STUDIES ON SOME ASPECTS OF THE BIOLOGY AND BEHAVIOUR PATTERNS OF ANOPHELINE MOSQUITOES

BY

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SUPERVISOR'S CERTIFICATE

I certify that the thesis entitled **Studies on some aspects of the biology and behaviour patterns of Anopheline mosquitoes** submitted by Mr. **Summerland Lyngdoh** for the **Degree of Doctor of Philosophy** of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any degree of any other University.

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PREFACE

PREFACE

Malaria, a disease of antiquity, has been widely prevalent in the world causing distress among large communities and taking a great toll of life. It has also proved to be a formidable deterrent to the cultural and socio-economic progress of man in the tropical, sub-tropical and monsoon prone zones of the world. History is replete with devastation caused by this disease. Widespread prevalence of this disease in India, among other factors, was responsible for the slow economic, scientific and industrial progress during the last two centuries. The disease remained in the realm of mystery till twin discoveries of the parasites by Laveran in 1880 and the establishment of the role of female mosquito by Ross in 1897. Consequent to these and subsequent discoveries, it has become common knowledge that human malaria is caused by the protozoan parasites of the genus *Plasmodium*. The members of this group, unlike some pathogenic organisms, do not have an independent existence. All stages of the parasites spend their life either in man or in some species of mosquito genus *Anopheles*. This epoch making discovery has led to the understanding of the involvement of biological factors viz. Parasites, Mosquito vector and Host (man) and their dependence on environment for perpetuation of the disease.

Since the discovery of Malaria transmission was made, and because of its pre-èminence among the communicable diseases, intensive work was carried out on different aspects of the disease including its control. India has always been in the forefront of Malaria research and has been a pioneer in studying the feasibility of Malaria control with the use of residual insecticides extensively in rural areas. The major attempt in control was directed initially against mosquito larvae and subsequently as knowledge improved against adults as well. With the advent of DDT, a synthetic residual insecticide, there has been a steady progress towards the use of chemicals in public health. Thus, for the first time a cost-effective tool became available for mass application to interrupt Malaria transmission especially among rural and other communities. This led to experimentation in major malarious areas of the world. In India systematic studies were undertaken under the aegis of Health Directorate of the then Bombay Presidency (now Maharashtra and Gujarat States) and erstwhile Malaria Institute of India during 1945 to 1952, which established technical, operational and administrative norms for undertaking a mass campaign against the disease in the country. The success of the National Malaria Eradication

Programme (NMEP) in 1953-1957 in consonance with global thinking reflected by the deliberation of World Health Organisation (WHO) resulted in the creation of a separate Directorate of National Malaria Eradication Programme (NMEP) in 1958, with the sole objective for eradication of the disease from the country.

However, the rich *Anopheles* fauna of the country, diverse ecological-cum-terrain features, the different social and cultural strata of the population, the lack of peripheral health infrastructure as well as special characteristics inherent to such biological phenomena soon provided difficulties in attaining the goal. The resistance of mosquitoes to insecticides and resistance of parasites to anti-malarial drugs has combined to create a dangerous and potentially explosive resurgence of the disease. This, on the other hand, has also created a formidable problem and is becoming great challenge to the field of Malaria Eradication.

With reasons stated above, the resurgence of Malaria occurred in a big way in 1976, and a revised strategy known as Modified Plan of Operation (MPO) was launched in April 1977, with the following objectives, viz., prevention of deaths due to Malaria, reduction of morbidity due to Malaria, and maintenance of Industrial and Green Revolution due to freedom from Malaria, as well as retention of achievements gained so far. Under the Modified Plan of Operation (MPO) and area with 2-API or more are earmarked for regular rounds of insecticides spray. In area below 2-API, provision is made for limited operation depending on the size of foci. Spray operation is carried out regularly, that is, 2 to 3 rounds depending on the occurrence of the disease and prevalence of the vectors.

As far as North-Eastern Region of India is concerned and according to the Indian Council of Medical Research (ICMR) reports which stated that about 18 districts of the region are accounted for about 80 per cent of *P. falciparum* infections. With a view to check the spread of falciparum Malaria, *P. falciparum* containment programme (PfcP) was launched in October 1977, in the above 18 districts. As seen from the various reports about the occurrence of the resistance strain in parasites towards drugs, as well as insecticides resistance of the vector species throughout the world, the North Eastern Region has also not escaped from this global problem. The first report of *P. falciparum* resistance to chloroquine in India came from Diphu of Karbi Anglong Districts of Assam (ICMR-Reports), so also it was further reported from many other districts of the region. As far as the State of Meghalaya is concerned, there is a compact belt consisting of areas of difficult hilly and semi-hilly terrain with high rainfall and poor communication. Therefore, complete knowledge about the disease as well as the vector species and their control is not highly upto date.

Hence, a virtual absence of scientific knowledge on some of these aspects, prompted us to undertake the present investigation. It is, therefore, earnestly hoped that the information embodied in this thesis would have relevance to the socio-economic development and scientific awareness of these areas of the country by evolving a better understanding on the occurrence and species compositions among the *Anopheles* mosquitoes population.

The present study pertains to all the *Anopheles* species present with its seasonal fluctuation in relation to the peak occurrence of the disease reported from the study areas during the period from May 1992 to April 1994. The results obtained during the present study are presented in this thesis entitled **Studies on Some Aspects on the Biology and Behaviour Pattern of *Anopheles* Mosquitoes.**

GENERAL INTRODUCTION

In the present day world, where the economy of a nation depends on judicious prospecting, conservation and proper management of renewable and non-renewable resources, the role of Biological studies is manifestly becoming significantly important. Today the field of biology is no longer an academic subject only as it concerns every one directly or indirectly related to food, energy crisis, pollution or health (Nasar, 1977). The study of insects has great significance on the life of mankind. Some of these insects are very useful and beneficial to man, whereas a majority are not useful or dangerous to human race. The struggle between man and insects began long before the dawn of civilization has continued without cessation to the present time, and will continue, no doubt, as long as human race endures.

Anopheles mosquito have been, and still are the subject of a tremendous amount of study and the literature on them is extremely large and varied. They are among the best known groups of insects, because of their bio-medical and agricultural importance to man as vector and pest respectively. Malaria a vector-borne disease known to be transmitted through certain species of *Anopheles* mosquitoes was and still are major public health problem in the world, adversely affecting the socio-economic development, particularly of her poor people. In pre-historic times malaria was common in southern valley of the Nile and mention about spleenamagaly and fever was seen in Ebers papyrus of 1570 B.C. Other malarious regions were Mesopotamia, Suriname, Babylon in 3500-3000 B.C. Malaria is restricted to 15°C Summer Isotherms on both sides of the globe and spread over 12 epidemiological zones viz. North America, Central America, South America, North European and Asiatic, Mediterranean desert, Ethiopian, Indo-Persia, Indo-Chinese, Malaysia, Chinese and Australia (McDonald, 1957). Malaria receded from throughout Europe, North America and Australia during the 19th Century.

Problem of Malaria is multi-faceted and global in character. It has been co-existing and taking toll of human life since the time immemorial, and the well organized malaria control for the past four decades has not diminished its magnitude upto the expectation. The failure has often led to the attitude of pessimism with regard to an ultimate solution even among the scientists. World-wide intensive research on various aspects of the disease has resulted in new knowledge and more clear concept on many aspects, but the task ahead is still complex and difficult.

World Malaria Situation

Out of the total world population of 5,300 million, 3,100 million (59%) live in the areas free from malaria. About 1,700 million (32%) live in areas where transmission is re-established and the situation is unstable or deteriorating. Over 500 million are living mainly in Tropical Africa, in an area where malaria remains basically unchanged. More than 40% of the world population living in about 99 countries is exposed to the risk of contracting malaria (WHO, 1992). The global malaria incidence is estimated to be 120 million clinical cases per annum and 1.1 million deaths mainly in children. Countries in tropical Africa are estimated to account for more 80% of all cases. Excluding African region, 90% of the reported cases annually originate from 19 countries. About 75% of these are concentrated in 9 countries. viz., India, Brazil, Argentina, Sri Lanka, Thailand, Indonesia, Vietnam, Cambodia and China. Most of these countries have reported parasite-resistance (*Plamodium falciparum*) to chloroquine and vector resistance to various insecticides.

Malaria in India

The evidence of existence of feveric related diseases are found in writings of Vedic (1500-500 B.C.) and Brahmanic (800 B.C.-100 A.D.) periods. The ayurvedic sages Charaka and Susruta noted both tertian and quantan fevers. In Punjab alone, periodic epidemics in 1890-1908 caused 0.25 to 0.31 million deaths. Sinton (1935) who studied the various aspects of the disease in India concluded that "the problem of the very existence in India is the problem of malaria, which constitutes one of the most important causes of economic misfortune endangering poverty lowering the physical and intellectual standards." In 1935 Sinton and Chopra (in Malaria and Its Control in India, 1984, NMEP) estimated that in Indian sub-continent there were at least 100 million cases of malaria occurring annually, of which, 1 million died. In 1947, the economic loss due to malaria was about Rs.7,500 million per annum.

It was in the year 1897-1898, when Sir Ronald Ross, saw occysts on the stomach wall of an anopheline for the first time and realized their significance, very little was known about mosquitoes. Since then tremendous achievements of the earlier workers have provided a solid foundation on the ecological and biological information, much of which is still relevant till today. The recognition of anopheline species and naming them scientifically in India also soon followed after the discoveries of Sir Ronald Ross in 1897-1898. The first series of papers dealing with taxonomy of Indian anophelines species commenced with that by Grassi (1899) and was followed by those of Giles (1901), Liston (1901), Theobald (1901-1902), James (1902), Gogill (1903), James and Liston (1904-1911), Donitz (1961) and others. The larvae of Indian Anophelines was studied extensively by Puri resulting in his comprehensive monograph on the subject (Puri, 1931). The pioneering work of Puri on Anopheline larvae and of Christopher and others on the adult and egg stages culminated in 1933 in the publication of a volume on anopheline in the

Fauna of British India series. In general this is till the standard work of reference till today. Further, a number of species of these groups have been identified in India in later years (Wattal *et al.*, 1962; Reid, 1968; Harrison and Scanlon, 1975), but much more work remains to be done to determine their distribution.

Reviews by authors such as Bates (1949), Muirhead Thomson (1965), Horsfall (1972) and others to name a few have also dealt exhaustively with several aspects of biology on a global bases. The work of Reid (1968), Harrison and Scanlon (1975) have referred to many features of the biology in South-East Asian countries. In India itself, the small volume Vector of Malaria in India by several authors, published by the then Indian Society for Malaria and other Mosquito-Borne Diseases (1961) has fairly well dealt with biology of several species as known till then.

Considering the importance of *Anopheles* mosquitoes intensive research works were carried out in the control aspects, in accordance with previous knowledge acquired, with regard to their ecology, biology and behaviouristic aspects. The first effort to control malaria was started in 1902 at Mian Mir, a cantonment near Lahore (now in Pakistan) by minor and inexpensive methods suggested by Sir Ronald Ross. Control efforts were mainly directed on mosquito breeding in canal and irrigation ponds. Later in Bombay city the malaria was controlled by preventing breeding of *Anopheles* *culicifacies* (Bentley, 1911). These control methods against the various species of *Anopheles* were also attempted in cities like Delhi, Bangalore and Pune.

Similarly, successful malaria control was achieved using environmental management, uses of oil, larvivorous fish, fumigation and pyrethrum in Sardar Canal Project (Clyde, 1931), Irwin Canal Project (Rao, 1945) and Cauvery Meltus Project (Russel and Knipe, 1942). Other examples include deweeding to *Anopheles fluviatilis* (Viswanathan, 1946), and growing shade loving plants over tea garden drains to control *Anopheles minimus*. Russel and Knipe (1939) and Covell (1941) demonstrated the utility of pyrethrum space sprays for controlling *Anopheles culicifacies*. But it was found to be unsuccessful against *Anopheles fluviatilis* (Viswanathan and Rao, 1943) due to outdoor resting habits.

The advent of DDT and its introduction as residual insecticides in 1944, the emphasis shifted to anti-adult measures. Senior-White (1945) Viswanathan and Parikh (1946) were the first to spray DDT for civilian use. The success of these trials led to the introduction of rural malaria control programme in 1946 (Viswanathan and Rao, 1947, 1948, 1949), pilot demonstration project (Afridi and Dilip Singh, 1947, Ramakrishnan *et al.*, 1948) and demonstration units for the use of DDT (Isaaris *et al.*, 1953; Jaswant Singh *et al.*, 1953; Klerks, 1951; Srivastava, 1950).

Following the success of these trials, National Malaria Control Programme (NMCP) was launched in 1953. About 70 million people were covered by residual spraying under this programme. The programme achieved a remarkable success (Rao, 1955; Jaswant Singh *et al.*, 1957). Further, the Government of India with other collaborating agencies such as USAID and WHO (NMCP, 1953-1958) launched "National Malaria Eradication Programme" (NMEP) in 1958, where the objective of this programme was total ending of transmission, elimination of reservoir of infection and prevention of re-establishment. Malaria situation drastically improved with 0.049 million reported cases in 1961 in compared with 20 million cases in 1956 (Ray *et al.*, 1988).

India, however, made spectacular success in achieving control of vector-borne diseases, largely because of wide spectrum toxic effect of DDT on mosquitoes. However, this effect did not last long and mosquitoes species precipitated resistant population with diminish impact on Malaria control. Large scale developmental plans brought in radical changes in the ecosystem. Industrial and agricultural activities set in motion large scale movement of people from malarious to non-malarious area and vice-versa and brought in its wake many alterations of the ecosystems. Man's greed for exploitation of natural resources brought him in contact with untouched virgin ecosystem and thereby alter the food chain and behaviour of many living organisms.

Subsequently, malaria cases increased to 0.69 million in 1970. Situation deteriorated further reaching a peak of 6.45 million in 1976. The steady resurgence of malaria prompted the government of India to implement the Modified Plan of Operation (MPO) in April 1977, with the objective of elimination of mortality, reduction of morbidity, protection of green revolution and maintenance of gains achieved (Pattanayak and Roy, 1980). Numbers of Malaria cases decreased to 2.7 million in 1980. From 1981-84 it remained at a static level of about 2 million per annum. Cases slightly declined in the following years to fluctuate between 1.8 to 1.6 million in 1985-1987 (NMEP Annual Reports). In 1989, cases reported were 2 million, which again shows a steady rise, and has remained at that level afterwards. In 1990, total deaths recorded were 290 including 147 in Orissa and 65 in Rajasthan (NMEP Annual Reports). Thus, it shows that even after the implementation of Modified Plan of Operation (MPO), the malaria incidence could not be reduced below 2 million cases per year.

The reasons for the set back in residual spraying has been elucidated by several authors viz. Ray (1977), Kalra (1978), Ramachandra Rao (1979), Sharma and Mahotra (1986). The causes of failure of eradication campaign as emphasized by these authors were technical (like drug resistance in parasites, vectors resistance to insecticide and human resistance to spraying), operational and administrative.

Reuben (1980), further stressed that under the strong selection pressure exerted by residual insecticides over many years the behaviour of *Anopheles* mosquitoes has in many cases undergone a change.

Based on the advances already made on the studies of biology, ecology, behaviour and control aspects of various *Anopheles* species, the researches now are required to be intensified in India and it should also be centered around determination of the availability and distribution of the various species under controlled environmental conditions (Dey, 1977). Also it is necessary to study each local situation afresh to see whether the old assumptions regarding the identity of *Anopheles* species and their biology are correct (Reuben, 1980). But in order to do so, firstly, it becomes necessary to understand the basic structure and function of their natural ecosystem. Secondly, it involves a thorough knowledge regarding their general biology. But till now there is no work reported on hilly, semi-hilly and foot-hill terrains of Meghalaya state, where the sub-tropical climatic conditions prevail.

Although the seed of awareness for the utility of Science and Technology has been sown in Meghalaya from the beginning of the Seventh Five-Year Plan, yet the State has not been able to developed on adequate Science and Technology based plus its awareness at par with the rest of the country. Over 80% of the population resides in the rural areas, isolated from the main stream of development. There are limited agricultural land in Meghalaya but 85% of the population subsists on agriculture [1991-92 Draft Proposals, Vol.I, Government of Meghalaya, Eighth Five-Year Plan (1990-1995)].

Keeping this in view, studies on certain aspects of the biology and behaviour pattern of *Anopheles* mosquitoes from Byrnihat areas, Ri-Bhoi District, Meghalaya were undertaken. The study includes **two** major parts - (i) Biological studies, which includes their habitat structure - breeding places and their analysis, population dynamics, seasonal prevalence and certain aspects of their ecosystem, and (ii) Ultra-structural studies - since many morphological features of taxonomic and behavioural significance cannot be resolved even with the best available optical microscope because of its low resolving power, the ultrastructural studies were undertaken using the Scanning Electron Microscope which has high resolving power and large depth of field. Further, it appears that a first step in understanding the complexity of behaviour shown by any species is a thorough examination of its sensory system. The study, therefore, includes investigation on morphology of different types of sensilla, viz. sensilla trichodea, sensilla basiconica, sensilla caeloconia, hair plate sensilla, pit-sensilla, plate organs, campaniform sensilla etc. in different body parts in two representative species from the study area. It is hoped that the informations presented from the investigation could be of help for the basic understanding of a sub-tropical ecosystem as well as the structural and functional aspects of the population, in this region of our country.

