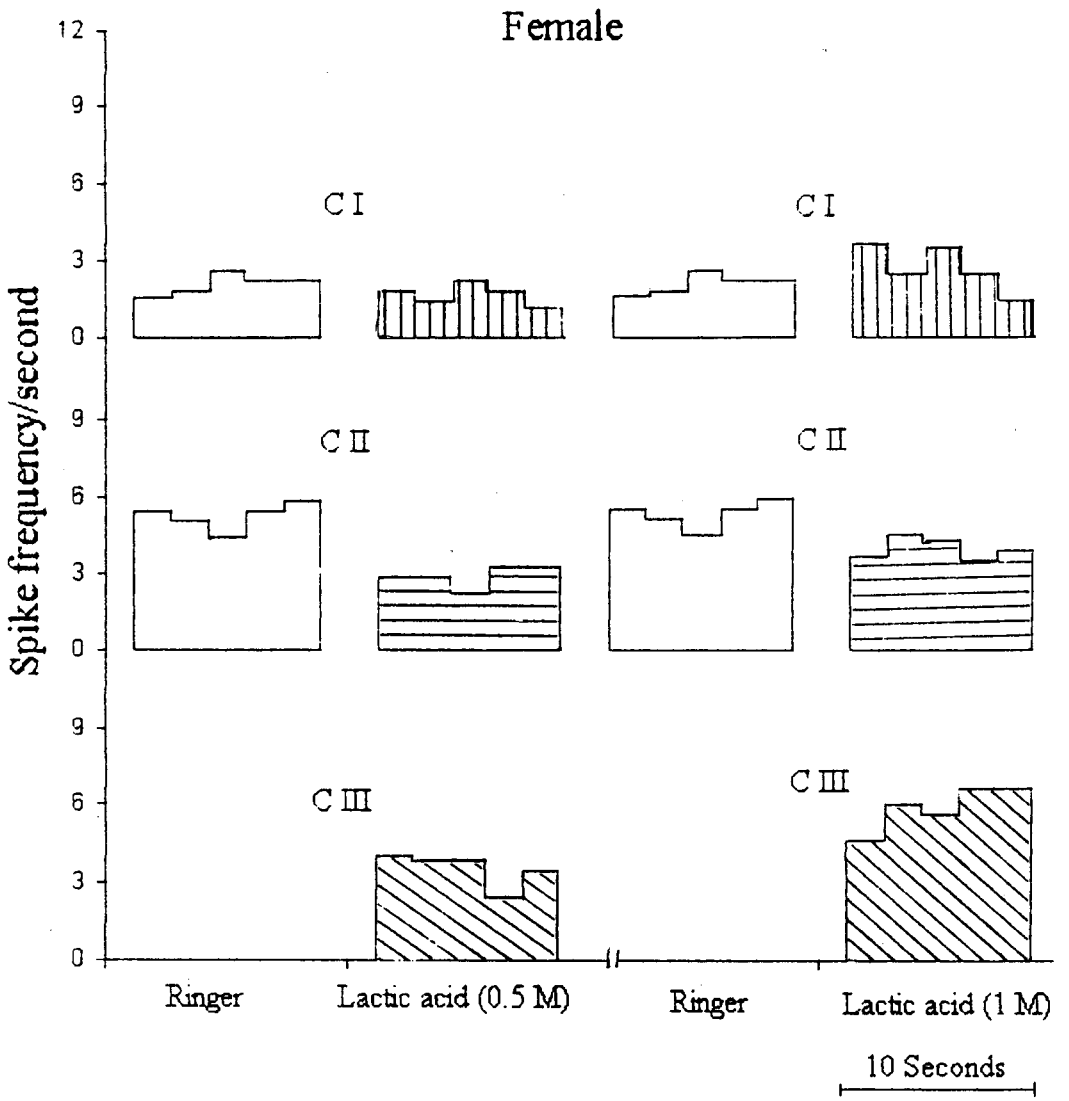

FIGURE- 49. Across sensilla pattern for lactic acid from female labrum.

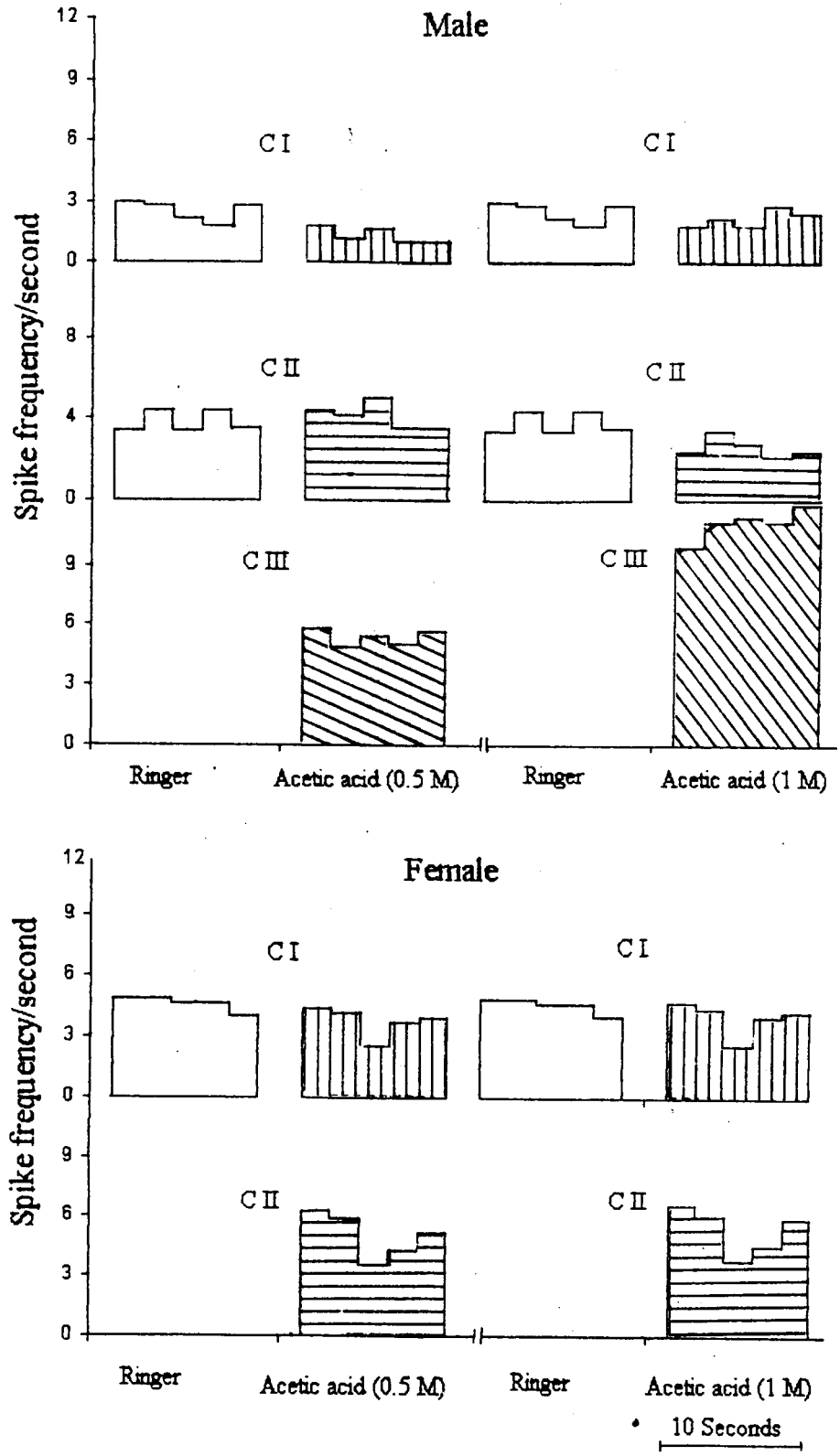

FIGURE- 50. Across sensilla pattern for acetic acid from male and female labellum.