Geographical Distribution

Anopheles mosquitoes are distributed widely throughout the world, some species live at the altitude of over 19,000 ft., while other survive as far as 3,760 ft. below sea level. The world *Anopheles* fauna comprises of 466-species, out of which only 66 species are known to be vectors of malaria. Some of the characteristic features of the anopheline fauna of these regions (Oriental region) have been briefly described by many authors viz. Christopher (1933), Freeborn (1949), Lane (1949), De Meillon (1949), Bates *et al.* (1949), Puri (1949), Stones *et al.* (1959), Rusell *et al.* (1963), Stones and Delfinado (1973), Knight and Stone (1977). In India, the survey of the *Anopheles* species have been extensively carried out, as out of 53 *Anopheles* species recorded, 11 species are currently recognized as vectors viz. *Anopheles annularis*, Van der Wulp, 1884; *Anopheles culicifacies*, Giles, 1901; *Anopheles stephensi*, Liston, 1901; *Anopheles minimus*, Theobold, 1901; *Anopheles tesselatus*, Theobold, 1901; *Anopheles aconitus*, Donitz, 1902; *Anopheles fluviatilis*, James, 1902; *Anopheles philippinensis*, Ludlow, 1902; *Anopheles varuna*, Iyengar, 1924; *Anopheles balabacensis*, Baisis, 1936.

In the North-Eastern Region, with the inclusion of West Bengal States and which is known as the Shillong Region around 32 species of *Anopheles* are present viz.

Anopheles - *aconitus*, *aitkenii*, *annandalei*, *annularis*, *balabacensis*, *barbirostris*, *culcifacies*, *fluviatilis*, *gigas*, *hyrcanus*, *insulaeflorum*, *jamesii*, *jeyproriensis*, *karwari*, *kochi*, *leucosphyrus*, *lindesayi*, *maculatus*, *majidi*, *minimus*, *pellidus*, *philippinensis*, *ramsayi*, *splendidus*, *stephensi*, *subpictus*, *sundaicus*, *tesselatus*, *turkhudi*, *umbrosus*, *vagus* and *varuna* (Bull. Nat. Soc. Ind, Mal Mosq. Dis., 9,2, March, 1961).

Out of which, the following species has been recognized as vectors, namely - *A. philippinensis* *A. minimus*, *A. annularis*, *A. balabacensis*, *A. aconitus*, *A. fluviatilis* (NICD Div. of Med. Ent. and Vector Control).

Flight and dispersal characteristic of anophelines have an important bearing on the distribution pattern (Ramachandra Rao, 1985). These behaviour patterns are controlled not only by the innate abilities of the mosquitoes but also by the nature of the environment, such as, availability and preferences of breeding places, physiological processess which regulate the direction of flight, host preference or effectiveness of the control measures which forces the dispersal (James and Liston, 1911). According to the observation made by Jaswant Singh and Mohan (1951), flight and dispersal which may appear to be purposeless flight may have an evolutionary significance connected with survival. Thus, the process of adaptation of any species may not be complete, due to various environmental changes, even though some may have become acclimatised to the conditions with several allotropic forms.

It has been observed by the present author that the topography of the North Eastern Region of the country consists areas of difficult hilly terrains, semi-hilly terrains, foot-hills and the plains with varieties of forest cover, high rainfall, poor communication and with rivers and streams meets either with river Brahmaputra (India) or Megna river (Bangladesh) - Fig.1. Thus, the distribution pattern of terrain system and the tendency of mass dispersal (Ramachandra Rao, 1984; Jaswant Singh and Mohan, 1951; James and Liston, 1911) could be the further evidences for the distribution and dispersal.

Taxonomic Status

PHYLUM	-	ARTHROPODA
CLASS	-	INSECTA
ORDER	-	DIPTERA
SUB-ORDER	-	NEMATOCERA
FAMILY	-	CULICIDAE
GENUS	-	ANOPHELES

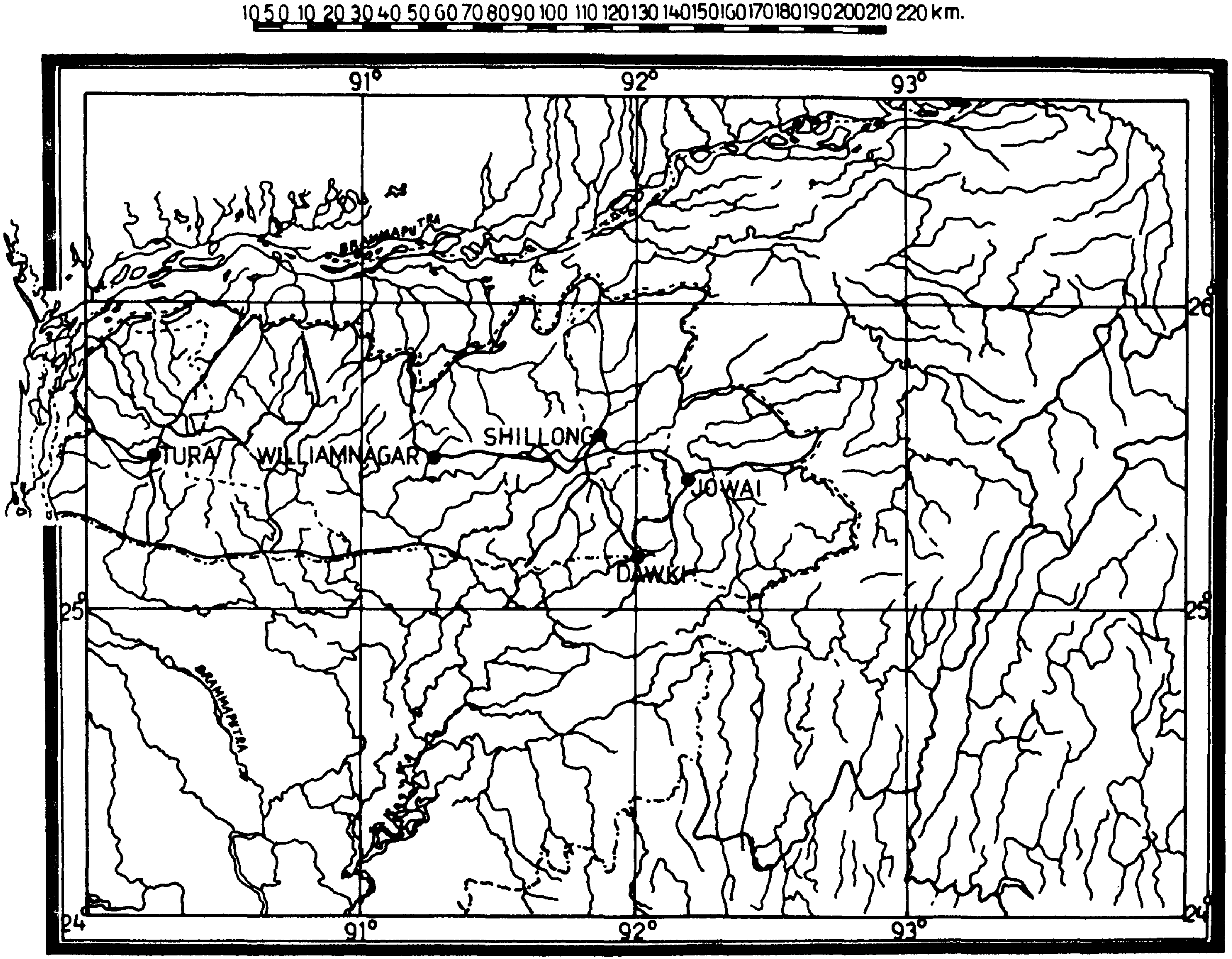

Fig. 1. Map showing the distribution of the lotic system of North-eastern India.

Part-I

BIOLOGY

INTRODUCTION

Biological study of any group of organisms requires an intimate knowledge of the nature of environment in which they live, feed and reproduce (Gupta, 1980). The basic information on the biology of anopheline mosquito in India was available from the extensive works done by different authors in the early decades of the century. The tempo of research was maintained into the 30s and 40s by workers of the Malaria Institute of India and by such stalwarts as Drs. Paul Russel of the Rockfeller Foundation, T. Ramachandra Rao and D.K. Vishwanathan. Research on ecology virtually came to a halt after the early successes of the DDT residual spraying programme of the NMCP (National Malaria Control Programme). Now, it has received fresh impetus with the recognition of the necessity for an integrated approach to malaria control, instead of total reliance on chemical methods. The tempo of investigations on the biology has increased in the recent years and considerable advances were being made (Muirhead-Thomson, 1940; Russell and Rao, 1940, 1942; Russell *et al.*, 1944; Vishwanathan *et al.*, 1952; Quaraishi, 1965; Shalaby, 1966, 1971; Sen *et al.*, 1973; Rajagopal *et al.*, 1976, 1979; Ramachandra Rao, 1978, 1979; Chandrahas *et al.*, 1979; Batra *et al.*, 1979; Banchan, 1979; Garrett-Jones *et al.*, 1980; Curtis and Rawling, 1980; Kalra, 1980; Panicker *et al.*, 1981; Bhatnagar *et al.*, 1982; Rosenberg, 1982; Rawling and

Davidson, 1982; Maheswary, 1982; Reuben, 1984; Mani *et al.*, 1984; Tiwari *et al.*, 1984; Das and Baruah, 1985; Sharma *et al.*, 1986; Dutta and Baruah, 1987; Nagpal and Sharma, 1987; Snellan, 1987; Hati *et al.*, 1987; Chatterjee *et al.*, 1988; Sharma, 1990, 1991, 1992; Mutinga et *al.*, 1992; Wajihullah et *al.*, 1992; WHO, 1992).

This awareness has led to the concern over deterioration of the ecosystem as a result of Man's greed for exploitation of natural resources and poor land utilization. Further, long span uses of insecticides has stimulated research in the basic dynamic of the ecosystem and its biotic communities. In addition, the influences of *man* on any population, is also one of the important parameters in the content of ever-increasing rate of urbanization and industrialization which needs consideration (Clerk *et al.*, 1978). Therefore, before any assessment of population or control effects can be made, or national remedial action is suggested or formulated, there must be a sound detailed knowledge of the natural characteristic of regional ecosystem as a reference standard. This knowledge can be resulted only from investigating the local situation afresh to see whether the old assumptions regarding the identity of various species and their ecology are correct (Reuben, 1980), or to re-investigate the habitat as a unit ecosystem (Evan, 1956) rather than just the specific conditions in the system (Hynes, 1970, Slack, 1955).

Ecological changes have great bearing on the breeding and species composition, resulting in the appearance, disappearance or introduction of various mosquito species (Tandon and Haiti, 1978). It is of considerable interest to confirm the earlier findings in respect of mosquito species composition in different ecologically distinct niche before evolving any suitable abatement/control programme.

The factors which influence and determine the occurrence of certain species of animal in the particular regions of the world have been the subject of much discussion by biologists ever since the times of Darwin and Wallace. Generally speaking, in the course of evolutionary history, many new forms arise either by natural variation and selection or mutation. Their survival and dispersal depend upon their ability to withstand the environment. Certainly the forms which had arisen and have been eliminated are many times more than those which have survived. Modern evolutionary theory based on intimate understanding of ecology and genetics can offer reasonably satisfactory explanation for many of the known features of distribution of species of mosquitoes as well as of their bionomics (Huxley, 1942; Frizzi, 1947; Wattal *et al.*, 1960; Doraisamy, 1963; Reid *et al.*, 1966; Avirachan *et al.*, 1969; Aslamkhan and Baker, 1969; Bryan, 1973; Haridi, 1973; Bhat, 1975; Knight and Stone, 1977; Saifuddin *et al.*, 1978; Subha Rao and Adak, 1978; Miles, 1979).

Physical variables like terrain, soil, water, air and forces such as solar isolation, gravity and molecular energy within a habitat present a continuous gradient of physical conditions (James and Liston, 1911; Iyenger, 1929, 1931; Mehta, 1934; Nursing *et al.*, 1934; Ramachandra Rao and Russell, 1938; Russell and Mohan, 1940; Renn, 1941; Bates, 1949; Muirhead Thomson, 1940b; Vishwanathan *et al.*, 1943, Pal, 1945; Reid and Wharton, 1949; Brooke Worth, 1953; Bhombore *et al.*, 1956; Venkat Rao, 1961a; Quraishi, 1965; White, 1974a; Colluzi *et al.*, 1975; Rajagopal, 1976; Reisen and Aslamkhan, 1976, 1978; Ansari *et al.*, 1977; Reisen and Siddiqui, 1978). These gradients elicit a series of responses within the constituent population from time to time, thereby, resulting in their adjustment to the conditions or facing extinction.

Physiography and climate play and important role in the seasonal prevalence of most species. The exact mechanism of this habit is not known, but it is another illustration of how climate and terrain can influence seasonal abundance of anophelines. In this connection, reference may be made with the case of *A. sundaicus* in India, as it has disappeared from the entire Orissa and Andhra Pradesh coast where it was prevalent till the early 1950s (Rao, 1984).

It is an established fact that distribution of aquatic fauna largely depends on the chemical properties of water. In the case of some anophelines mosquito larvae, it has been noted by a number of workers that, besides other factors, even very small amount of free ammonia has marked inhibitory effects on their breeding. In this connection Senior-White (1948) has shown that, except in the case of rossi-group, saline ammonia is inhibitory to anopheline breeding in amount exceeding one part per million. This effect was clearly seen in cases of most anopheline mosquitoes of India (Challam, 1924; Iyengar, 1931; Dev Raj Mehta, 1934; Senior-White and Adhikari, 1939). The mode of toxicity as well as concentration lethal to a variety of organisms have also been well documented (Warren, 1962; Campbell, 1973). While many environmental disturbances have no instant detectable effects on aquatic invertebrates in the short terms, they may prevent normal reproduction and cause eventual local extinction of a species (Lehmkulhl, 1979). Heavy metal and toxic substances may drastically reduce reproduction rates in species exposed to sublethal levels. Dissolved slats and pH affects organisms at abnormally high and low levels but most mechanisms are unknown (Sen, 1938b; Lehmkuhl, 1979).

The interaction and changes between temperature and humidity in a natural habitat have great influence on the life of many insects. Changes of this kind caused by wanton destruction of forest, unplanned urbanization and human behaviour, formed an effective alteration in the temperature and humidity. Mosquito in general is a haterothermal or cold-blooded animal and its body temperature varies according to the temperature of environment. Most insects which withstand sub-zero temperature do so by super-cooling, thus preventing their body water from freezing during winter (Salt, 1961). The aquatic stages of mosquitoes shows an increasing sensitivity to high temperature (Davis, 1932; Muirhead-Thomson, 1940c; Pal, 1945; Barr, 1952; Lal, 1953; Nielsen and Haeger, 1954; Haufe and Burgers, 1956; Bar-Zeev, 1957b; Love and Welchel, 1957). Not a great deal is known about the sensitivity of mosquito to low temperature. But it is known that prolonged exposure of mosquito to only moderately low temperature are detrimental (Smith, 1901, 1902; Shute, 1929; Hearle, 1929; Mac Gregor, 1932; Bliss and Gill, 1933; Huffakar, 1944; Haufe, 1952; Lal, 1953). The survival of adult mosquitoes at moderate and high temperature also depends in most cases upon both temperature and humidity. At optimum humidity the survival is inversely proportional to the temperature (Ingram, 1954) and at moderate temperature the mortality is roughly inversely proportional to the relative humidity even though it does not appear to be a direct relationship to longevity (Lewis, 1933; Mehta, 1934; Karamchandani, 1935; Afridi *et al.*, 1940; Muirhead-Thomson, 1941b; Russell and Rao, 1942c; Pal, 1943; Bar-Zeev, 1957b). High temperature also affect the survivality of adult mosquito, as a result of desiccation consequent on evaporation (Mellanby, 1932; Karamchandani, 1935). But more precise studies on such relationship are needed in natural communities where the fluctuation do occur from season to season.

The population of insect community in a particular habitat fluctuates to a greater or lesser extent in time and space. The study of such natural population, therefore, requires an intimate knowledge on the concepts of population and the environment in which they live. Gauge (1961) defined population as a collection of individuals persisting through a familiar but vaguely delimited time span with an ill defined area or space, whereas their environment include abiotic milieu - the non-living organic matter, plants and animals inclusive of other members of the population. On the other hand, Solomon (1949) emphasized that population and environment are inseparable and every population requires a real ecological setting for its function, rather than an imaginary ecosystem minus the population, called the environment. By this statement he redefined the ecosystem concept originally proposed by Tansley (1935) to explain the inter-relationships between the functional aspects of a population and the sets of abiotic conditions. Such approach could be applied to study of population dynamics as a "life system" which consists of population itself and its effective environment wherein the external influences such as the biotic and abiotic factors are observed and measured.

The ability of an established community, called an epicentre, to recolonize and impoverished area with regard to total number of species present, becomes especially critical when the ecosystems exist under the strain of either a chronic or acute source of toxic stress (Cairns, Jr. *et al.*, 1980). Density of a species is among the most important factors in the ecological studies, for it determines the numbers in which a species is encountered in specific time in a given area. Sir Ronald Ross (1911) stated, the transmission of malaria by *Anopheles* mosquitoes are the function of the numbers in which they occur. Subsequently, in India attempts were made to correlate the number of female mosquitoes that could be captured within a given time with malaria transmission or absence of it in an area. Careful studies by Russell and Rao (1942), showed that malaria transmission corresponds with a period of high density of the vector. Similarly, "critical densities" have been determined for other *Anopheles* species also (Vector of Malaria in India, 1961). In general, the concept of critical density is a valid one as long as it is remembered that other factors, notably longevity (Macdonal and Davidson, 1935) may alter its absolute value under particular circumstances (Reuben, 1980). Thus, identification of such component becomes imperative for the clear understanding of their ecosystem. Further, as mentioned earlier, influences of man on any population, is also one of the important parameters in the content of ever-increasing rate of urbanization and industrialization which needs consideration (Clark *et al.*, 1978).

Keeping in view the above facts, the present study on biology of the various *Anopheles* mosquitoes was being undertaken. The study includes collection of information on the topography, climate, geology, lithology, vegetation cover and landuse, nature of perturbations, the physico-chemical variables of the environment and with special emphasis on the population dynamics and seasonal prevalence.

STUDY AREA

General Consideration

Meghalaya is one of the seven north-eastern states of India with a population of 13,35,819 as per census of enumeration of 1981. Nearly 81.96 per cent of the population of the state live in rural areas. The state has a total of 4902 villages, of which, 65 per cent are very small with an average population of less than 200. The population of Meghalaya is predominantly tribal which constitutes nearly 80.6 per cent. the Scheduled Caste population is barely 0.41 per cent and others is 19.01 per cent of the total population of the State. It has an area of approximately 22,500 sq. kms. (Government of Meghalaya, Eighth Five-Year Plan, 1990-95, Vol.I). The state lies between 25°5" and 26°10" North latitudes and 98°47" and 97°47" East longitudes. It is a strip of land spread along the northern boundary of Bangladesh and is bounded by that country on the south and as well as the west. The length of the International boundary is about 496 kms. The State is surrounded on the other sides by Assam (Fig.2). Access to the State is mainly by road from Assam. The land surface of the State mostly comprises steep hills, and deep gorges, the valley land being very small.

Meghalaya was under the National Malaria Control Programme (NMCP) since 1953, National Malaria Eradication

Programme (NMEP) since 1958, and since 1977 onward the area is under Modified Plan Operation (MPO). As a result of the successive programmes implemented, yet there was still resurgence of the disease with slight fluctuation. As already mentioned by the programme implementation authorities, the main drawback in the control of the disease was due to several factors, viz. administrative, operational and technical. As pointed out, in the area of the Garo Hill Districts, the villages live in *jhum* huts in deep jungle area for rice cultivation virtually during the whole transmission seasons and their houses in the villages are kept locked. Therefore, the coverage of insecticides spray is greatly affected. Whereas, hundred of villages in Khasi, Jaintia Hills and Ri-Bhoi districts are rearing silkworms and bee-hives from time immemorial, very little insecticides coverage is possible in these villages as people are not only refusing but are very hostile to DDT sprays, as it indirectly affects their livelihood. Further, the increased in population, various development works under successive Five-Year Plans, road construction, minor irrigation and unscientific mining of minerals by the private parties, have all contributed to the creation of fertile foci for breeding of mosquitoes, thereby contributing to the spreading of malaria.

Byrnihat Primary Health Centre

Byrnihat primary health centre, the area chosen for the study, is one of the 34 Primary Health Centre in Meghalaya. The centre form a part of the administrative block of the Ri-Bhoi district. This primary health centre spread over wide areas and covers about 106 villages of diverse ecological conglomeration and with a population of about 21,219. It is known to be a malaria endemic area and as seen in Table-1, the occurrence of the disease is quite prominent. This Primary Health Centre (PHC) is bordered by Assam of eastern Himalayas in the North-West and East Khasi Hills of Meghalaya on the South. The only river known as Umtrew originates in the West Khasi Hills and merges into Brahmaputra (Assam) on the North-East.

Topography

Approximately the geographical location of the land is 26°1'58" North 91°53'02" East and the altitude of 182 metres means sea level (Fig.2). The Primary Health Centre could be divided into three topographically different regions, viz. hilly region covered with forests, foot-hill regions and low-lying flood plains used mainly for rice cultivation during the monsoon and remain as pasteur lands for the rest of the years. In addition of streams there are several natural and

Table-1

Monthly Epidemiological Report of Byrnihat Area obtained during the Period of Investigation

Sl. No.	Month and Year	Active Case Detected (ACD)			Passive Agencies (PA)			Total	
		Collected	Examined	Positive	Collected	Examined	Positive	Collected	Postive
1.	May 1992	221	221	9	33	33	1	254	10
2.	June 1992	160	160	10	70	70	13	230	23
3.	July 1992	168	168	3	20	20	1	188	4
4.	Aug. 1992	195	195	12	19	19	6	214	18
5.	Sept.1992	151	151	11	3	3	-	154	11
6.	Oct. 1992	189	189	6	54	54	7	243	13
7.	Nov. 1992	126	126	17	21	21	6	147	23
8.	Dec. 1992	146	146	19	18	18	1	164	20
9.	Jan. 1993	120	120	22	21	21	5	141	27
10.	Feb. 1993	120	120	12	15	15	2	135	14
11.	Mar. 1993	65	65	4	29	29	3	94	7
12.	Apr. 1993	65	65	-	17	17	-	82	-
13.	May 1993	40	40	-	70	70	13	110	13
14.	June 1993	158	158	10	82	82	14	240	24
15.	July 1993	229	229	8	36	36	4	265	12
16.	Aug. 1993	164	164	8	2	2	-	166	8
17.	Sept.1993	169	169	4	203	203	6	372	10
18.	Oct. 1993	174	174	21	61	61	5	235	26
19.	Nov. 1993	113	113	12	39	39	5	152	17
20.	Dec. 1993	65	65	1	46	46	5	111	6
21.	Jan. 1994	32	32	3	8	8	2	40	5
22.	Feb. 1994	60	60	7	18	18	2	78	9
23.	Mar. 1994	61	61	3	8	8	-	69	3
24.	Apr. 1994	94	94	4	12	12	3	106	7
	Total	3085	3085	206	905	905	104	3990	310

Source : District Medical and Health Officer (DM & HO), Nongpoh, Ri-Bhoi District, Meghalaya

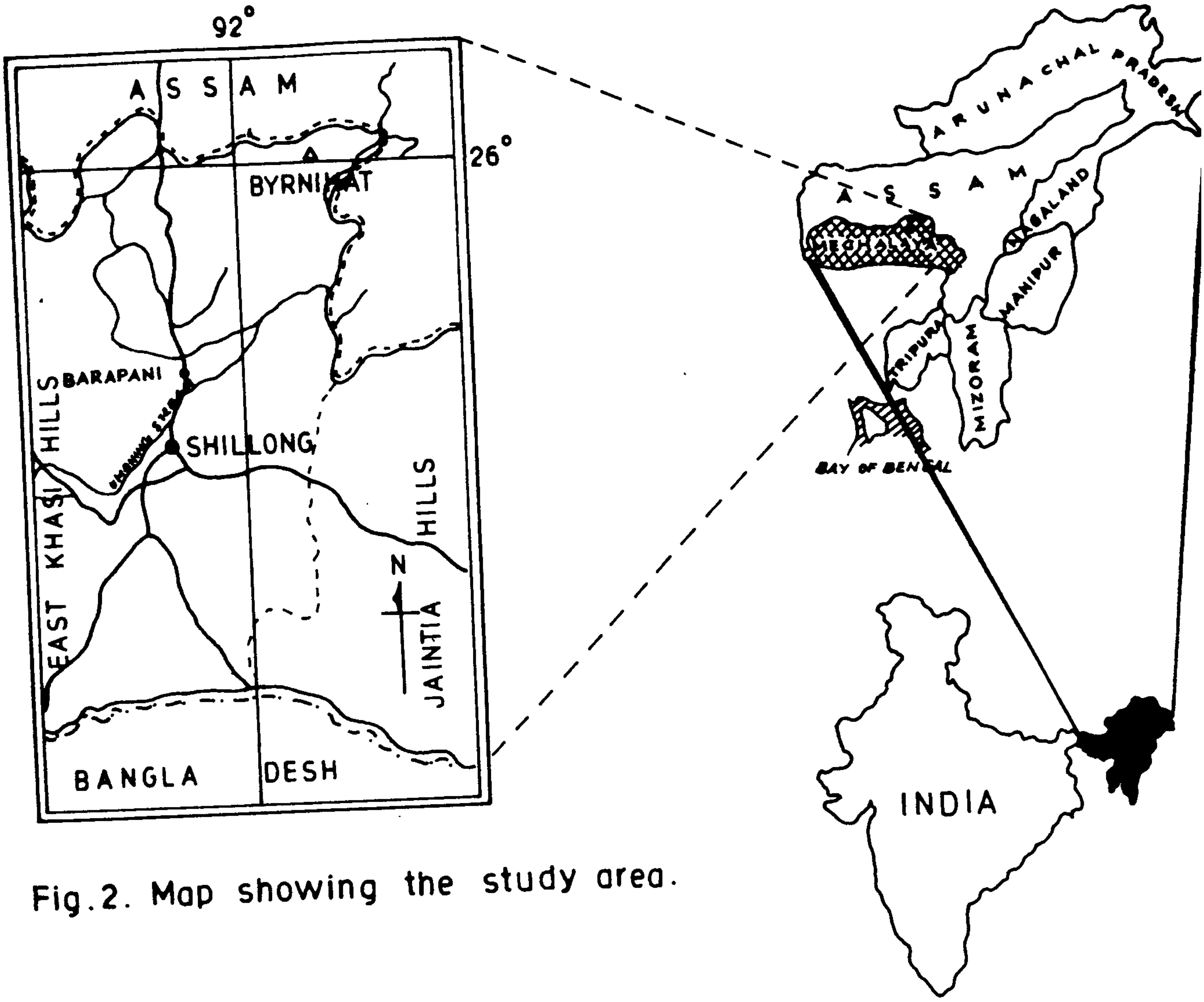

Fig.2. Map showing the study area.

man-made ponds used mainly for cultivation, domestic purpose and rearing of fishes.

Climate

The region under study has a typical monsoon climate with high rainfall (Fig.3). In fact, Mawsynram, Cherrapunjee, Pynursla belt in Khasi Hills along the southern border records a rainfall varying from 1,000 to 15,000 mms per year. In the present study, based on temperature regimes, each annual cycle has been divided into three seasons :

i) Wet season : May to October

ii) Cool dry season : November to February

iii) Hot season : March to April.

The summer temperature in Byrnihat areas goes as high as 29°C, while in winter it falls to 16°C. Flash floods and spates in the streams during the rainy season due to high rainfall is quite frequent but permanent flooding of the drainage basin for longer period never occurs.

Geology

The highland comprising the Khasi, Jaintias, Garos and detached Mikir Hills is known as the Shillong Plateau, which forms the North-Eastern part of the continental mass of India. Physiographically, it represent a remnant of an ancient plateau of pre-Cambrian Indian Peninsular shield

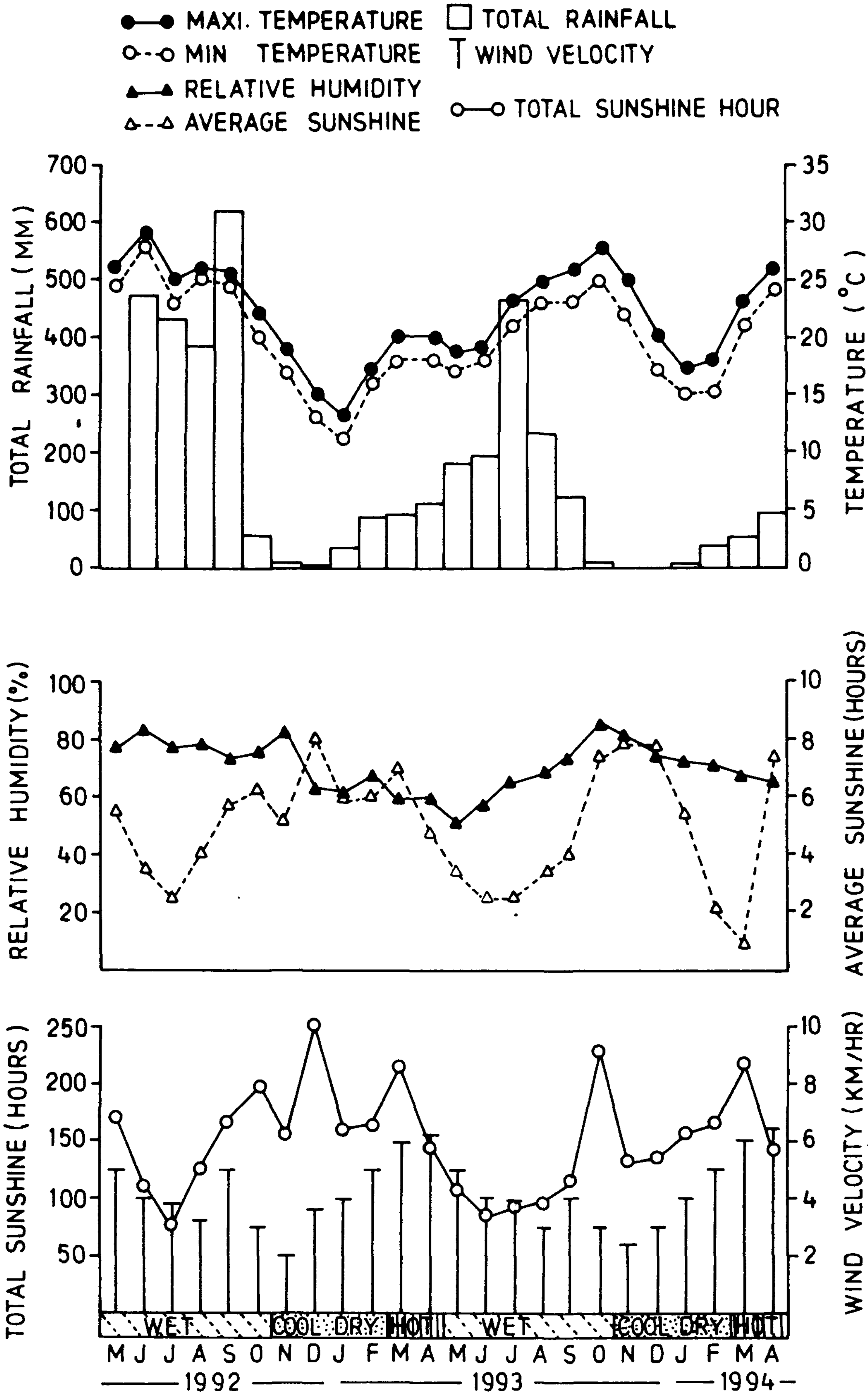

Fig. 3. CLIMATIC ASPECTS OF BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION

uplifted to its present height of 610 m. to 1544 m. above the sea level. The karnal of the plateau is the exposed Archean gneisses and schists covered in this area by Pre-Cambrian quartzites and phyllites. This was later marked by the intrusion of younger granites and basic/ultrabasic schists. This ancient pene-planned surface of the plateau is still preserved with marks of different cycles of denundation. It is hidden beneath the mezoic traps along the central, southern fringe and cretaceous, tertiary and post-tertiary sediments. The polycyclic erosional surface at various levels indicate that the present physiographic configuration of the plateau was obtained through different geological events since Mesozoic to present study.

The tectonic history of the plateau begins with the effusion of plateau basalts (Sylhet traps) through fractures and faults in the basement and uplifts and subsidence of the adjacent basement blocks. These were followed by upper cretaceous - tertiary sedimentation into the relatively down lifted portion along the faults. The tectonic force has been known to be vertically dominated and controlled by differential movements along the basement fractures. Further details of the tectonic theory is not discussed here as it is outside the purview of the present study.

Lithology

The rocks around Byrnihat area are mainly Gneisses, Schists and Granites (D.G., G.S.I. Report, 1974), (Fig.4). The clay rocks consist of phyllites, mica schists and carbon phyllites, while the sandy type includes Green stones, granites and upper cretaceous sandstones. The sandstone are mainly coarse grained. The chemical composition of the rocks in terms of various oxides is as follows :

SiO_2 : 51.94%, Al_2O_3 : 6.70%, TiO_2 : 6.64%, Na_2O : 1.10%, K_2O : 0.05%, CaO : 8.95%, MgO : 4.76% and the rest includes other trace element oxides.

Soil

By and large the soil in the state is acidic in nature and comparatively rich in organic matter and nitrogen but poor in phosphorus. They are mostly lateritic type derived largely from granites, schist rocks and vary from sandy, red and clayed loam to sandy lime. The soil in the basin are more fertile than the upland soils since much of the bases and organic matter from the top soil of the latter gets washed away due to high rainfall. The sediments are mostly sandstones and shales (mudstones) and well-defined fossiliferous limestones. The percentage of organic carbon is nearly 5 to 10 and pH between 4.5 to 6.5.