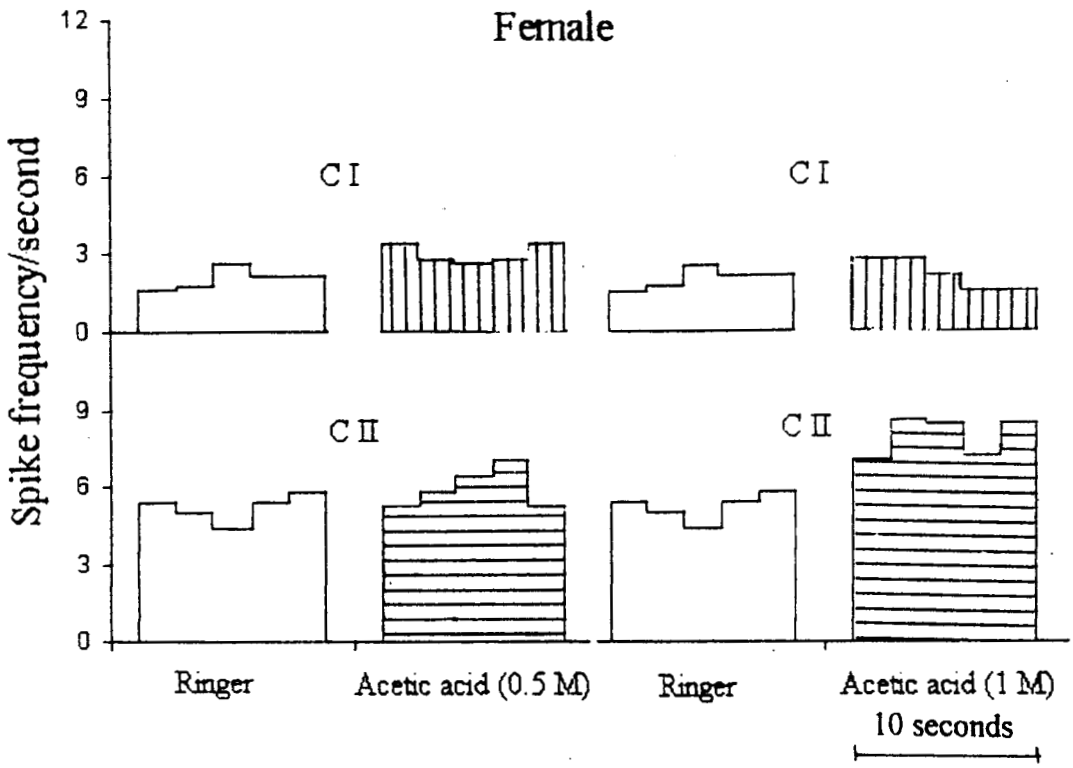

FIGURE- 51. Across sensilla pattern for acetic acid from female labrum.

3.4. DISCUSSION

3.4.1. MORPHOLOGY

Electrical activities produced by various chemosensory cells can be used as a measure of the functions of chemoreceptor systems. In females, labrum bears three pairs of sensilla; a pair of apical sensilla, a pair of subapical sensilla and a pair of companiform sensilla (Lee and Craig, 1983b). The apical sensilla located at the tip of the labrum of the female mosquito are innervated by five neurones each. The subapical sensilla are found a short distance behind the apical sensilla. They too are innervated by five neurones each (Lee, 1974). On the basis of their structure both the apical (Cell I) and subapical sensilla (Cell II) can be classified as contact chemosensilla (Anna *et al.*, 1993). In the present work, electrophysiological recordings from the labrum of females showed three spike heights (Cell I - Apical Sensilla, Cell II - Subapical Sensilla, Cell III - Companiform Sensilla), which responds to the stimulus tested and thus gives strong support to Lee's argument. Dethier (1955) and Hudgson (1957) reported that action potentials of different sizes can be recorded if the hair is stimulated mechanically or if the receptor site is stimulated with distilled water or with sugar, salt or protein solutions.

3.4.2. ELECTRO PHYSIOLOGY

The present study describes electrophysiological characteristics of contact chemoreceptors in the labrum and labellum of female and labellum of male *A. subalbatus*, that are sensitive to lactic acid, dextrose and acetic acid. Three types of receptor cells were observed as is shown in Table 13, 15 and 16. One type (Cell I) was sensitive to all the stimuli tested but was of relatively low sensitivity and low spontaneous activity. The second type (Cell II) also was more broadly tuned and

was characterized by a relatively high spontaneous firing rate. The third type (Cell III) was highly specific which was not sensitive to some of the stimuli tested in both the sexes. This cell type was relatively moderate in their firing activity. All the cells responded by increasing their rate of firing in response to stimulus presentation. Inhibited cells were observed in some cases when compared to control (Insect Ringer). Similar results were obtained (Bowen, 1992 and Anna *et al.*, 1993) in the electrophysiological studies of receptor neurones on the antennae of females of *Culex pipiens* that are sensitive to terpenes.

3.4.3. RESPONSE TOWARDS DEXTRSE, LACTIC ACID AND ACETIC ACID

Ranking potencies of the stimulus for males and females follow the order. Dextrose > Lactic acid > Acetic acid. Dextrose being the most stimulatory at the entire range tested. Lactic acid was less effective than dextrose. Acetic acid showed burst activities on stimulation in females. However, as far as olfactory response is concerned, females showed maximum response to lactic acid (Smith *et al.*, 1970). Thus the host attractant, lactic acid becomes most active stimulus as far as female antennal olfactory receptors were concerned. However, as far as contact chemoreceptors are concerned, it may be most sensitive to food substances than attractants. Here, this may be the reason why females showed maximum response to dextrose than lactic acid in the present study.

The present results are in agreement with the observations of Clements (1992) that mosquitoes possess chemosensilla on the labrum to identify the food substances and bear sugar sensitive sensilla. He also observed that afferent nerve impulses were generated when the labral chemosensilla in *Culiseta inornata* were stimulated with sucrose solution. Salama (1966) observed that most pentoses, hexoses, di and tri-saccharides are stimulatory in *Aedes aegypti*.

The electrophysiological studies indicate that sensilla on the labrum in *A. subalbatus* contain chemoreceptors that respond to the three tested stimuli. The records obtained from those chemoreceptors in response to insect ringer showed spikes of only two types. Anna *et al.* (1993) also observed spikes of two types in response to saline and nucleotides from the apical setiform sensilla on the labrum of *C. pipiens*. However, in the present study spikes of three types were obtained with the test stimulus and spikes of two types were obtained with Insect Ringer. All the cells responded generally by increasing their rate of firing in response to stimulus presentation. Inhibited cells were observed in some cases. When dextrose was given as test stimulant, the spike frequency of cell I increased significantly in females. In contrast to this, Cell I of males showed significant decrease in spike frequency, when stimulated with dextrose. Similar results were obtained in the case of Cell II of both the sexes. But in the cases of Cell III, females were not responding to dextrose where as males showed response to dextrose. The results show that dextrose has an inhibitory effect on the activity of Cell I and Cell II in males. However, dextrose evoked stimulatory effect on Cell III of males. In contrast to this, Cell I and Cell II of females showed excitatory effect on dextrose stimulation. From the above mentioned results, it is evident that the contact chemosensory cells respond differently in males and females to dextrose. It is noteworthy that dextrose is a major energy source in both male and female mosquitoes.