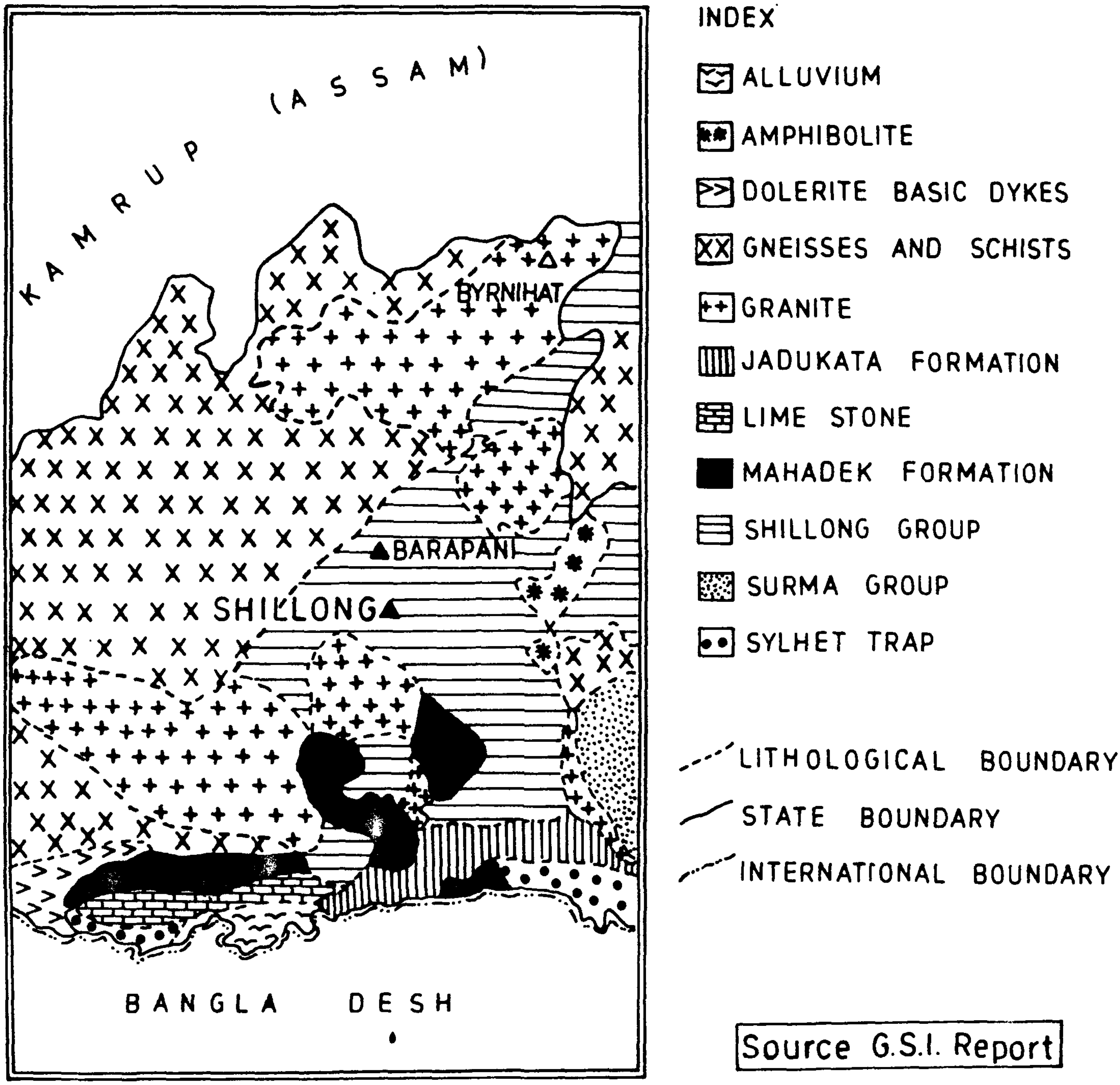

Fig. 4. Lithological map of the study area.

Vegetation and General Land Use

The natural vegetation of the area is a forest, which has been grouped by Champion and Seth (1968) in most tropical forest. The natural forest is now confined to small areas. Whereas large one of this region is under secondary growth of forest on the abandoned *jhum* land. Depending on the fallow age, degree of disturbance and species composition of two forest stands showed great variations.

The overall practice of agricultural activities are dependent on rainfed agriculture. The situation of degraded forest, agricultural land, rainfall, common practices of *jhum* and *Bun* cultivation etc. cause serious degradation of soil and hence immediate programme of operation is needed to conserve soil moisture. Keeping in view the problems and potential of rainfed farming, the development objectives of National Watershed Development Programme for Rainfed Agriculture (NWDPRA) in the state as a whole for the Eighth Five-Year Plans are as follows :

1) To conserve, improve and utilise scientifically the neutral endowments like land, water, plant, animal and human resources for ensuring an economically sustainable and perpetually productive bio-system.

2) To secure stability and growth of agriculture production for ensuring food security and to meet the growing

demand of human and livestock population for food, fodder, fuel, fibre and drinking water.

3) To generate employment and economic earning opportunities for providing livelihood security to the rural people in these under-privileged areas, particularly the small, marginal and tribal farmer.

4) To promote, restoration of ecological balance through scientific land and water management.

5) To reduce regional inequalities through a wider spread of agricultural growth.

Study Villages at Byrnihat PHC

Villages for the Entomological study are selected on the basis of malaria situation and accessibility, as such it represents the most obvious areas of the land. Four villages were selected from all the habitat under study and represent finally as a unique single sampling site. The four villages selected are Harilibhogan, Norbong, Garo-basti and Amjok (Figs.5,6,7,8). It was hoped that the areas chosen would cover the main range of condition found in various zones.

Fig.5 Photograph showing the study area at Harilibhogan.

Fig.6 Photograph showing the study area at Norbong.

Fig.7 Photograph showing the study area at Garo-basti.

Fig.8 Photograph showing the study area at Amjok.

MATERIALS AND METHODS

Limnological Study

Physico-Chemical Analysis

Water samples from the larval breeding sites were collected every month from May, 1992 to April, 1994 (24 months). All the samples immediately after collection were passed through a 0.45 M filter (Crowther and Hynes, 1977) and brought to the laboratory. Various parameters were analysed and estimated within 24 hours.

In addition to air and water temperature readings which were measured in the field using a mercury bulb thermometer (Misra, 1968) are maximum and minimum air temperature, whereas relative humidity, rainfall, wind velocity of the area under consideration were collected regularly from the local meteorological station during May, 1992 to April, 1994.

pH and conductivity were measured with the use of a Toshniwal pH meter (Model CAT. CL.-43) and Elico conductivity bridge (Type CM-82) respectively. Carbon-dioxide by phenolphthalin-N/44 NaOH and total alkalinity using the standard method (APHA, 1965). Ammonia nitrogen by Nessler's reagent (Mackereth, 1963), phosphate-phosphorous by Stannous chloride and molydate method (APHA, 1965) and oxidazable organic matter by permanganate method.

Anopheles Mosquitoes Population Study

The monthly collection of the Anopheles mosquitoes for the purpose of their relative abundance and seasonal prevalence were made for 24 months (May, 1992 to April, 1994).

<u>Anopheles larvae ecosystem study</u>

The larvae breeding sites where collection is made vary in size and shape and their surface is apt to fluctuate with seasonal environmental changes. It also vary from unpolluted oxygenated water of temporary or permanent character viz. burrow pits, ponds, swamps, rice-field, irrigation canals, especially in those containing aquatic vegetation, particularly green algae to water jar and other miscellaneous containers. The principle containers in the miscellaneous group were plate used as flower-pot stands, foot-paths, small side drains, tyres (unused) and other where temporary accumulations of water exists especially during rainy seasons.

The collecting device adapted for the present study depends upon the type and size of breeding places investigated :

- a ladle with a diameter 10 cm was used for collecting the larvae from the large water bodies of temporary or permanent character (WHO, 1975). A total of 20 dips was done for every sites under study (Fig.9).

- from the miscellaneous habitats, were the application of the above method is not possible, the larvae were collected by using a glass-dropper (Fig.10).

Larval-pupal counts of mosquitoes were made every month during the study period, by straining the water from the container examined through a plankton net, then the larvae under study were identified to a genus level with the help of a standard keys (Christopher, 1933, Barraoud, 1934; Puri, 1953; Das *et al.*, 1990).

Analysis of the Larval Habitat (Miscellaneous)

The analysis of variance was carried out using two different levels, that is, at two criteria of classification and three criteria of classification, by using the following formulas :

1) Two criteria of classification : We classified our sample of N values of X according to some quality A into K classes and, according to another quality B, into n classes so that N=nk. Then the sample variate value in the ith A-class and jthfB-class are identify as X_{ij}. And$\bar{X}_j$ are the mean of the jth row and X_i the mean of the ith column. We then have the following identity :

$$\sum_{i=1}^{k} \sum_{j=1}^{n} (X_{ij}-\bar{X})^2 = \sum_{i=1}^{k} \sum_{j=1}^{n} \{(X_{ij} - \bar{X}_i - \bar{X}_j + \bar{X}) + (\bar{X}_i - \bar{X}) + (\bar{X}_i - \bar{X})\}^2$$

$$= \sum_{i=1}^{k} \sum_{j=1}^{n} (X_{ij} - \bar{X}_i - \bar{X}_j + \bar{X})^2 + \sum_{i=1}^{k} \sum_{j=1}^{n} (\bar{X}_i - \bar{X})^2 + \sum_{j=1}^{n} \sum_{i=1}^{k} (\bar{X}_j - \bar{X})^2$$

$$= \sum_{i=1}^{k} \sum_{j=1}^{n} (X_{ij} - \bar{X}_i - \bar{X}_j + \bar{X})^2 + \sum_{i=1}^{k} n (\bar{X}_i - \bar{X})^2 + \sum_{j=1}^{n} k(\bar{X}_j - \bar{X})^2$$

2) Three criteria of classification - The sample values of normal variate are classified according to three criteria, A, B, C into l groups of m rows and n columns so that N=lmn where N is the total number of sample values. X_{ijk} denote the value of the variates in jth row and kth column of the ith group, where i=1,2,3, ... l, j=1,2,3, ... m and k=1,2,3, ... n.

We then have the following identity :

$$\sum_{i=1}^{l} \sum_{j=1}^{m} \sum_{k=1}^{n} (X_{ijk} - \bar{X})^2$$

$$= mn\sum_{i=1}^{l} (X_i = \bar{X})^2 + kl\sum_{j=1}^{m} (\bar{X}_j = \bar{X})^2 + lm\sum_{k=1}^{n}$$

$$(\bar{X}_k - \bar{X})^2 + l\sum_{j=1}^{m} \sum_{k=1}^{n} (\bar{X}_{jk} - \bar{X}_j - \bar{X}_k + \bar{X})^2 +$$

$$n\sum_{i=1}^{l} \sum_{j=1}^{m} (\bar{X}_{ij} = \bar{X}_i - \bar{X}_j + \bar{X})^2 + \sum_{i=1}^{l} \sum_{j=1}^{m} \sum_{k=1}^{n}$$

$$(\bar{X}_{ijk} - \bar{X}_{jk} - \bar{X}_{ik} - \bar{X}_{ij} + \bar{X}_i + \bar{X}_j + \bar{X}_k - \bar{X})^2$$

where $\bar{X}$ = overall mean.

Anopheles Adult Ecosystem Study

Adult female mosquitoes normally feed at night on human/animal baits and rest during the day. The resting places vary in character and differ considerably with the different species of mosquitoes. Some are found to rest indoor (Endophilic) or outdoor (Exophilic). The condition (shelter), which seem to attract the adults mostly are those which combine darkness/diffused light with relatively high humidity and minimum air movement.

The indoor shelter includes human dwelling/cattle shed. They are found to rest preferably in the corners/ceilings, behind hanging objects, under furnitures, in shelves, earthen pots, baskets, in heaps of dry cowdung, cokes and in piles of fire-wood.

The outdoor shelter includes hallow tree stumps, unoccupied dense objects, dense shrubs, tall grasses, unused cistern and the like.

The Anopheles mosquitoes resting on the surface or feeding on host species (cattle/man) were collected by using an aspirator tube (Fig.11). It is made up of glass tube and about 30-45 cms. in length, 8-12 mm internal diameter and having a piece of mosquito netting trapped over one end, and a 50 cm long rubber tuning is fitted over the end of the glass tubing provided with mosquiting and a small plastic tubing is inserted into the opposite end to form a mouth-piece (Belkin *et al.*, 1965).

The Anopheles mosquitoes collected were then anesthetize using chloroform and identified with the help of standard keys (Christopher, 1933; Barraud, 1934; Wattal and Kalra, 1961; Das *et al.*, 1990).

To get a correct assessment of the seasonal prevalence and relative abundance of adult *Anopheles* species occurring in an area, four villages were selected, and in each village ten catching stations (5 human/mixed dwelling and 5 cattle sheds) are established. The collection (involving two workers) were made from each village once a month (for a period of 24 months) and for a period of 40 minutes/Man/catching station. The Anopheles mosquitoes were recorded species-wise every month and totalled together.

Man Hour Density (M.H.D.)

From the data collected the density of Anopheles and of individual species are expressed in terms of ManHour Density (MHD), obtained following this formula:

$$MHD = \frac{N}{M \times N'} \times 60$$

where N = Total nos. of mosquitoes species collected,
M = Time spent (minutes) by each worker,
and N' = Total nos. of workers.

<u>Statistical Analysis of Anopheles Adults for Different Localities</u>

In order to compare the species abundant of different localities, we have calculated some of the statisitical analysis, using the following formulas:

$$\text{Mean} = \frac{(\Sigma x_i)}{n} = \frac{\text{Sum of all observations}}{\text{Nos. of observations}}$$

$$\text{SD (Standard Deviation)} = \frac{\Sigma\ (x_i - \bar{x})^2}{n}$$

$$\text{Kurtosis} = \frac{\{\Sigma(x_i - \bar{x})\}^4}{(SD)^2}$$

For all the above analysis, meaning of the signs shown are as follows -

x_i = Values of the ith variable,

$\bar{x}$ = Mean of the same variable,

$(SD)^2$ = Square root of standard deviation

n = Nos. of observations.

$$\text{Coefficient variation} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

$$\text{Skewness} = \frac{\text{Mean} - \text{Mode}}{SD}$$

Fig.9 Photograph showing the sampling method for collection of larvae from rice-fields.

Fig.10 Photograph showing the sampling method for collection of larvae from miscellaneous structures.

Fig.11 Photograph showing the sampling method for collection of adult.

RESULTS

Limnological Aspect (Individual Level)

Physico-Chemical Analysis

The present investigation includes a study of nine physico-chemical factors for twenty-four months (May, 1992 to April, 1994) at four stations (Harilihogan, Norbong, Garo-basti and Amjok) at Byrnihat area having distinctive climatic conditions.

I. Rainfall : At Byrnihat areas, the rainfall commenced in early April and continued upto September (Fig.3). However, the months of July and September experienced higher precipitation, whereas the months of November and December almost being a rainless period.

II. Water Temperature : The water temperature showed a distinct summer maxima and winter minima with small differences caused by rain and prolonged cloudy weather, the peak and fall were prominent in all the study area. Individually, the stations - Horilibhogan, Norbong, Garo-basti and Amjok had a fluctuation range of 28°-14°C, 80°-13°C, 31°-15°C and 27.5-11°C approximately (Fig.12).

III. Hydrogen ion concentration (pH) : The pH of the water fluctuated considerably throughout the year. Comparatively, the pH was mostly leaning towards the alkaline range of 5.8-6.8, 6.2-7.8, 6.3-7.7, 5.9-7.2 respectively (Fig.13) at Harilibhogan, Norbong, Garo-basti and Amjok.

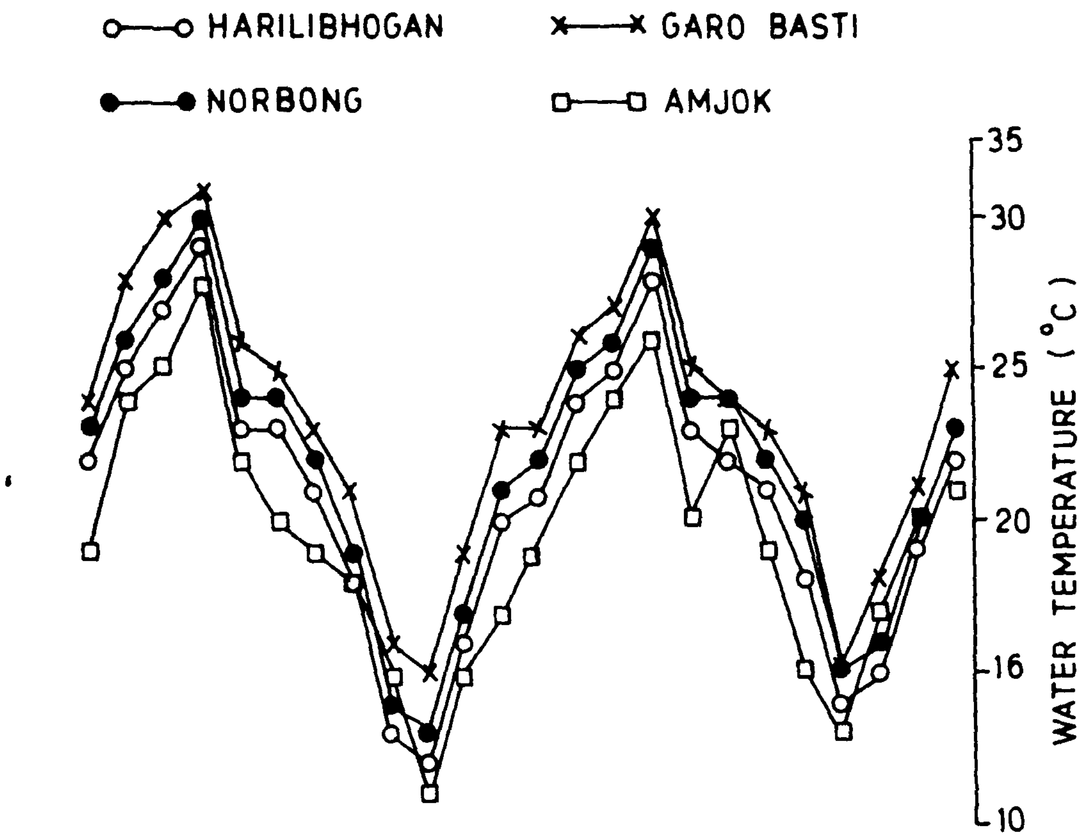

Fig. 12. WATER TEMPERATURE AT FOUR DIFFERENT STATIONS.

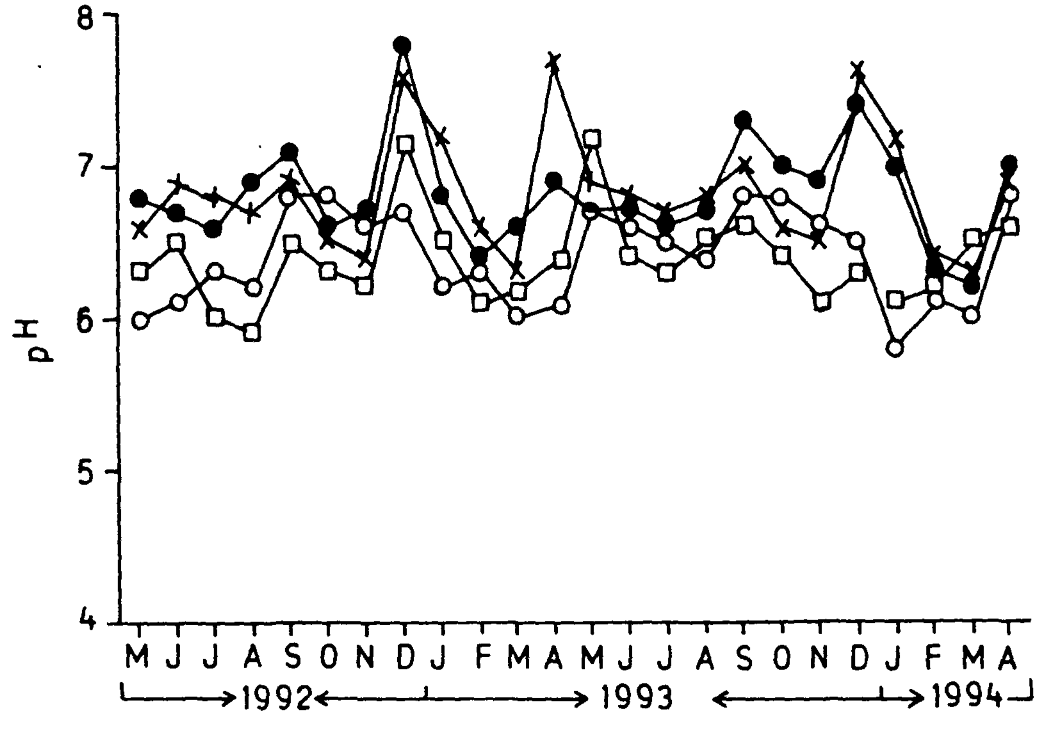

Fig. 13. pH VALUES AT FOUR DIFFERENT STATIONS.

IV. Carbon-dioxide : It was varying in general from 1.5 to 5.0 mg/l. The stations at Harilibhogan, Norbong, Amjok and Garo-basti had a fluctuating range of 3.0 to 5.0 mg/l, 2.7 to 4.8 mg/l, 2.4 to 4.7 mg/l, 1.5 to 4.6 mg/l, respectively (Fig.14). As seen, Amjok recorded a quite low carbon-dioxide concentration comparing to the other stations under study.

V. Specific Conductivity : It varies between 35.5 to 62.3, 22.2 to 56.1, 21.2 to 56.4 and 33.3 to 48.8 µmho/cm at Harilibhogan, Norbong, Garo-basti and Amjok (Fig.15). During the first annual cycle Amjok experienced a quite low conductivity.

VI. Total alkalinity : Both phenolpthelein (carbonate) alkalinity and methyl orange (Bi-carbonate) alkalinity was recorded at all the stations, although the former quantity was lesser than the latter. Total alkalinity at Harilibhogan, Norbong, Garo-basti and Amjok varied from 17.3 to 38.2 mg/l, 18.8 to 47.2 mg/l, 19.9 to 50.0 mg/l and 15.1 to 33.3 mg/l respectively (Fig.16).

VII. Phosphate-Phosphorous : Comparatively, it fluctuated in general from 0.23 mg/l to 1.30 mg/l and had a summer maxima and winter minima at all the stations. Individually, phosphate-phosphorous content varied in the range of 0.43 to 1.10 mg/l, 0.23 to 0.90 mg/l, 0.32 to 1.30 mg/l and 0.34 to 0.92 mg/l at the stations - Harilibogan,

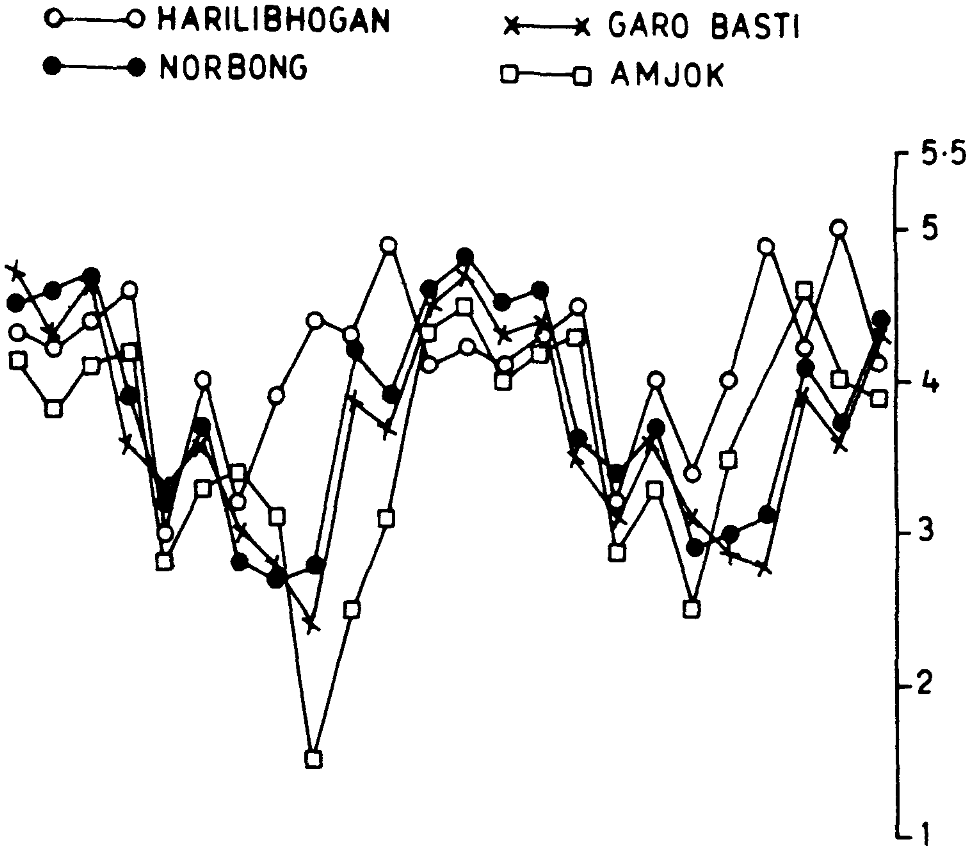

Fig. 14. CARBON DIOXIDE VALUES AT FOUR DIFFERENT STATIONS.

Fig. 15. SPECIFIC CONDUCTIVITY VALUES AT FOUR DIFFERENT STATIONS.

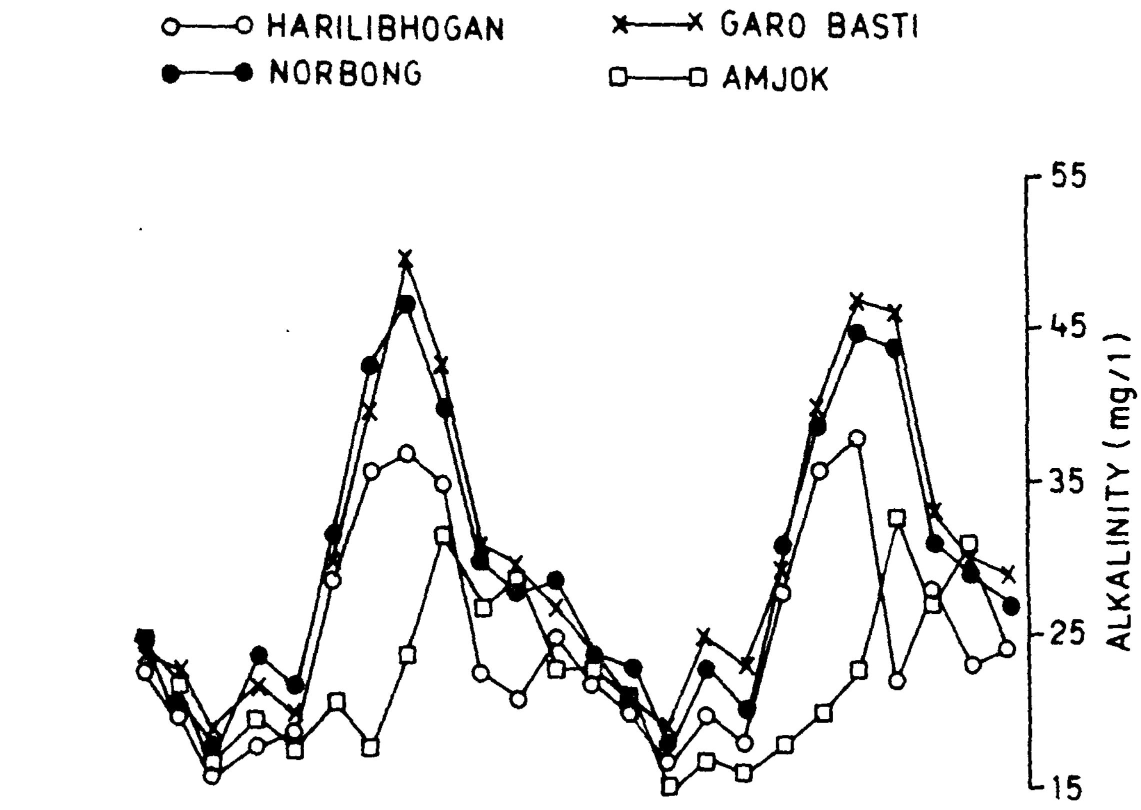

Fig. 16. TOTAL ALKALINITY VALUES AT FOUR DIFFERENT STATIONS.

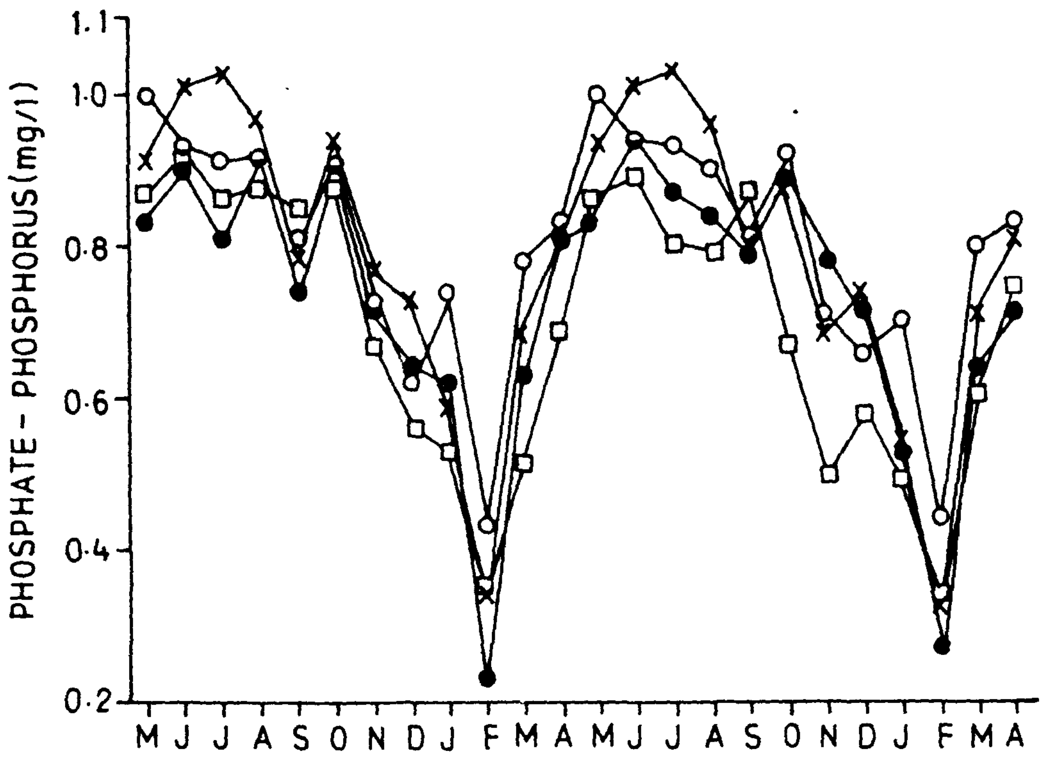

Fig. 17. PHOSPHATE - PHOSPHORUS CONCENTRATION AT FOUR DIFFERENT STATIO

Norbong, Garo-basti and Amjok (Fig.17). Garo-basti recorded the maximum phosphate-phosphorous value in the months of June 1992 and July 1993.

VIII. Ammonia-nitrogen : In general it fluctuated from 0.052 mg/l to 0.097 mg/l. Individually, Harilibogan, Norbong, Garo-basti and Amjok recorded its fluctuation range of 0.051 to 0.093 mg/l, 0.052 to 0.097 mg/l, 0.051 to 0.091 mg/l and 0.052 to 0.09 mg/l respectively (Fig.18).

IX. Oxidisable organic matter : Comparatively it was found to be higher and revealed summer maxima and winter minima at all the stations. Oxidisable organic matter content at Harilibhogan, Norbong, Garo-basti and Amjok are seen to fluctuate in the range of 2.74 to 8.6 mg/l, 3.0 to 8.4 mg/l, 2.62 to 8.4 mg/l and 2.71-7.5 mg/l respectively (Fig.19).

Anopheles Population Study

Byrnihat and the surrounding area are divided into three main seasons in a year: a wet season which begins in May and last upto October, a cool dry season from November upto the end of February and a hot dry season during March and April. There is usually a gradual transition between seasons. Figure-3 shows the region under study has a sub-tropical monsoon climate with high rainfall. Usually, during