When lactic acid is used as test stimulant, Cell I of males in response to both the concentrations were excited. But in females, Cell I was excited only at high concentration (1M). In the case of Cell II, lactic acid was excitatory only at 1M concentration in males. In females 0.5M lactic acid was inhibitory in the Cell II level and no significant change in response to higher concentration in the Cell III level occured. In females, both concentrations showed excitatory effect. The

results show that Cell III of females is specific for lactic acid. This may be because lactic acid acts as a host attractant for female mosquitoes (Smith *et al.*, 1970). The present results also showed a significant response to lactic acid by Cell I and Cell II of males, eventhough males were not expected to be attracted by host animals since male mosquitoes are non-blood feeders. However, this response may be because male mosquitoes may need to be attracted to the places where female mosquitoes are likely to be seen, for mating. This contention is in agreement with Davis (1977), who observed that male mosquitoes have antennal sensilla sensitive to certain host related chemical stimuli like lactic acid. He suggested that host related stimuli act as a long distance mate location signal.

It was also found that Cell III of female was sensitive to lactic acid where as Cell III of male was not sensitive to it. This may be because, the presence of high sensitivity host attractant receptors is a requirement for the expression of host seeking behaviour and that the responsiveness of these receptors is modulated throughout the life cycle in such a way as to determine the behavioural mode of the female (Bowen, 1991). Apart from the difference in Cell III response, there was not much difference in spike activity observed between male and female to lactic acid as a stimulant. Host responsiveness in mosquitoes is closely correlated with the state of peripheral sensory system (Davis, 1984a, 1984b; Bowen *et al.*, 1988). Actively host seeking females posses highly sensitive lactic acid receptors where as non-host seeking females are characterized by receptors of low sensitivity (Davis and Takahashi, 1980).

When acetic acid is used as test stimulant, cell I of males in response to both the concentrations were inhibited when compared to the control. But in females cell I of labrum as well as labellum was excitatory when compared to control. In the case of cell II, males showed inhibition with 0.5 M acetic acid however, with 1 M

acetic acid cell II of males was excited when compared to control. In the case of cell II of females, labrum and labellum showed excitation with both the concentrations of acetic acid. Similar results were observed in males and females with their total cell frequency which was also excitatory. Moreover, the result showed that cell III responds to acetic acid only in males. The unique feature observed with acetic acid was the burst activity through out the period of recording which was not observed with the other two tested stimuli. That may be the reason why repellents were effective not only on the onset of repellent application but through out the period. But for this sustained activity, these chemicals will not act as repellents. Bowen (1992) reported that acetic acid was excitatory with antennal receptors of female *Culex pipiens*. In the present study, all the cells were excited when stimulated with acetic acid except cell I and cell II of male. In male *Aedes aegypti*, acetic acid showed inhibitory response from antennal receptors (Davis 1976). The present study showed that the contact chemoreceptors have both excitatory (cell III of male and all the cells of female) as well as inhibitory (cell I and cell II of male) effect with insect repellents.

3.4.1. QUALITY MESSAGE - ACROSS SENSILLA PATTERN

Earlier studies on electrophysiology were very few and focused mostly on electrophysiological correlates of either the host-seeking behaviour of female/mate locating behaviour of male. As far as is known, the present study is the first of its kind to investigate the electrophysiology of contact chemoreceptors on the labrum as well as labellum of the female and the labellum of the male mosquito, *A. subalbatus* in response to different chemical substances in order to understand the neuronal code of quality message perse in both male and female of the same species.

It is known in vertebrates that there is 'across-neuron pattern' to signal the quality of chemicals. 'Across neuron pattern' conveying the quality message of glucose, amino acid and salts on the gastric chemoreceptive neurons in frog (Ramakrishna and Sharma, 1975) and taste quality information in the neurons of olfactory bulb and the nucleus solitarius in the brain stem of rat (Doetsch and Erickson, 1970) had been studied. However, no such studies regarding 'across neuron' or 'across sensilla' pattern has been reported in invertebrates. The present study showed the across sensilla pattern of the mosquitoes towards dextrose, lactic acid and acetic acid. It seems to be the first report about the across sensilla pattern among insects in particular and in invertebrates in general.

The relative frequency among the neurons seems to be a function of the quality of test solution. The responses to dextross, lactic acid and acetic acid are different from each other and have relatively specific features. In dextrose, spikes of different heights are more or less uniformly active and continue to fire for relatively longer durations. Almost similar results were obtained for lactic acid also, however, the duration of activity was somewhat lesser than that of dextrose. In the case of acetic acid, intermittent burst activity was observed throughout the period eventhough the pattern of activity was the same as that of other tested stimuli i.e., all spike heights were observed in this case also but could be easily distinguished from the response to other test solutions. For example, in the case of dextrose, cell III is predominent in males, but for females, cell I is predominent with labral response. For lactic acid, Cell II is prodominent in males but cell III showed predominance in the case of female labral response. In acetic acid, males showed predominance in the Cell II level. In contrast to this females (labral) showed predominance in the Cell I level.

As far as the duration of response is concerned, dextrose appears to be similar to lactic acid. Neurons seem to be active for relatively longer durations under the influence of dextrose than those involved in signalling lactic acid. The signalling is of still lesser duration in the case of acetic acid. Thus, each test solution, though specific and different from others, excites many cells to varying degrees, producing a pattern of relative frequency of activity across these cells. So the present study projects the across sensilla pattern towards dextrose, lactic acid and acetic acid in mosquitoes. It is assumed that such across sensilla pattern may exist in other insects as well as invertebrates also. It is worthwhile to undertake similar investigations in other species of invertebrates.

CHAPTER 4

Summary and Conclusions

1) The mosquito, *Armigerus subalbatus* was selected for the present study because, it is the largest and the most common mosquito of Kerala.

2) Behavioural studies were conducted to analyse the olfactory response of both the sexes of *A. subalbatus* of different age groups and to study the age related responses to different attractants/repellents.

3) In behavioural studies, some attractants and repellents for *A. subalbatus* were traced.

4) Among the ornamental, medicinal and common plants tested, none of them was an attractant to mosquitoes of any of the age groups. But mosquitoes showed repellence towards six of the plants i.e., *L. aspera*, *V. nigendu*, *C. citratus*, *O. sanctum*, *P. latifolia* and *A. indica*.

5) Among the organic chemicals tested, acetone 1,4-dioxan, phenol, petroleum ether, lactic acid, butan-1-ol and amyl alcohol were found as attractants and acetic acid was found to be a repellent.

6) Among the organic chemicals tested, ammonia and ammonium chloride were found as attractants but no repellents were found among this group.

7) Among the natural products, honey, sweat and urine were observed to be attractants. No repellents were traced from natural products.

8) Both the sexes showed preference to acetone and honey. However, for lactic acid, sweat and urine (metabolic wastes) females showed more preference than that of males.

9) Significant age wise increase in response was observed in most of the attractants\repellents tested.

10) Significant sex wise difference in response was observed to all attractants/repellents tested.

11) Six different combinations of oviposotion attractants were tested to find out the best among them. Arecanut mixture was found as the best oviposition attractant.

12) Electrophysiological studies were conducted to analyse the response of contact chemoreceptors. Both labral and labellar response to dextrose (food), lactic acid (atttractant) and acetic acid (repellent) were studied.

13) Labrum of male does not show any response to the tested stimuli as well as control. But female labrum showed response to test and control. These results indicate that males lack contact chemosensilla on the labrum.

14) Both males and females showed labellar response to test as well as control.

15) Newly emerged mosquitoes (0-24h age) do not show labral and labellar response, which indicate that in newly emerged mosquitoes contact chemoreceptors were not sensitive to either to their food, attractant or repellent.