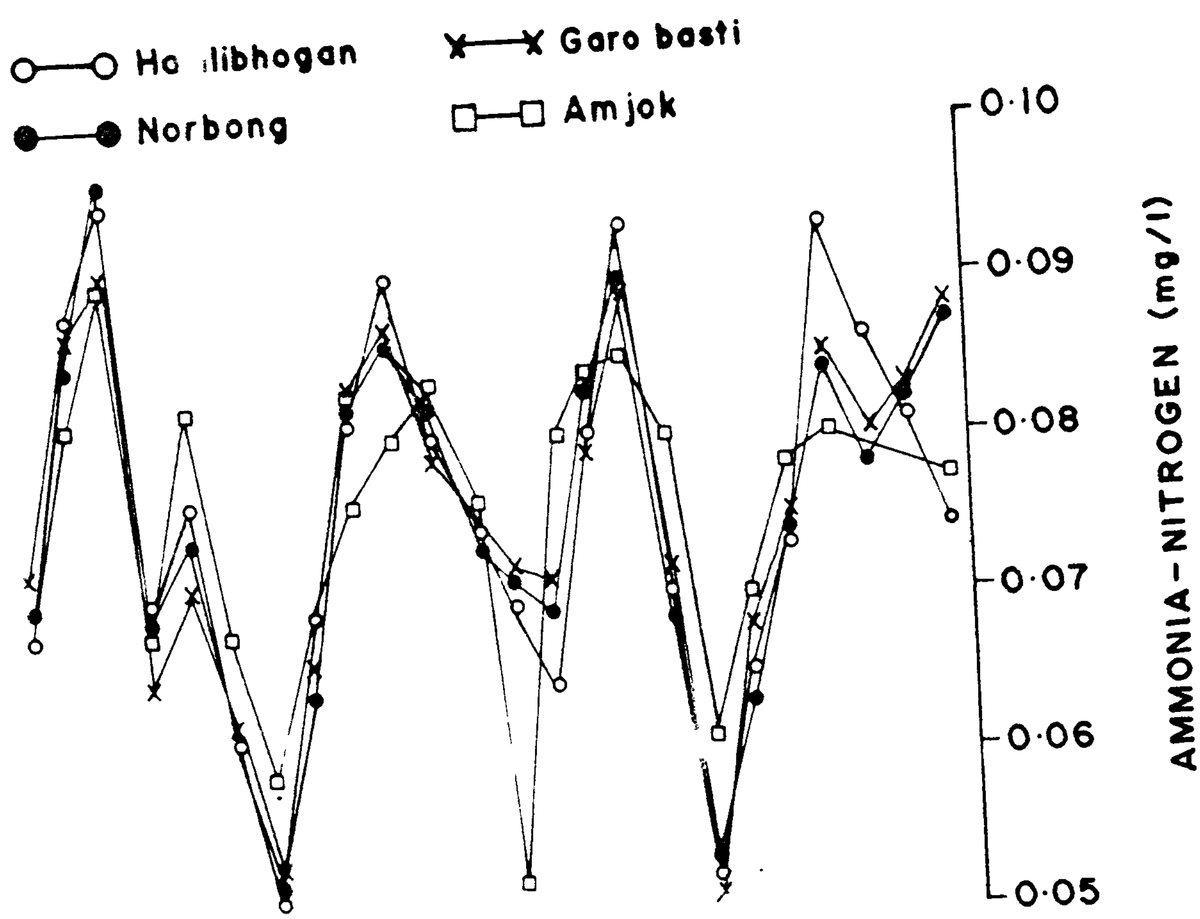

Fig. 18. Ammonia-nitrogen concentration at four different stations

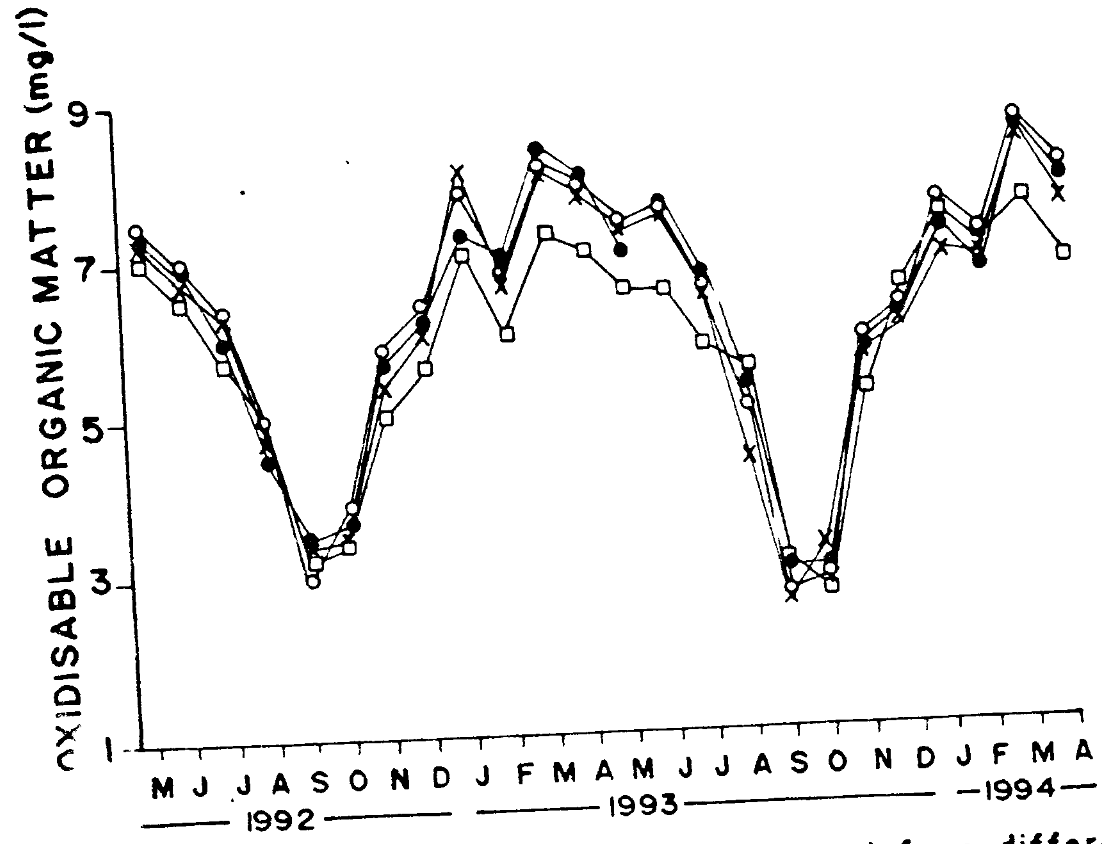

Fig. 19. Oxidisable organic matter values at four different stations

rainy season, there is a lowering of temperature and the increased of humidity. Such a condition, as observed, favour the abundance of adult mosquitoes population. Also, in view of the distinct seasons, the physico-chemical variables under study, such as, water temperature, pH, carbon-dioxide, specific conductivity, total alkalinity, oxidisable organic matter values, ammonia nitrogen concentration and the phosphate phosphorous concentration shows positively significant correlations. In this way the effect of climate on larval population is highly significant, which actually determined the population of larvae. And since the larvae habitat vary in many ways, it has been possible to produce a simple classification - these are the rice-field and miscellaneous structures.

Larvae Ecosystem Study

The population study of the larvae exhibited seasonal fluctuation in terms of the total numbers collected, were the maxima being in the rainy summer and minima in the rainless winter. A summary of results of survey conducted in four localities during the periods of investigation is given in Table-2. Both the potential larvae habitats (rice-field and miscellaneous structure) in which larvae were actually collected and present are shown. It seems likely that the period of larvae abundance correspond during the months of

Table-2

Village Wise Survey of Anopheline Habitats over 24 Months

Month & Year	HARILIBHOGAN				NORBONG				GARO-BASTI				ANJOK			
	Rice-field		Miscella-neous		Rice-field		Miscella-neous		Rice-field		Miscella-neous		Rice-field		Miscella-neous	
	NOD	TNL	NOP\NOE	TNL	NOD	TNL	NOP\NOE	TNL	NOD	TNL	NOP\NOE	TNL	NOD	TNL	NOP\NOE	TNL
May'92	20	10	10\27	36	20	25	9\13	18	20	15	7\9	29	20	25	5\10	25
Jun'92	20	12	8\12	18	20	15	8\12	25	20	14	8\9	32	20	16	6\7	20
Jul'92	20	25	10\25	30	20	20	8\17	14	20	15	6\8	30	20	10	8\9	38
Aug'92	20	22	18\22	41	20	20	10\12	25	20	25	9\15	24	20	10	5\10	25
Sep'92	20	14	6\14	33	20	20	11\17	28	20	18	4\18	14	20	15	3\7	12
Oct'92	20	11	5\12	23	20	25	5\10	30	20	16	6\17	16	20	19	3\5	10
Nov'92	20	12	4\10	7	20	4	8\12	10	20	7	1\5	4	20	3	1\3	3
Dec'92	20	10	4\10	8	20	3	1\6	2	20	8	1\3	2	20	3	1\7	2
Jan'93	20	3	1\3	3	20	2	1\4	4	20	6	1\3	3	20	7	1\4	7
Feb'93	20	8	2\8	8	20	4	2\5	4	20	4	1\4	6	20	6	1\3	2
Mar'93	20	7	3\7	15	20	7	2\5	6	20	11	3\9	5	20	15	1\6	2
Apr'93	20	8	6\8	16	20	6	2\7	9	20	16	6\12	16	20	12	5\11	17
May'93	20	15	9\15	40	20	17	8\10	34	20	29	6\12	15	20	25	3\6	36
Jun'93	20	16	12\16	33	20	19	14\18	32	20	25	7\13	30	20	26	8\14	22
Jul'93	20	18	12\18	32	20	22	9\15	39	20	30	8\10	32	20	15	8\15	30
Aug'93	20	34	16\34	28	20	25	10\18	24	20	25	9\11	32	20	29	9\15	31
Sep'93	20	26	14\16	38	20	32	8\11	22	20	26	5\8	15	20	25	9\12	24
Oct'93	20	15	8\15	30	20	30	8\17	36	20	15	6\14	26	20	29	10\12	25
Nov'93	20	2	5\12	26	20	6	4\7	9	20	7	8\12	10	20	9	3\9	15
Dec'93	20	5	3\6	10	20	3	2\7	3	20	2	1\3	1	20	5	1\3	2
Jan'94	20	6	2\4	5	20	2	1\2	5	20	6	3\6	5	20	3	1\2	4
Feb'94	20	12	5\12	12	20	9	2\4	6	20	4	1\7	2	20	6	3\7	6
Mar'94	20	12	4\12	18	20	18	2\10	10	20	12	4\9	6	20	18	5\8	12
Apr'94	20	11	9\11	27	20	15	6\14	22	20	16	2\5	18	20	19	8\11	16
		314		537		349		417		352		373		350		386

NOD = Number of Dips; NOP\NOE = Number of Positive\Number of Examination; TNL = Total Number of Larvae.

May to October, which is the rainfall season. The dry period from November to April without or with minimum rainfall correspond to a period of very low larval density.

The total numbers of 3078 larvae were collected over 24 months (May, 1992-April, 1994), of which, the total numbers encountered at the individual level are Harilibhogan 851 (27.65%), Norbong 766 (24.89%), Garo-basti 725 (23.55%) and Amjok 736 (23.91%). The details larvae collected from the study areas are summarised in Table-2. There is not much significant differences of the total numbers of larvae collected in all the four stations and also with respect to the types of habitats from where they are collected. The total larvae collected from both rice-field and miscellaneous habitats separately indicates that the composition are Harilibhogan 314 (23%) and 537 (31.35%), Norbong 349 (25.57%) and 417 (24.35%), Garo-basti 352 (25.79%) and 373 (21.77%), Amjok 350 (25.64%) and 386 (22.53%), respectively.

In comparison, the monthly average data of the larvae collected over all the stations at the individual level, during the period of investigation, reveals that there is close variations among all the stations under study. It was found out that the numbers of larvae collected from the rice field, the average values vary between 1.5-18.2, 2.1-32.6, 2.1-30, 3-29, at Harilibhogan, Norbong, Garo-basti and Amjok (Fig.20). Whereas in the miscellaneous habitats, the

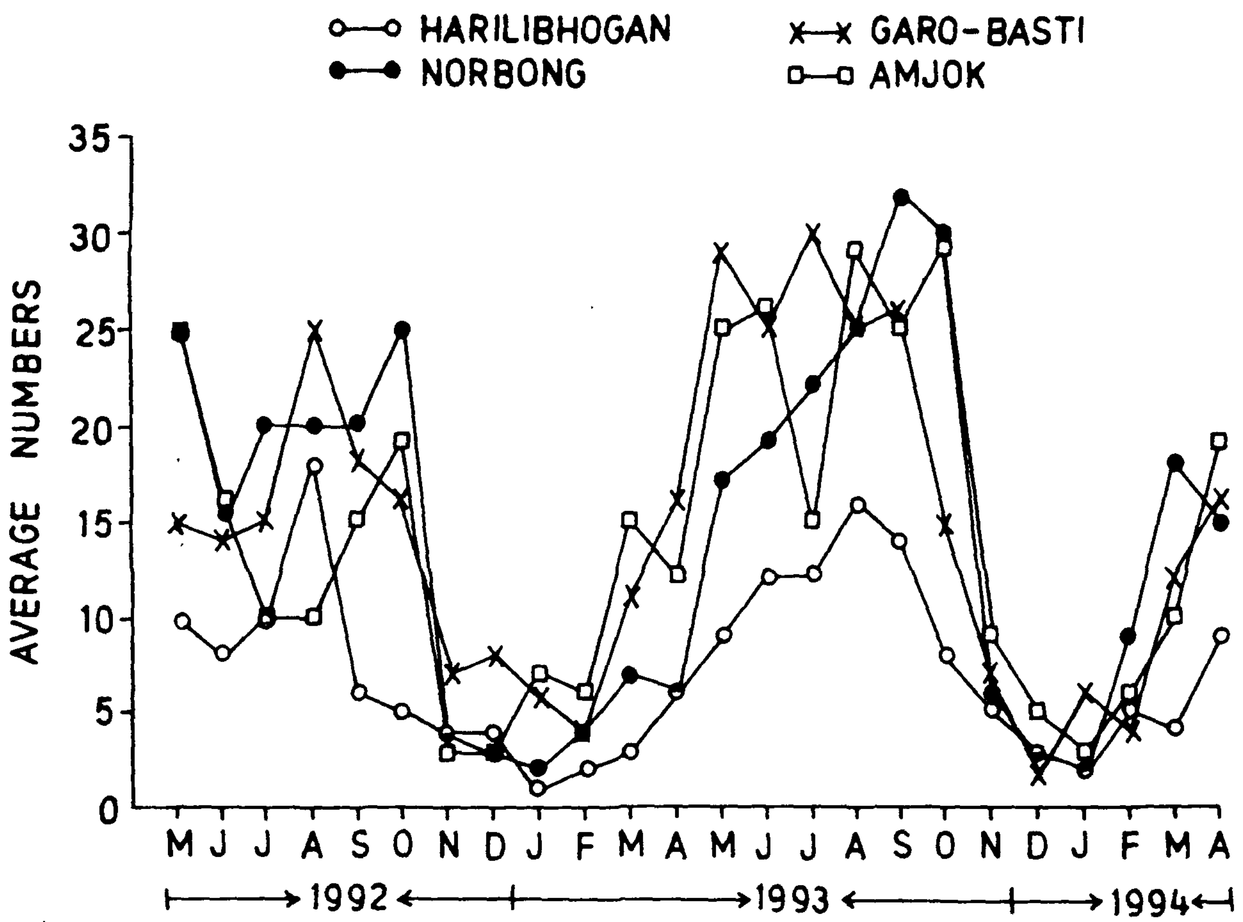

FIG. 20. AVERAGE NUMBERS OF LARVAE IN RICE-FIELD BY MONTHS IN THE FOUR VILLAGES.

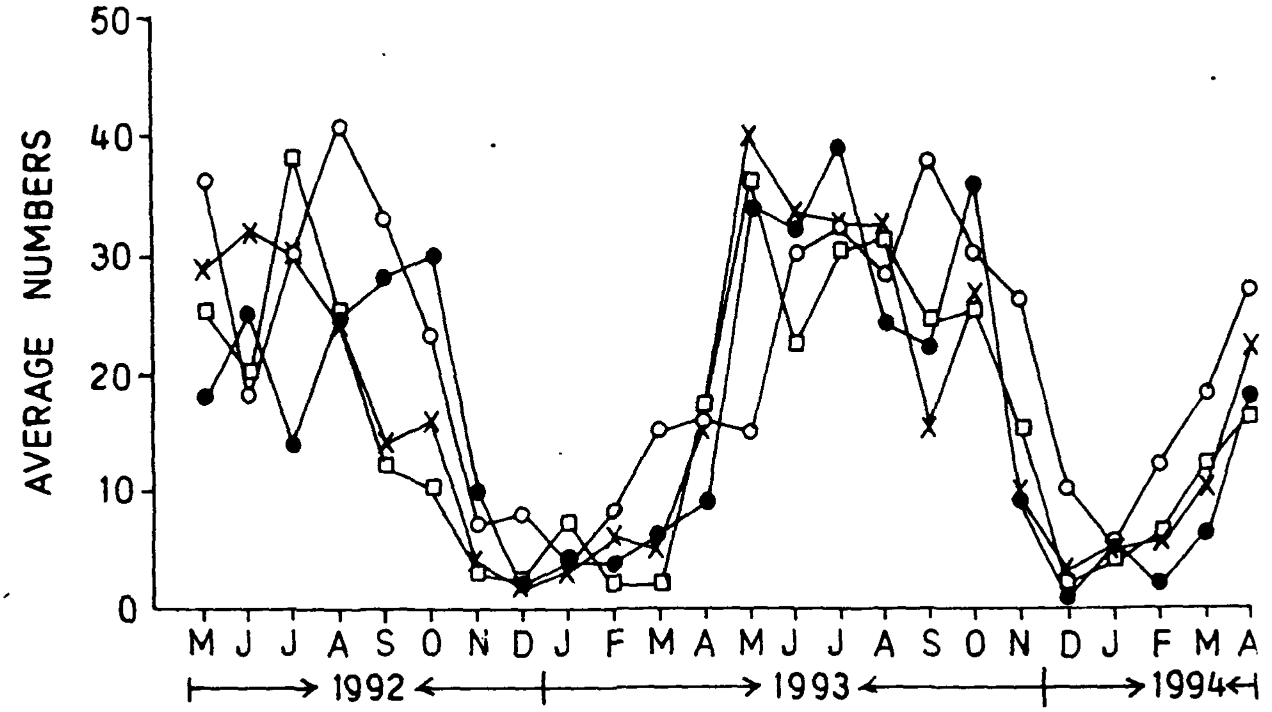

FIG. 21. AVERAGE NUMBERS OF LARVAE OCCUPYING MISCELLANEOUS BY MONTHS IN THE FOUR VILLAGES.

fluctuation range occurs between 3.9-41, 1-39, 3-33.3 and 3-37, at Harilibhogan, Norbong, Garo-basti and Amjok (Fig.21).

Analysis of Larval Habitats (Miscellaneous)

The data were collected in a form suitable for analysis of variance; Table-3 shows the main analysis. since the stations under study and the numbers of occupied habitat within them differed, we are not in this analysis interested in the differences between stations, which may be artificial. Similarly, differences between occupied habitats (Rice-field and miscellaneous) are also in a sense artificial. Since the numbers of different kinds available are not equal. The items in the analysis which are of particular interest and which are presented in this study, are the effect of months and all the interactions. The analysis of variance for data for all the stations shows no significant interaction between villages and months, but a significant effect of months (Table-3).

Since the analyses of the larval habitats in the four stations combined show apparently random fluctuation in the numbers of occupied habitats, each of the four stations has been analysed separately to determine whether there was any pattern from month to month. However, despite the greater variability in the numbers of available and apparently

Table-3

Analysis of Variance for Fields for all Localities

Source of Variation	d.f.	S.S.	M.S.	F
Months (M)	23	9900.99	430.48	23.77*
Field (F)	3	11592.34	3864.11	213.37*
Villages (V)	3	232.78	77.59	4.28*
V x F	9	500.20	55.58	3.07*
V x M	69	443.72	6.43	0.36*
M x F	69	6393.16	92.65	5.12*
Residential	207	3748.04	18.11	
Total	383	32811.23		

* Significant at $p<0.01$

suitable miscellaneous habits, there is always large numbers of these which were unoccupied.