16) Towards dextrose, three cell types (cell I, cell II and cell III) responded from the male labellum and two cells(cell I and cell II), responded from female labellum and labrum.

17) When lactic acid was used as stimulant, two cell types (Cell I and cell II) responded from the labellum of both male and female. But three cells responded from the female labrum.

18) Towards acetic acid three cells responded from the male labellum whereas, only two cells responded from the labrum as well as labellum of females.

19) When test stimuli (dextrose, lactic acid or acetic acid) were compared to the control (Insect Ringer) significant increase in spike frequency was observed from the labellum of both the sexes and labrum of females.

20) With the increase in concentration of the test stimulus (from 0.5M to 1M), significant increase in response was observed.

21) Across sensilla patterns from labrum and labellum were analysed for dextrose lactic acid and acetic acid. The pattern is different in males and females for the same chemical.

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Appendices

APPENDIX - I

1. **Tables of statistical analysis (attractants)**

ACETONE

	Col	Mean	sd	se	Sum
1	♀ 0-48h	7.9	0.99443	0.31447	79
2	48-96h	8.5	0.70711	0.22361	85
3	>96h	9.1	0.8756	0.27689	91
4	♂ 0-48h	8.9	1.19722	0.37859	89
5	48-96h	9.4	0.69921	0.22111	94
6	>96h	9.7	0.48305	0.15275	97

1,4 DIOXAN

	Col	Mean	sd	se	Sum
1	♀ 0-48h	7.3	0.82327	0.26034	73
2	48-96h	7.5	1.08012	0.34157	75
3	>96h	7.7	0.94868	0.3	77
4	♂ 0-48h	6.8	0.78881	0.24944	68
5	48-95h	7.1	1.1005	0.34801	71
6	>96h	7.3	0.94868	0.3	73

PHENOL

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.9	0.73786	0.23333	69
2	48-96h	7.8	0.63246	0.2	78
3	>96h	8.6	0.5164	0.1633	86
4	♂ 0-48h	6.4	0.5164	0.1633	64
5	48-96h	7	0.8165	0.2582	70
6	>96h	7	0.8165	0.2582	70

PETROLEUM ETHER

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.2	1.0328	0.3266	62
2	48-96h	6.9	0.8756	0.27689	69
3	>96h	6.5	0.84984	0.26874	65
4	♂ 0-48h	6.3	0.67495	0.21344	63
5	48-96h	6.5	0.84984	0.26874	65
6	>96h	6.9	0.73786	0.23333	69

LACTIC ACID

	Col	Mean	sd	se	Sum
1	♀ 0-48h	5.1	0.31623	0.1	51
2	48-96h	7.9	0.56765	0.17951	79
3	>96h	9.3	0.48305	0.15275	93
4	♂ 0-48h	6.1	0.8756	0.27689	61
5	48-96h	6.4	1.07497	0.33993	64
6	>96h	6.1	0.8756	0.27689	61

BUTAN-1-OL

	Col	Mean	sd	se	Sum
1	♀ 0-48h	7	1.1547	0.36515	70
2	48-96h	7.2	0.78881	0.24944	72
3	>96h	7.2	0.78881	0.24944	72
4	♂ 0-48h	7.2	0.91894	0.29059	72
5	48-96h	7.6	0.69921	0.22111	76
6	>96h	7.3	0.82327	0.26034	73

AMYL ALCOHOL

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.2	0.91894	0.29059	62
2	48-96h	6.5	0.84984	0.26874	65
3	>96h	6.1	0.73786	0.23333	61
4	♂ 0-48h	6.4	0.69921	0.22111	64
5	48-96h	6.5	0.84984	0.26874	65
6	>96h	6.7	0.67495	0.21344	67

AMMONIA

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.2	1.0328	0.3266	62
2	48-96h	7	0.4714	0.14907	70
3	>96h	6.9	0.31623	0.1	69
4	♂ 0-48h	6.4	0.69921	0.22111	64
5	48-96h	6.6	0.84327	0.26667	66
6	>96h	6.5	0.70711	0.22361	65

AMMONIUM CHLORIDE

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.1	0.99443	0.31447	61
2	48-96h	6.5	0.70711	0.22361	65
3	>96h	6.1	1.1005	0.34801	61
4	♂ 0-48h	6.7	0.94868	0.3	67
5	48-96h	7	0.8165	0.2582	70
6	>96h	7.1	0.56765	0.17951	71

HONEY

	Col	Mean	sd	se	Sum
1	♀ 0-48h	7.3	0.82327	0.26034	73
2	48-96h	8.1	0.73786	0.23333	81
3	>96h	9.1	0.99443	0.31447	91
4	♂ 0-48h	8.1	0.73786	0.23333	81
5	48-96h	8.9	1.19722	0.37859	89
6	>96h	9.8	0.42164	0.13333	98

URINE

	Col	Mean	sd	se	Sum
1	♀ 0-48h	4.3	0.82327	0.26034	43
2	48-96h	6.9	0.73786	0.23333	69
3	>96h	8.3	0.48305	0.15275	83
4	♂ 0-48h	6	0.94281	0.29814	60
5	48-96h	6.2	0.91894	0.29059	62
6	>96h	6	0.8165	0.2582	60

SWEAT

	Col	Mean	sd	se	Sum
1	♀ 0-48h	5.3	0.48305	0.15275	53
2	48-96h	6.1	0.99443	0.31447	61
3	>96h	7.4	0.69921	0.22111	74
4	♂ 0-48h	6	1.1547	0.36515	60
5	48-96h	6.9	0.99443	0.31447	69
6	>96h	6.1	0.99443	0.31447	61

2. ANOVA TABLES (ATTRACTANTS)

ACETONE					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	3995.27	1997.635	2717.21	3.07
Sex	1	397.6597	397.6597	540.9	4
Age	2	397.6278	198.8139	270.43	3.07
Error	54	39.69971	0.73518		

1,4 – DIOXAN					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2657.433	1328.717	1455.39	3.07
Sex	1	264.8625	264.8625	290.11	4
Age	2	264.7972	132.3986	145.02	3.07
Error	54	49.30005	0.912964		

PHENOL					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2681.533	1340.767	2861.71	3.07
Sex	1	265.7958	265.7958	567.31	4
Age	2	265.7639	132.882	283.62	3.07
Error	54	25.30005	0.468519		

PETROLEUM ETHER					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2149.794	1074.897	1507.65	3.07
Sex	1	213.968	213.968	300.11	4
Age	2	214.1917	107.0958	150.21	3.07
Error	54	38.5	0.712963		

LACTIC ACID					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2432.945	1216.472	2182.39	3.07
Sex	1	233.6681	233.6681	419.21	4
Age	2	235.7195	117.8597	211.44	3.07
Error	54	30.09985	0.557405		

BUTAN-1-OL					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2630.536	1315.268	1719.72	3.07
Sex	1	262.2764	262.2764	342.93	4
Age	2	262.2834	131.1417	171.47	3.07
Error	54	41.30005	0.764816		

AMYL ALCOHOL					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2050.811	1025.406	1628.59	3.07
Sex	1	204.3555	204.3555	324.56	4
Age	2	204.3	102.15	162.24	3.07
Error	54	34	0.62963		

AMMONIA					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2182.867	1091.433	2120.05	3.07
Sex	1	217.3	217.3	422.09	4
Age	2	217.4833	108.7417	211.22	3.07
Error	54	27.80005	0.514816		

AMMONIUM CHLORIDE					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2176.131	1088.065	1422.66	3.07
Sex	1	216.7653	216.7653	283.42	4
Age	2	216.2556	108.1278	141.38	3.07
Error	54	41.2998	0.764811		

HONEY					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	3692.095	1846.047	2536.57	3.07
Sex	1	365.5347	365.5347	502.26	4
Age	2	367.3583	183.6792	252.38	3.07
Error	54	39.2998	0.727774		

SWEAT					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2056.275	1028.137	1599.99	3.07
Sex	1	197.1125	197.1125	306.75	4
Age	2	200.3889	100.1945	155.92	3.07
Error	54	34.69995	0.642592		

URINE					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2011.311	1005.656	1201.45	3.07
Sex	1	197.9306	197.9306	236.47	4
Age	2	199.0333	99.51667	118.89	3.07
Error	54	45.19995	0.837036		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the difference is highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

3. Tables of statistical analysis (repellents)

LEUCAS ASPERA

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.8	0.91894	0.29059	68
2	48-96h	7.3	0.48305	0.15275	73
3	>96h	7.5	0.52705	0.16667	75
4	♂ 0-48h	6.9	0.8756	0.27689	69
5	48-96h	7.1	0.56765	0.17951	71
6	>96h	7.8	0.63246	0.2	78

VITEX NEGUNDO

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.9	0.73786	0.23333	69
2	48-96h	7.5	0.52705	0.16667	75
3	>96h	7.3	0.48305	0.15275	73
4	♂ 0-48h	6.5	0.52705	0.16667	65
5	48-96h	7	0.8165	0.2582	70
6	>96h	7.1	0.73786	0.23333	71

CYMBOPOGON CITRATUS

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6	0.66667	0.21082	60
2	48-96h	6.6	0.5164	0.1633	66
3	>96h	6.8	0.63246	0.2	68
4	♂ 0-48h	6.1	0.56765	0.17951	61
5	48-96h	6.6	0.5164	0.1633	66
6	>96h	6.8	0.78881	0.24944	68

OCIMUM SANCTUM

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.1	0.31623	0.1	61
2	48-96h	6.3	0.48305	0.15275	63
3	>96h	6	0.8165	0.2582	60
4	♂ 0-48h	6	0.66667	0.21082	60
5	48-96h	6.4	0.5164	0.1633	64
6	>96h	6.5	0.52705	0.16667	65

PREMNA LATIFOLIA

	Col	Mean	sd	se	Sum
1	♀ 0-48h	6.7	0.82327	0.26034	67
2	48-96h	7	0.4714	0.14907	70
3	>96h	7.9	0.73786	0.23333	79
4	♂ 0-48h	6.3	0.48305	0.15275	63
5	48-96h	6.7	0.82327	0.26034	67
6	>96h	7	0.8165	0.2582	70

AZHADIRACHTA INDICA

	Col	Mean	sd	se	Sum
1	♀ 0-48h	7.9	0.31623	0.1	79
2	48-96h	8.1	0.73786	0.23333	81
3	>96h	7.1	0.73786	0.23333	71
4	♂ 0-48h	7.4	0.5164	0.1633	74
5	48-96h	7.6	0.69921	0.22111	76
6	>96h	6.8	0.78881	0.24944	68

ACETIC ACID

	Col	Mean	sd	se	Sum
1	♀ 0-48h	8	0.66667	0.21082	80
2	48-96h	8.6	0.5164	0.1633	86
3	>96h	8.7	0.67495	0.21344	87
4	♂ 0-48h	8.7	0.67495	0.21344	87
5	48-96h	9	0.94281	0.29814	90
6	>96h	9.1	0.99443	0.31447	91

4. ANOVA TABLES (REPELLENTS)

LEUCAS ASPERA					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2136.248	1068.124	2518.73	3.07
Sex	1	425.3024	425.3024	1002.90	4
Age	2	425.5428	212.7714	501.73	3.07
Error	54	22.8999	0.424072		

VITEX NEGUNDO					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	1807.585	903.7927	2335.14	3.07
Sex	1	359.3643	359.3643	928.49	4
Age	2	360.2238	180.1119	465.36	3.07
Error	54	20.90015	0.38704		

CYMBOPOGON CITRATUS					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	1659.276	829.6381	2502.83	3.07
Sex	1	330.431	330.431	996.84	4
Age	2	330.5048	165.2524	498.53	3.07
Error	54	17.8999	0.33148		

OCIMUM SANCTUM					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2073.786	1036.893	2058.54	3.07
Sex	1	411.6571	411.6571	817.26	4
Age	2	412.3667	206.1833	409.34	3.07
Error	54	27.19995	0.503703		

PREMNA LATIFOLIA					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2411.286	1205.643	2818.37	3.07
Sex	1	479.3357	479.3357	1120.52	4
Age	2	480.2095	240.1048	561.28	3.07
Error	54	23.1001	0.42778		

AZHADIRACHTA INDICA					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	3239.138	1619.569	2776.40	3.07
Sex	1	645.5833	645.5833	1106.71	4
Age	2	645.5381	322.769	553.32	3.07
Error	54	31.5	0.583333		

ACETIC ACID					
Source	df	SS	MSS	F	Tab. F
Sex X Age	2	2249.571	1124.786	2372.59	3.07
Sex	1	447.4429	447.4429	943.82	4
Age	2	448.3524	224.1762	472.87	3.07
Error	54	25.6001	0.474076		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the difference is highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

APPENDIX - II

I TABLES OF STATISTICAL ANALYSIS (LABELLAR RESPONSE)

1 DEXTROSE

CELL I

	Col	Mean	sd	se	Sum	N
1	A	1.26	0.2881	0.12884	6.3	5
2	B	0.68	0.33466	0.14967	3.4	5
3	C	0.92	0.31145	0.13928	4.6	5
4	D	2.28	0.27749	0.1241	11.4	5
5	E	1.28	0.21679	0.09695	6.4	5
6	F	0.78	0.22804	0.10198	3.9	5

CELL II

	Col	Mean	sd	se	Sum	N
1	A	1.92	0.73959	0.33076	9.6	5
2	B	1.1	0.7874	0.35214	5.5	5
3	C	1.56	0.43359	0.19391	7.8	5
4	D	0	0	0	0	5
5	E	2.62	1.94859	0.87144	13.1	5
6	F	3.18	0.80436	0.35972	15.9	5

CELL III

	Col	Mean	sd	se	Sum	N
1	A	0	0	0	0	5
2	B	2.36	0.95289	0.42615	11.8	5
3	C	2.82	1.01833	0.45541	14.1	5
4	D	0	0	0	0	5
5	E	0	0	0	0	5
6	F	0	0	0	0	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	A	3.18	0.76616	0.34264	15.9	5
2	B	4.14	1.64104	0.73389	20.7	5
3	C	5	1.39104	0.62209	25	5
4	D	2.28	0.27749	0.1241	11.4	5
5	E	3.9	2.02485	0.90554	19.5	5
6	F	3.96	0.60249	0.26944	19.8	5

A. Insect Ringer
B. 0.5M Dextrose
C. 1M Dextrose
] Male

D. Insect Ringer
E. 0.5M Dextrose
F. 1M Dextrose
] Female

2 LACTIC ACID

CELL I

	Col	Mean	sd	se	Sum	N
1	G	1.26	0.2081	0.12884	6.3	5
2	H	2.12	0.68702	0.30725	10.6	5
3	I	4.04	0.61074	0.27313	20.2	5
4	J	2.28	0.27749	0.1241	11.4	5
5	K	2.44	0.19494	0.08718	12.2	5
6	L	1.99	1.75441	0.62028	15.92	8

CELL II

	Col	Mean	sd	se	Sum	N
1	G	1.92	0.73959	0.33076	9.6	5
2	H	2.28	0.72938	0.32619	11.4	5
3	I	4.04	0.72664	0.32496	20.2	5
4	J	0	0	0	0	5
5	K	4.04	0.49295	0.22045	20.2	5
6	L	0.78	0.22804	0.10198	3.9	5