Harilibhogan

The analysis of variance for Harilibhogan shows significant interaction of both months and field (Table-4). There is thus no doubt that the number of occupied miscellaneous habitat varies from month to month and Table-2 shows that in November and December there was a mark decrease in the total numbers of occupied habitat and a slow recovery from May and June onwards. The reduction occurred at the beginning of the cool season : the population remained low during the cool and hot dry season, and a slow recovery at the beginning of the wet season. Considering the data at a glance, shows that the months of August and September, the percentage value of occupied miscellaneous habitat was 82% and 88% respectively and the minimum of 25% in the month of February.

Norbong

In the analysis of Norbong data, once again there is a significant interaction of month and field (Table-4). From the data obtained during the course of investigation show much less striking changes than those from Harilibhogan. The population showed a decreased from November but recovered

Table-4

Analysis of Variance of Fields for Individual Localities

Station	Source of Variation	d.f.	S.S.	M.S.	F
Honilibhogan	Months	23	2771.49	120.50	3.54*
	Field	3	3357.45	1119.15	32.84*
	Residual	69	2351.30	34.08	
	Total	95	32137.00		
Norbong	Months	23	3284.74	142.31	3.91*
	Field	3	2711.78	903.93	24.72*
	Residual	69	2523.47	36.57	
	Total	95	28733.00		
Garo Basti	Months	23	2464.74	107.16	3.29*
	Field	3	2942.87	980.96	30.14*
	Residual	69	2245.88	32.55	
	Total	95	25831.00		
Amjok	Months	23	2559.74	111.29	3.23*
	Field	3	3082.70	1027.57	29.85*
	Residual	69	2375.55	34.43	
	Total	95	26361.00		

* Significant at $p<0.01$

again by May onward (Table-2). The percentage of occupied miscellaneous showed that the months of August, June and September, has the maximum with 83%, 78% and 73% respectively, whereas the months of December and March has the minimum of 17% and 20%.

Garo-basti

Similarly like Norbong and Harilibhogan the analysis of variance showed significant interaction of month and field (Table-4). Changes in the numbers of occupied miscellaneous habitats, despite their expected similarity differ from the two stations above. As observed, the numbers of habitat examined, and the numbers of larvae encountered comparatively exhibited that was lesser (Table-2). However, the reduction in the number of occupied habitat shows that the months of February, September, November has the minimum of 14%, 22% and 20%, whereas, the month of May, June, July and August shows the maximum of 78%, 89%, 75%, and 82% respectively.

Amjok

Even though the data show significant interaction between month and field (Table-4), there was no evidence of a positive correlation between the total number of occupied miscellaneous habitat and the total number of occupied miscellaneous habitat and the total numbers of larvae encountered (Table-2). The reduction in the numbers of occupied habitat also shows a declining trend from November upto March and a slow recovery from April onwards. Considering the percentage of occupied habitat, the maximum was observed in July and September at 89% and 83% respectively and with a minimum of 14% and 33% in the months of November and December.

Adult Ecosystem Study

During the course of investigation employing a total of 128 Man Hour for 24 months extending from May 1992 to April 1994, altogether 2587 mosquitoes consisting of 23 species were captured. Inspite of two rounds of residual spraying carried out annually between April and August, season-wise collections of Anopheles adults were still high. Month-wise analysis revealed a marked seasonal variation in the numbers collected. Table-5 reveals the numbers collected was abundant from May to October, a declining trend from September and with a slow recovery from April onwards. Collection were made from indoor resting places (human dwelling), outdoor - Cowsheds (with thatched roofs only) and their surrounding. Majority of the mosquitoes collected were from the outdoor structures. Details of mosquitoes captured in the Byrnihat area are summarised in Fig.22, of which, the maximum number among all the *Anopheles* species collected

Table-5

Total Number of *Anopheles* species encountered at Byrnihat Area during the Period of Investigation

Species	1992								1993												1994			
	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1. *A. maculatus*	20	13	29	18	1	5	-	-	8	3	5	16	38	7	11	9	-	2	7	1	1	4	6	9
2. *A. karwari*	5	4	5	5	9	1	2	-	14	2	1	3	5	1	3	14	-	4	1	-	10	-	-	-
3. *A. aconitus*	24	14	12	12	8	9	-	5	-	5	5	13	29	13	16	13	5	7	-	2	-	4	6	10
4. *A. vagus*	65	21	21	30	4	2	-	2	2	1	2	24	34	21	27	8	3	4	-	1	-	4	5	16
5. *A. splendidus*	15	8	-	2	3	10	3	-	3	2	4	12	20	6	-	-	8	6	3	-	-	3	3	9
6. *A. hyreanus*	9	15	5	8	30	26	27	3	-	4	7	9	24	7	7	22	17	18	23	-	1	4	4	5
7. *A. phillipinensis*	32	29	29	30	48	11	-	-	-	5	3	22	51	16	26	38	34	8	-	-	-	3	5	13
8. *A. jayporiensis*	11	7	6	9	2	5	-	1	-	-	3	21	21	10	5	2	3	3	-	-	-	1	1	9
9. *A. gigas*	5	8	-	5	4	6	6	-	-	2	4	7	12	7	4	2	11	2	9	-	-	4	1	3
10. *A. majidi*	2	2	-	-	-	4	2	-	3	-	1	6	8	1	-	-	3	1	8	-	4	-	-	-
11. *A. annularis*	22	8	6	14	8	4	-	-	3	-	1	13	31	7	4	12	-	3	1	-	4	2	-	8
12. *A. fluviatilis*	12	1	6	-	-	7	-	-	-	-	-	10	12	13	-	-	2	-	-	-	-	-	-	-
13. *A. aikeni*	7	8	12	-	-	6	4	-	3	-	-	-	15	8	-	-	-	6	14	-	-	-	-	3
14. *A. sundaicus*	2	1	5	4	-	-	-	-	-	-	-	-	3	2	2	5	6	-	-	-	-	-	-	1
15. *A. jamesi*	4	1	3	7	2	-	2	-	-	-	-	-	-	2	2	6	-	-	4	-	-	-	-	-
16. *A. kochi*	-	1	1	4	22	2	5	-	15	1	2	8	9	3	9	32	7	5	9	-	11	-	-	5
17. *A. balabacensis*	-	1	1	5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18. *A. stephensi*	-	2	2	2	-	1	-	-	-	-	-	-	1	2	1	2	-	-	-	-	-	-	-	1
19. *A. culicifacies*	-	2	-	-	-	2	-	11	2	-	-	-	7	1	1	1	3	-	-	7	-	-	-	1
20. *A. subpictus*	-	1	-	-	-	1	-	-	-	-	-	-	2	1	2	2	2	-	-	-	-	-	-	1
21. *A. tesselatus*	-	8	3	2	-	3	-	-	10	-	1	-	4	1	5	1	-	-	-	-	9	1	-	2
22. *A. barbitrostris*	-	2	5	6	2	1	2	-	-	1	2	4	2	1	2	-	15	6	-	9	-	1	-	4
23. *A. varuna*	-	-	-	1	3	-	-	-	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-

Code of *Anopheles* Species

1. *A. maculatus*
2. *A. karwari*
3. *A. aconitus*
4. *A. vagus*
5. *A. splendidus*
6. *A. hyrcanus*
7. *A. phillipinensis*
8. *A. jayporiensis*
9. *A. gigas*
10. *A. majidi*
11. *A. annularis*
12. *A. fluviatilis*
13. *A. aikeni*
14. *A. sundaicus*
15. *A. jamesi*
16. *A. kochi*
17. *A. balabacensis*
18. *A. stephensi*
19. *A. culicifacies*
20. *A. subpictus*
21. *A. tesselatus*
22. *A. barbitrostris*
23. *A. varuna.*

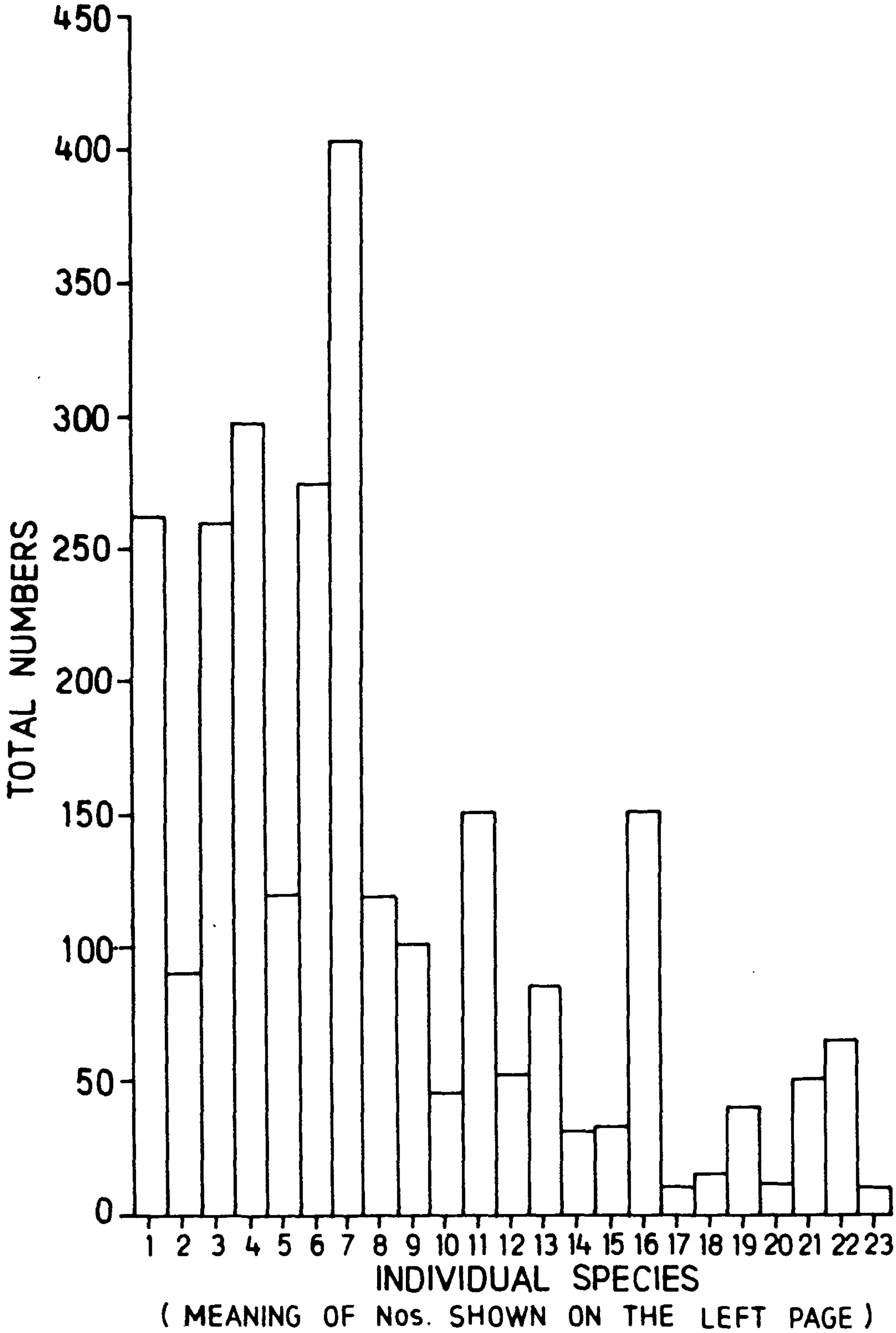

FIG. 22. TOTAL NUMBERS OF ANOPHELES SPECIES ENCOUNTERED AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

shows *Anopheles phillipinensis* 403 (15.58%), *A. vagus* 297 (11.48%) and *A. hyrcanus* at 275 (10.63%).

The trend of the relative abundance of *Anopheles* species at Byrnihat area reveals that preferred abundance of the diversity vary from season to season. Fig.23 reveals that occurrence of different species of which, the maximum of 20 species was observed on June and a minimum of 5 species in the month of December. The total population of *Anopheles* species collected also exhibited a seasonal fluctuation with the maximum at 280 (10.82%) in the month of May and a minimum of 21 (0.81%) and 22 (0.85%) in the months of December and February (Fig.24).

Month-wise analysis of total average on the types of species occurring revealed a marked seasonal variation. Comparatively the total average shows a maximum of 82.50 in the month of May and a minimum with 5.50, 6.50 in the months of December and February (Fig.25). The monthly total Man Hour density (MHD) of the species occurring also exhibit a maximum in May (61.50) and a minimum in December (4.13) respectively (Fig.26).

The total Man-Hour Density (MHD) of the individual species encountered during the period of investigation vary from species to species. Figure-27 shows the different species of mosquitoes which were encountered during the period of investigation. The maximum Man-Hour Density are of