CELL III

	Col	Mean	sd	se	Sum	N
1	G	0	0	0	0	5
2	H	0	0	0	0	5
3	I	0	0	0	0	5
4	J	0	0	0	0	5
5	K	0	0	0	0	5
6	L	0	0	0	0	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	G	3.18	0.76616	0.34264	15.9	5
2	H	4.4	1.2083	0.54037	22	5
3	I	8.08	1.27945	0.57219	40.4	5
4	J	2.28	0.27749	0.1241	11.4	5
5	K	6.48	0.41473	0.18547	32.4	5
6	L	3.96	0.60249	0.26944	19.8	5

G. Insect Ringer
H. 0.5M Lactic Acid
I. 1M Lactic Acid
] Male

J. Insect Ringer
K. 0.5M Lactic Acid
L. 1M Lactic Acid
] Female

3 ACETIC ACID

CELL I

	Col	Mean	sd	se	Sum	N
1	M	1.26	0.2881	0.12884	6.3	5
2	N	0.66	0.11402	0.05099	3.3	5
3	O	1.1	0.41833	0.18708	5.5	5
4	P	2.28	0.27749	0.1241	11.4	5
5	Q	3.4	0.54772	0.24495	17	5
6	R	2.8	0.44721	0.2	14	5

CELL II

	Col	Mean	sd	se	Sum	N
1	M	1.92	0.73959	0.33076	9.6	5
2	N	1.32	0.43243	0.19339	6.6	5
3	O	2.08	0.5933	0.26533	10.4	5
4	P	0	0	0	0	5
5	Q	5.2	1.30384	0.5831	26	5
6	R	5.8	2.48998	1.11355	29	5

CELL III

	Col	Mean	sd	se	Sum	N
1	M	0	0	0	0	5
2	N	2.66	0.58992	0.26382	13.3	5
3	O	5.28	1.03296	0.46195	26.4	5
4	P	0	0	0	0	5
5	Q	0	0	0	0	5
6	R	0	0	0	0	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	M	3.18	0.76616	0.34264	15.9	5
2	N	4.64	0.8735	0.39064	23.2	5
3	O	8.46	1.16319	0.52019	42.3	5
4	P	2.28	0.27749	0.1241	11.4	5
5	Q	8.6	1.67332	0.74833	43	5
6	R	8.6	2.50998	1.1225	43	5

M. Insect Ringer
N. 0.5M Acetic Acid
O. 1M Acetic Acid
] Male

P. Insect Ringer
Q. 0.5M Acetic Acid
R. 1M Acetic Acid
] Female

2. ANOVA TABLE (LABELLAR RESPONSE)

1. DEXTROSE

CELL – I					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	21.04689	10.52345	134.92	3.4
Sex	1	14.60844	14.60844	187.29	4.26
Conc.	2	15.65267	7.826333	100.34	3.4
Error	24	1.872005	0.0780		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	58.44122	29.22061	30.23	3.4
Sex	1	29.76578	29.76578	30.8	4.26
Conc.	2	32.75033	16.37517	16.94	3.4
Error	24	23.19601	0.966501		

CELL – III					
Source	df	SS	MSS	F	Tab. F
Conc.	1	0.529	0.529	0.543959	5.32
Error	8	7.7800	0.9725		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	155.8999	77.94995	47.94	3.4
Sex	1	140.1978	140.1978	86.23	4.26
Conc.	2	144.3645	72.18227	44.4	3.4
Error	24	39.01996	1.625832		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values except cell III of male. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

2. LACTIC ACID

CELL – I					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	82.76492	41.38246	148.03	3.4
Sex	1	64.44289	64.44289	230.53	4.26
Conc.	2	70.40814	35.20407	125.93	3.4
Error	24	6.709137	0.279547		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	105.0621	52.53106	165.71	3.4
Sex	1	49.90222	49.90222	157.42	4.26
Conc.	2	54.99211	27.49605	86.74	3.4
Error	24	7.608002	0.317		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	319.3029	159.6515	222.98	3.4
Sex	1	224.5533	224.5533	313.62	4.26
Conc.	2	242.713	121.3565	169.49	3.4
Error	24	17.18402	0.716001		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

3. ACETIC ACID

CELL – I					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	57.92545	28.96272	204.93	3.4
Sex	1	44.37822	44.37822	314	4.26
Conc.	2	36.21545	18.10772	128.12	3.4
Error	24	3.391983	0.141333		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	179.8942	89.94711	60.06	3.4
Sex	1	82.03911	82.03911	54.78	4.26
Conc.	2	89.33599	44.668	29.83	3.4
Error	24	35.94403	1.497668		

CELL – III					
Source	df	SS	MSS	F	Tab. F
Conc.	1	17.161	17.161	24.25583	5.32
Error	8	5.66	0.7075		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Sex X Conc.	2	512.1254	256.0627	129.32	3.4
Sex	1	356.0738	356.0738	179.84	4.26
Conc.	2	411.474	205.737	103.91	3.4
Error	24	47.52002	1.980001		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

Q

3. TABLES OF STATISTICAL ANALYSIS (LABRAL RESPONSE)

.1 DEXTROSE

CELL I

	Col	Mean	sd	se	Sum	N
1	A	1.04	0.30496	0.13638	5.2	5
2	B	2.18	0.51672	0.23108	10.9	5
3	C	4.56	1.10815	0.49558	22.8	5

CELL II

	Col	Mean	sd	se	Sum	N
1	A	2.42	0.67971	0.30397	12.1	5
2	B	3.54	0.29665	0.13266	17.7	5
3	C	6	1.82071	0.81425	30	5

CELL III

	Col	Mean	sd	se	Sum	N
1	A	0	0	0	0	5
2	B	0	0	0	0	5
3	C	0	0	0	0	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	A	3.46	0.68775	0.30757	17.3	5
2	B	5.72	0.50695	0.22672	28.6	5
3	C	10.6	2.38851	1.06818	53	5

A. Insect Ringer
B. 0.5 M dextrose
C. 1M dextrose

2 LACTIC ACID

CELL I

	Col	Mean	sd	se	Sum	N
1	D	1.04	0.30496	0.13638	5.2	5
2	E	0.84	0.2881	0.12884	4.2	5
3	F	1.32	0.16432	0.07348	6.6	5

CELL II

	Col	Mean	sd	se	Sum	N
1	D	2.42	0.67971	0.30397	12.1	5
2	E	1.42	0.22804	0.10198	7.1	5
3	F	1.94	0.86487	0.38678	9.7	5

CELL III

	Col	Mean	sd	se	Sum	N
1	D	0	0	0	0	5
2	E	1.74	0.73007	0.3265	8.7	5
3	F	2.94	0.70214	0.31401	14.7	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	D	3.46	0.68775	0.30757	17.3	5
2	E	4	1.04163	0.46583	20	5
3	F	6.2	1.44914	0.64807	31	5

D. Insect Ringer
E. 0.5M Lactic Acid
F. 1M Lactic Acid

3. ACETIC ACID

CELL I

	Col	Mean	sd	se	Sum	N
1	G	1.04	0.30496	0.13638	5.2	5
2	H	1.5	0.2	0.08944	7.5	5
3	I	1.12	0.66106	0.29563	5.6	5

CELL II

	Col	Mean	sd	se	Sum	N
1	G	2.42	0.67971	0.30397	12.1	5
2	H	2.96	1.1149	0.4986	14.8	5
3	I	3.96	1.46731	0.6562	19.8	5

CELL III

	Col	Mean	sd	se	Sum	N
1	G	0	0	0	0	5
2	H	0	0	0	0	5
3	I	0	0	0	0	5

TOTAL CELL

	Col	Mean	sd	se	Sum	N
1	G	3.46	0.68775	0.30757	17.3	5
2	H	4.46	1.02859	0.46	22.3	5
3	I	5.08	1.41669	0.63356	25.4	5

G. Insect Ringer
H. 0.5 M Acetic Acid
I. 1M Acetic Acid

4. ANOVA TABLE (LABRAL RESPONSE)

1. DEXTROSE

CELL – I					
Source	df	SS	MSS	F	Tab. F
Conc.	2	130.5446	65.27232	123.31	3.88
Error	12	6.352005	0.529334		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Conc.	2	267.9533	133.9767	103.99	3.88
Error	12	15.46002	1.288335		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Conc.	2	778.6567	389.3283	181.51	3.88
Error	12	25.73993	2.144994		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

2. LACTIC ACID

CELL – I					
Source	df	SS	MSS	F	Tab. F
Conc.	2	16.58133	8.290667	122.52	3.88
Error	12	0.812002	6.77E-02		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Conc.	2	56.25534	28.12767	66.86	3.88
Error	12	5.047993	0.420666		

CELL – III					
Source	df	SS	MSS	F	Tab. F
Conc.	1	3.6	3.6	7.017544	5.31
Error	8	4.104	0.513		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Conc.	2	327.5047	163.7523	134.3	3.88
Error	12	14.63199	1.219332		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

3. ACETIC ACID

CELL – I					
Source	df	SS	MSS	F	Tab. F
Conc.	2	21.71	10.855	57.13	3.88
Error	12	2.279999	0.19		

CELL – II					
Source	df	SS	MSS	F	Tab. F
Conc.	2	148.3847	74.19233	57.69	3.88
Error	12	15.43201	1.286001		

TOTAL CELL					
Source	df	SS	MSS	F	Tab. F
Conc.	2	284.0146	142.0073	120.41	3.88
Error	12	14.15204	1.179337		

Tab. F is at 0.05 level of significance

In all the cases, calculated values are greater than the table values. Hence the differences are highly significant

df – Degrees of freedom, SS – Sum of squires due to the corresponding factor
MSS – Mean sum of squires, F – MSS due to the factor / MSS due to the error
Tab. F – Table value of 'F'

APPENDIX - III

The gynandromorph of *Armigerus subalbatus* (Diptera: Culicidae)

Armigerus subalbatus (Coquillet), one of the most common mosquitoes in Kerala, is well known for its vicious man-biting habit[1]. It breeds predominantly in foul-smelling water and septic tanks in urban areas[2]. We describe here the morphological differences in mouth parts and the ratio between male, female and gynandromorph of *Armigerus subalbatus*.

The gynandromorph is a genotypic mosaic, which phenotypically appears as a combination of male and female tissue[3]. A line of demarcation between male and female organs is always apparent. The gynandromorphs are divided into three broad types[4]. (a) Anterior ♂-posterior gynander: where the anterior region is phenotypically male, head has antennae and palpi typically of a male. The posterior region is phenotypically female, with wings of the female type and abdomen with well-developed ovaries. (b) Anterior ♀-posterior gynander: where the anterior region is typically female and posterior region male. (c) Bilateral gynander: where the right side of the body resembles a male and the left side a female. Head, mouth parts and abdomen of the right side resemble those of a typical male. Head, mouth parts and abdomen of the left side resemble those of a female. Thus, one side of the body – either right or the left, is male-like and the other side is female-like.

Gynandromorphs have been found in the natural population of mosquitoes[3]. They have been described from eleven Culicine genera[3]. Most specimens have head of one sex and the abdomen of the other sex[3]. More than half of the mosquito gynandromorphs have been found in the genus Culex[4]. The gynandromorph of the genus Culex, Anopheles and Aedes have been reported earlier[3,5-7]. The genetic cascade of events resulting in gynanders has been worked out in some detail in Culicine mosquitoes. However, what role can this play in the regulation of sexual differentiation is poorly understood[3]. The gynandromorph of *Armigerus subalbatus* has not been reported so far. Hence an attempt has been made to record the sex ratio and morphology of the mouth parts of these mosquitoes.

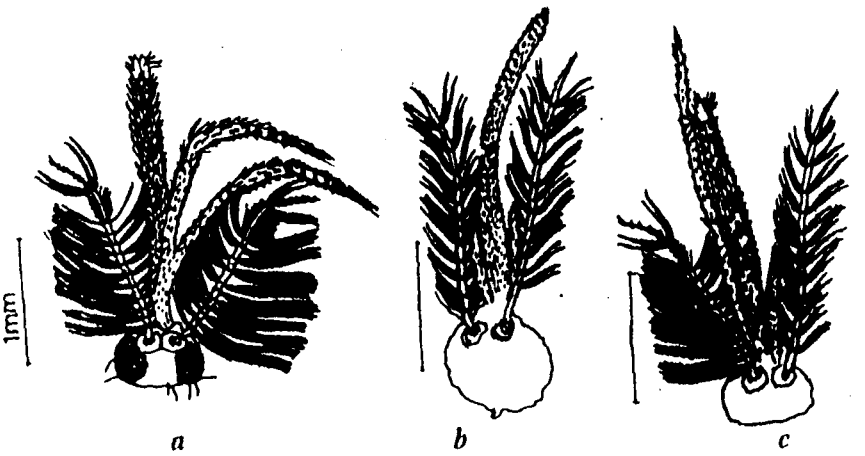

Figure 1. Head and mouth parts of *Armigerus subalbatus*. *a*, male, *b*, female, *c*, gynandromorph.

The gynanders described here were collected from the Calicut University campus during the screening of *Armigerus subalbatus* for chemoreceptive studies. Outdoor as well as indoor collections of adult mosquitoes were made during dawn (5 to 7 AM) and dusk (5 to 7 PM) hours of the day. These mosquitoes were identified and a culture was maintained in the laboratory. Observations were made using a stereozoom microscope.

In order to arrive at the ratio of gynanders, one thousand eggs of *Armigerus subalbatus* were collected from the field and reared to adults in the laboratory to observe the sex ratio. Out of this, 476 were males, 430 were females and three were gynanders.

The gynander described here is a typical bilateral gynander (Figure 1). The right side of the body resembled a female and the left side that of a male. Males are easily distinguished by their conspicuous plumose antennae which contrasts with the pilose antennae of the female. The right antenna of the gynander resembled that of the female with plumose type antenna, while the left antenna resembled that of a male, have a pilose-type structure. The proboscis is the least modified mouth part, being an intermediate between male and female. The maxillary palp of the right side was short and blunt as in female and with a large number of sensilla. Palp on the left side was extremely thin, long and pointed as in the male.

The differences between normal mosquito and a gynander were conspicuous to the naked eye and could be easily detected when the mosquito was resting. Mouth parts of the male are structurally adapted for the uptake of plant juices and those of the female used both to probe flowers and to pierce the skin. The chemosensory system of these gynanders is of great interest because it may function differently when compared to typical male or female sensory system, and merits study from the viewpoint of neurogenetics.

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HERBAL REPELLENTS AGAINST THE MOSQUITO *ARMIGERUS SUBALBATUS* (DIPTERA : CULICIDAE)

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Abstract: Armigerus subalbatus, one of the most common and vicious biting mosquitoes of Kerala, were observed for their repellent behaviour. Twenty aromatic plants were screened for their repellent efficacy out of which three plants showed repellance viz. Leucas aspera, Vitex negundu and Cymbopogon citratus. Mosquitoes showed maximum repellence towards L. aspera.

1. Introduction

Mosquitoes are still the world's number one vectors of human parasitic diseases. In recent years, repellent devices have become common in ruban and some rural areas of India due to mosquito menace. Smoke coils and mats containing pyrethroids are used widely to protect humans from the bites of mosquitoes (Feles et al, 1968; Hudson and Esozed, 1971; Charlwood and Jolley, 1984)\; Ansari et al, 1990). Although mosquito repellents containing pyrethroids generally are considered safe and there are no serious complaints of toxic reactions from their use, prolonged exposure to coil smoke may be harmful (Liu et al, 1987). Pyrethroids in coils or mats may continue as a standard method of mosquito protection as it has become a dire necessity. There is, therefore, need of a safer protection (Sharma and Ansari, 1994). Smoke of herbal leaves seems to be an effective mosquito repellent and this has distinct advantages over chemical repellent because it does not leave poisonous residues and does not pollute the environment (Pandian et al, 1989). As Kerala is well known for the megadiversity of mosquitoes, such a search is worth while (Narayanan and Pillai, 1996). Curtis et al (1990) reviewed the currently available synthetic and natural repellents. These repellents were mainly against the mosquitoes belonging to the Genus Aedes, Anopheles and Culex. No such work has been carried out on *Armigerus subalbatus*, which is the most common and vicious man-biting mosquitoes of Kerala (Reena and Ramakrishna, 1996; Pandian, 1994). We report here, the results of our study of the repellent action on *A. albatus* to three plant volatiles viz. *Leucas aspara, Vitex nigendu* and *Cymbopogon citratus*.

3. Materials and Methods

Adult mosquitoes of *A. Subalbatus* were collected from Calicut University campus and maintained in the laboratory. Mosquitoes of different age groups (0-12hr, 12-24hr and field collected) were used for the behavioural experiments. Tests were conducted during the peak hours of biting activity of this mosquitoes ie, at 6-7 am and 6-7 pm since it is a crepuscular biter (Pandian, 1994). The experiments were conducted in room conditions (RH 65-70% at 27 degree centigrade). Both male and female mosquitoes were tested in groups of 10. The average response was calculated in 100 mosquitoes of the same age group with each stimulant.

Mosquitoes to be testd for their response to air borne stimuli were introduced into a 13cm long and 8cm diameter glass cylinder, covered with a mosquito net at both the ends. Carrier air stream was directed into the cylinder by switching on a small fan, kept one meter away from the cylinder. Stimulus air stream was given by keeping a cotton ball of uniform size was soaked in the plant extract was kept in between the fan and the cylinder. Fresh leaves were crushed and the extract was taken as the stimulant. The carrier air stream was given continuously throughout the entire experiment. The stimulus air stream was given for one minute and at the end of that time, responding mosquitoes were counted. The cotton soaked in the plant extract was then removed, and the carrier air stream was allowed to flow through the cylinder for two minutes before the next stimulus was given, to clear the cylinder from the volatiles and to allow the mosquitoes to adapt to odour free condition. Behavioural responses were measured by observing the mosquitoes probing on either side of the screens or those making short flights from the sides of the cylinder towards the screens and then subsequently probing, were counted as responders. Sustained flight activity was not a behavioural option in this setup. There are three types of responses viz. attraction, repulsion and no response. If the mosquitoes fly against the stimulus air stream and probed on the net, the stimulus is counted as attractant. If the mosqitoes move towards the stimulus air stream flow, the responding stimulus is counted as repellants. All the mosquitoes in the absence of the stimulus air stream and non responding mosquitoes in the presence of stimulus air stream were immobile except for occasional grooming movements, are counted as non responders. Behavioural responses were assessed by this experiment. The tests were repeated without carrier air stream in the following manner. The cotton soaked in plant extract was kept 1cm away from one end of the screen.

If the mosquitoes fly away from the stimulus end where the soaked cotton was kept, to the opposite side, they are considered to have shown repellent behaviour to that given stimulus. Repellent efficacy was analysed by calculating the percentage of mosquitoes responded.

3. Results and Discussion

Twenty plants were tested (Table 1) for the behavioural responses out of which three were found as repellents viz. *Leucas aspera, Vitex nigendu* and *Cymbopogon citratus* (Table 2). From the table 2, it is found that male mosquitoes showed maximum repellence (78%) to *L. aspera*. In both sexes, responses increased with increase in age.

Table 1. Response of A. subalbatus to plant extracts NR-no response R-repellence

Number	Name of plants	Males	Females
1	*Coleus ambonicus*	NR	NR
2	*Eupatorium odoratum*	NR	NR
3	*Tagates erectus*	NR	NR
4	*Leucas aspara*	R	R
5	*Michelia champaka*	NR	NR
6	*Clerodendrum viscosum*	NR	NR
7	*Jasminum sambac*	NR	NR
8	*Chrysanthemum intybus*	NR	NR
9	*Ixora chinensis*	NR	NR
10	*Cymbopoga citratus*	R	R
11	*Acacia auriculiformis*	NR	NR
12	*Hibiscus rosa-sinensis*	NR	NR
13	*Impatiens balsamina*	NR	NR
14	*Anacardium occidentale*	NR	NR
15	*Casia fistula*	NR	NR
16	*Coriandrum sativum*	NR	NR
17	*Psidium gujava*	NR	NR
18	*Catheranthus roseus*	NR	NR
19	*Murraya koenigii*	NR	NR
20	*Vitex nigendu*	R	R

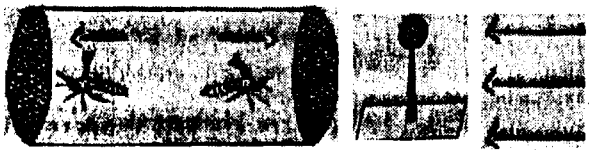
Mosquitoes of both sexes also showed very good response to *V. nigendu*. But in this case, females showed maximum repellence (75%). Here also, the response increased with the increase in age, but the females showed maximum response in 12-24 hr age group.

When *C. citratus* was kept as a stimulant, both males and females showed equal repellence (68%) and the response increased with an increase in age.

Table 2. Percentage of *A. subalbatus* showing repellant behaviour

Name of the stimulant	Females			Males		
	Age 0-12 hour	12-24 hour	field collected	Age 0-12 hour	12-24 hour	field collected
Leucas aspera	68	73	75	69	71	78
Vitex nigendu	69	75	73	65	70	71
Cymbopoga citratus	60	66	68	61	66	68

It appears from the table 2 that highest repellence (78%) was produced by *L. aspera* for an exposure of 1 minute. Among the tested plants, different age groups of *A. subalbatus* showed different responses to each plant extract. Lehane (1991) reported similar behavioural response in some blood sucking insects. We also assume that the response of mosquitoes to plant volatiles may increase with increasing age. The present study showed that *L. aspera* is a better repellent among the plants tested, but no significant difference was observed between the males and the females.



A B C

Figure 1. Behavioural setup to test odour preference/repellence

A - glass cylender
B - cotton ball soaked in plant extract
C - air stream —> attracted mosquitoes
<— repelled mosquitoes

The present study has shown that these plant extracts could be effectively utilised as moquito repellent. The efficacy of plant extracts was already reported (Carl, 1991, Curtis et al., 1990; Kalyan Sundaram and Babu, 1982; Sujatha et al., 1988). Similar studies conducted by Pandian et al (1989) showed that *L. aspera* and *V. nigendu* are potential repellants against *Culex quinquifasciatus*. However we found that the fresh plant extract itself is a good repellant of Armigerus. Since the plants studied in the present experiment are available in Kerala, these plants can be used as an effective mosquito repellent inexpensively. Wide distribution of *L. aspera* with its high repellence shows that it is a promising agent against *A. subalbatus*.

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