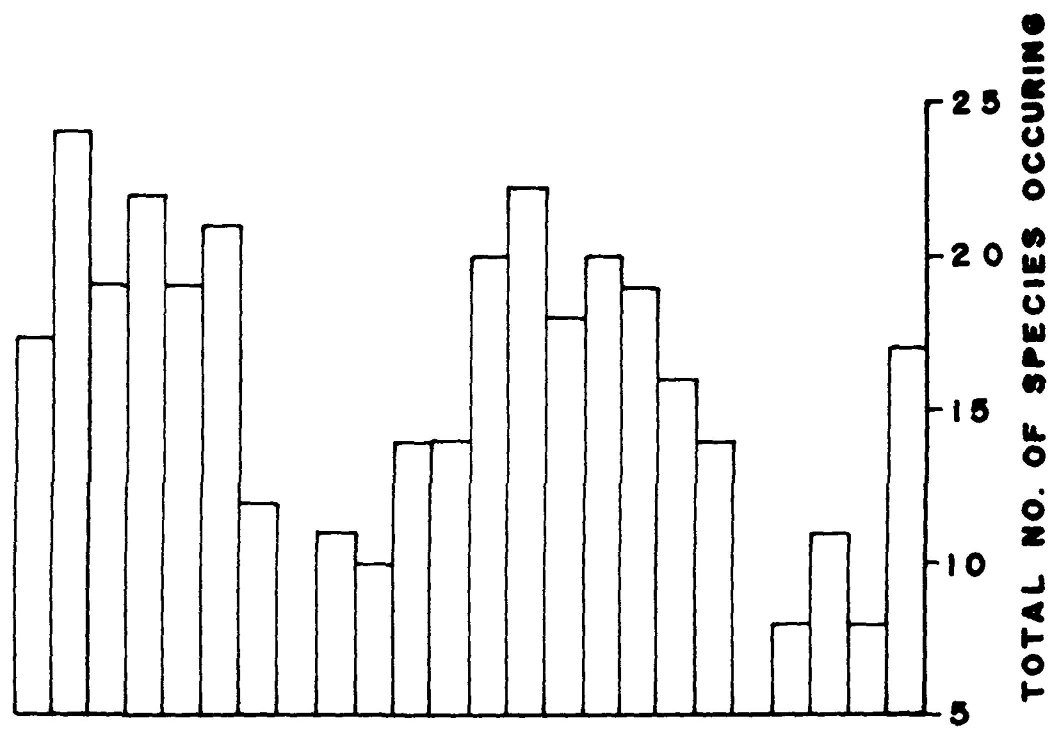

FIG.23. TOTAL NUMBERS OF ANOPHELES SPECIES OCCURRING AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

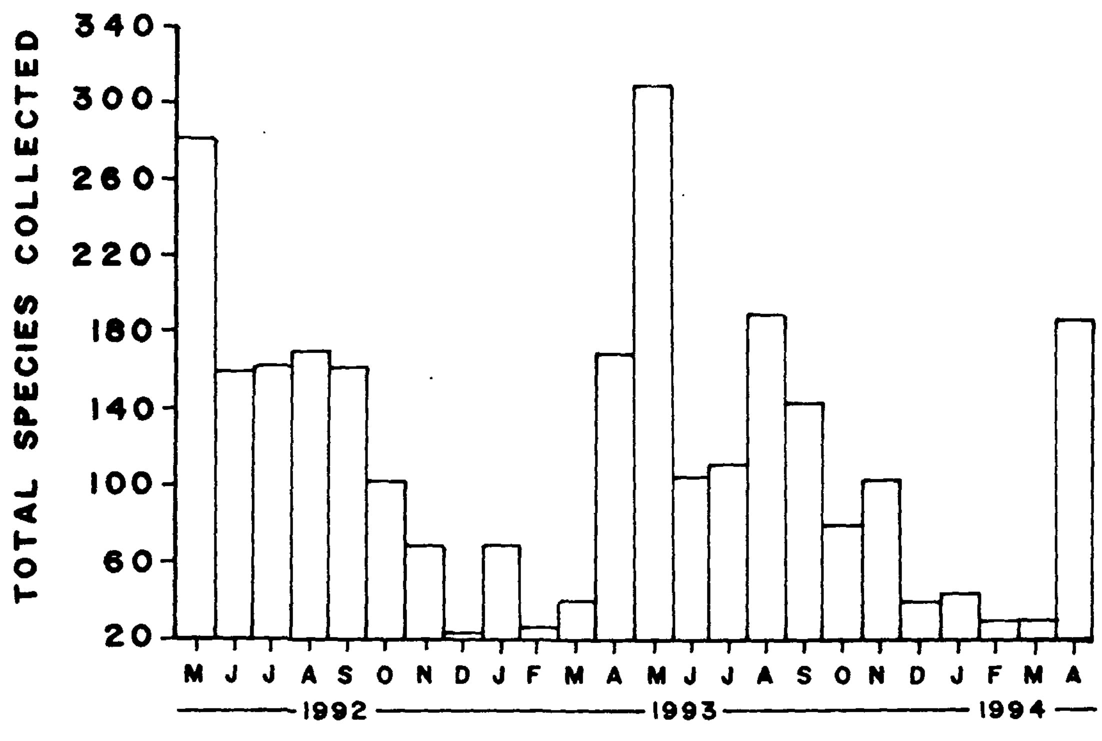

FIG. 24. TOTAL NUMBERS OF ANOPHELES SPECIES COLLECTED AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

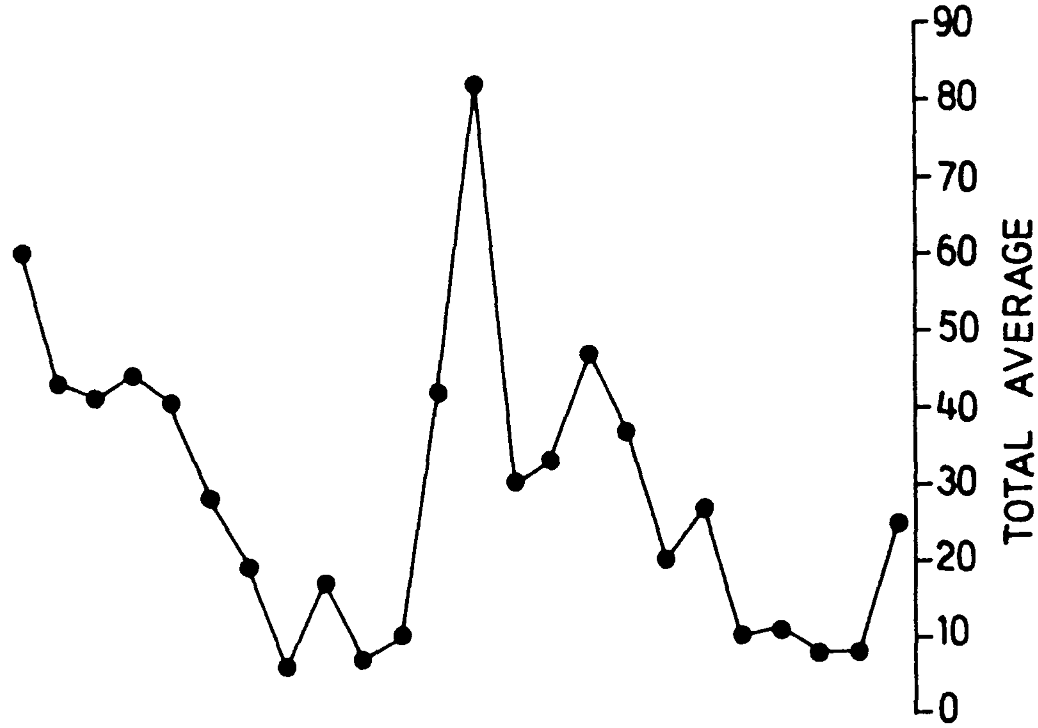

FIG. 25. TOTAL AVERAGE OF ANOPHELES SPECIES AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

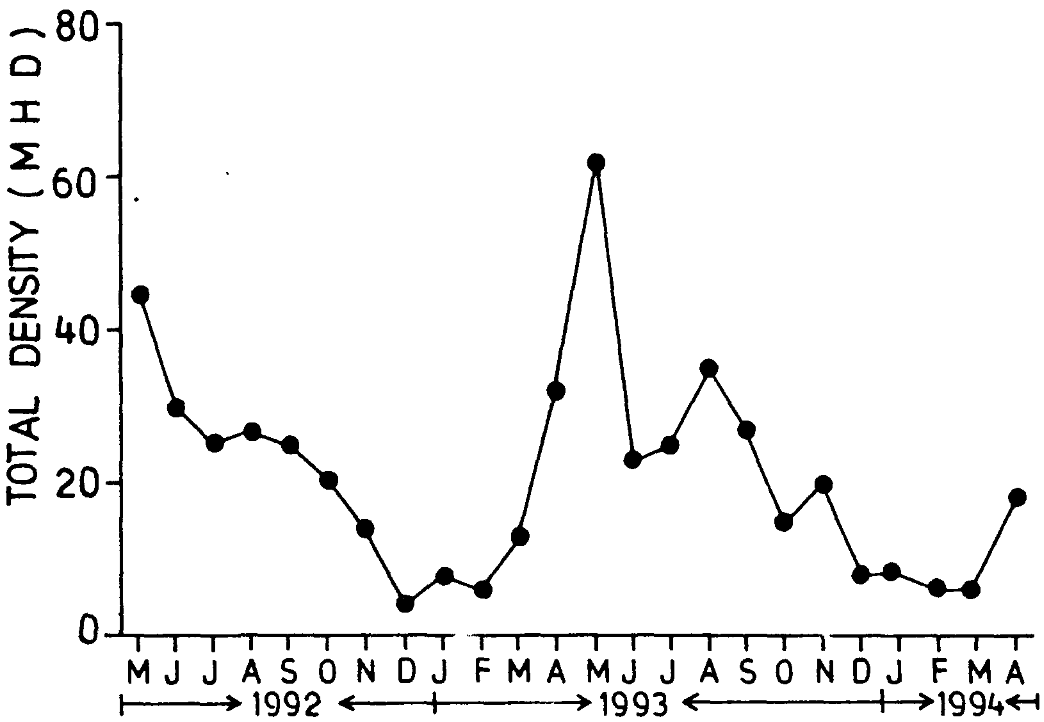

FIG. 26. TOTAL DENSITY (MAN HOUR DENSITY) OF ANOPHELS SPECIES AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

Code of *Anopheles* Species

1. *A. maculatus*
2. *A. karwari*
3. *A. aconitus*
4. *A. vagus*
5. *A. splendidus*
6. *A. hyrcanus*
7. *A. phillipinensis*
8. *A. jayporiensis*
9. *A. gigas*
10. *A. majidi*
11. *A. annularis*
12. *A. fluviatilis*
13. *A. aikeni*
14. *A. sundaicus*
15. *A. jamesi*
16. *A. kochi*
17. *A. balabacensis*
18. *A. stephensi*
19. *A. culicifacies*
20. *A. subpictus*
21. *A. tesselatus*
22. *A. barbitrostris*
23. *A. varuna.*

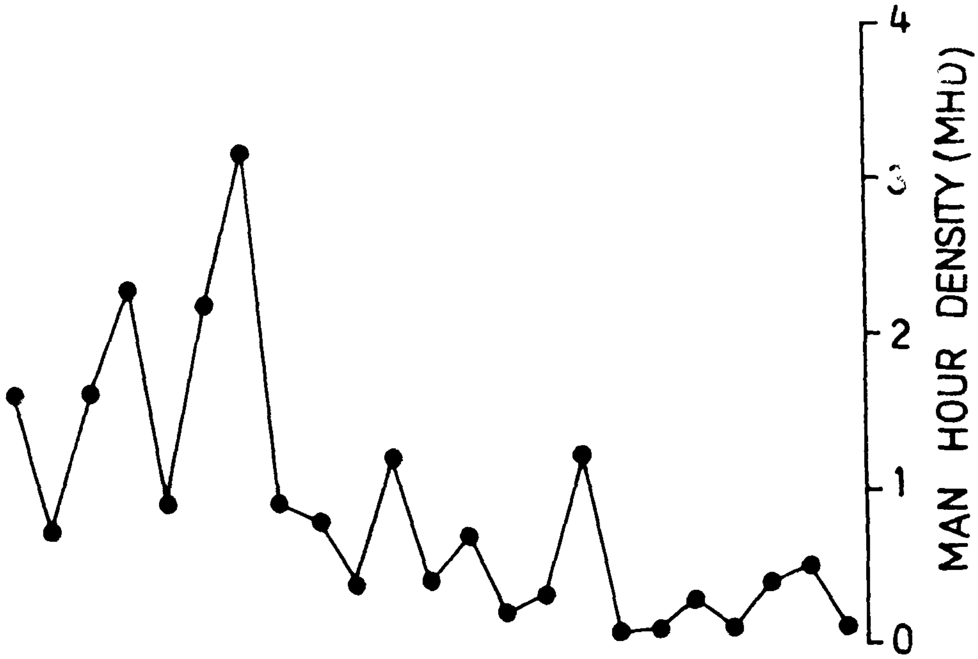

FIG. 27. TOTAL DENSITY (MAN HOUR DENSITY) OF ANOPHELES SPECIES AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

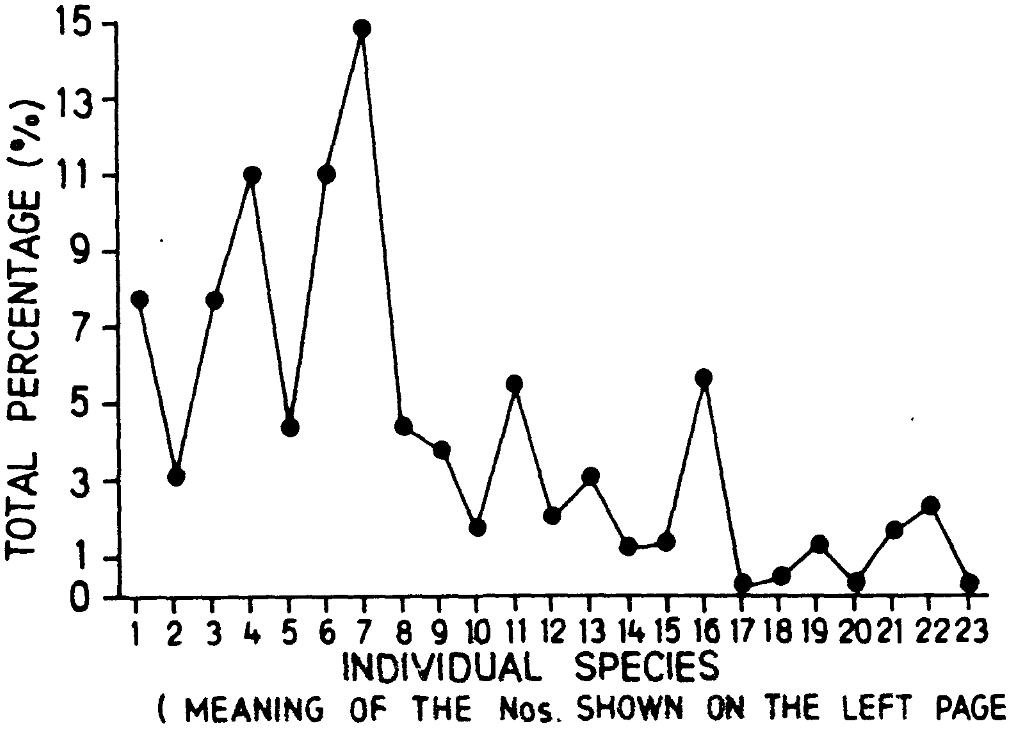

FIG. 28. TOTAL PERCENTAGE (%) OR RELATIVE ABUNDANCE OF ANOPHELES SPECIES AT BYRNIHAT AREA DURING THE PERIOD OF INVESTIGATION.

Anopheles phillipinensis (3.2), *A. vagus* (2.32) and *A. hyrcanus* (2.15) and the minimum are of *A. varuna* (0.06), *A. subpictus* (0.09) and *A. stephensi* (0.11) respectively (Fig.27). The total percentage composition of individual species collected are *Anopheles phillipinensis* 403 (15.58%), *A. vagus* 297 (11.48%) and a minimum are of *Anopheles varuna* at 8 (0.31%), *A. balabacensis* 9 (0.35%), *A. subpictus* 12 (0.46%) respectively (Fig.28).

Since collection of the adults in the four stations together show random fluctuation in the numbers of species occurring as well the total numbers collected, each of the four stations has also been analysed separately to determine the pattern of changes from month to month :

1) The total numbers of *Anopheles* species collected in the four different stations are listed in Table-6. A total of 32 man-hour were spent in each station and 2578 Anopheles mosquitoes belonging to 23 species were collected. At Harilibhogan, the known malaria vector *A. phillipinensis* formed 15.63% (96) followed by *A. hyreanus* slightly higher with 15.58% (97) are among the predominant species. At Norbong also *A. phillipinensis* formed the second largest species with 13.23% (90) and *A. vagus* formed the first largest species at 16.76% (114) respectively. Whereas at Garo-basti, *A. phillipinensis* formed the largest dominant species at 17.20% (108). And at Amjok the percentage

Table-6

Village Wise Distribution and Density of *Anopheles* Species at Byrnihat

Sl. No.	*Anopheles* Species	Harilibhogan 32(MHD)			Norbong 32(MHD)			Garo-Basti 32(MHD)			Amjok 32(MHD)		
		Total	MHD	%	Total	MHD	%	Total	MHD	%	Total	MHD	%
1.	*A. maculatus*	73	2.38	11.93	29	0.91	4.26	71	2.22	11.30	41	1.28	6.53
2.	*A. karwari*	33	1.03	5.39	14	0.44	2.06	21	0.66	3.34	21	0.66	3.34
3.	*A. aconitus*	30	0.94	4.90	32	1.00	4.76	36	1.13	5.73	104	3.56	16.56
4.	*A. vagus*	75	2.34	12.25	114	4.50	16.76	45	1.41	7.17	33	1.03	5.25
5.	*A. splendidus*	16	0.50	2.61	15	0.47	2.21	67	2.09	10.67	22	0.69	3.50
6.	*A. hyreanus*	97	3.03	15.85	72	2.25	10.58	61	1.91	9.71	45	1.41	7.17
7.	*A. phillipinensis*	96	3.00	15.68	90	2.81	13.23	108	3.38	17.20	109	3.40	17.36
8.	*A. jayporiensis*	23	0.72	3.76	48	1.50	7.06	30	0.94	4.78	19	0.59	3.03
9.	*A. gigas*	5	0.16	0.82	37	1.16	5.44	18	0.56	2.87	42	1.31	6.69
10.	*A. majidi*	6	0.19	0.98	15	0.47	2.21	13	0.41	2.07	11	0.34	1.75
11.	*A. annularis*	42	1.31	6.86	47	1.47	6.91	32	1.00	5.10	30	0.94	4.78
12.	*A. fluviatilis*	16	0.50	2.61	29	0.91	4.26	2	0.06	0.32	6	0.19	0.96
13.	*A. aikeni*	19	0.59	3.10	26	0.81	3.82	29	0.90	4.62	12	0.38	1.91
14.	*A. sundaicus*	4	0.13	0.65	7	0.21	1.03	10	0.31	1.59	10	0.31	1.59
15.	*A. jamesii*	12	0.38	1.96	7	0.21	1.03	3	0.09	0.48	11	0.34	1.75
16.	*A. kochi*	32	1.00	5.23	40	1.25	5.88	38	1.19	6.05	41	1.28	6.53
17.	*A. balabacensis*	7	0.22	1.14	1	0.03	0.15	0	0.00	0.00	1	0.03	0.16
18.	*A. stephensi*	0	0.00	0.00	3	0.09	0.44	8	0.25	1.27	3	0.09	0.48
19.	*A. culicifacies*	9	0.28	1.47	16	0.50	2.35	8	0.25	1.27	5	0.16	0.80
20.	*A. subpictus*	1	0.03	0.16	4	0.13	0.59	2	0.06	0.32	5	0.16	0.80
21.	*A. tesselatus*	9	0.28	1.47	8	0.25	1.18	10	0.31	1.59	23	0.72	3.66
22.	*A. barbitrostris*	7	0.22	1.14	26	0.81	3.82	10	0.31	1.59	22	0.69	3.50
23.	*A. varuna*	0	0.00	0.00	0	0.00	0.00	6	0.19	0.96	3	0.09	0.48
	Total	612	19.13	100.00	680	21.25	100.00	628	19.63	100.00	628	19.63	100.00

MHD = Man Hour Density; % = Relative Abundance composition of *A. phillipinensis* formed 17.36% (109) next to *A. aconitus* at 18.15% (114) respectively.

2) The actual monthwise numbers and species of adult Anopheles occurring in the four study areas viz., Harilibhogan, Norbong, Garo-basti and Amjok (Figs.29,30,31,32) fluctuated in different stations and in different months. At Harilibhogan, the total numbers of species occurring and collected varied from 15 types of species in the month of May and a minimum of 4 types of species in the months of December and February (Fig.29). At Norbong the fluctuation range shows a maximum of 16 species in the months of May and August and a minimum of 3 species in December (Fig.30). Whereas, a maximum of 16 species was observed in the month of August and a minimum of 3 species in the month of December was observed at Garo-basti (Fig.31). Similarly, at Amjok also a maximum number of 16 species occurred in the month of August and a minimum of 3 species in the month of December respectively (Fig.32).

3) The monthly total numbers of *Anopheles* species of four different stations shows a marked seasonal variations with wet seasons maxima and dry cool seasons minima, the peak and fall were prominent in all the study stations. At the individual levels the stations - Harilibhogan, Norbong, Garo-basti and Amjok had a fluctuation range of 73-5, 97-5, 78-3 and 80-5 respectively (Fig.33).

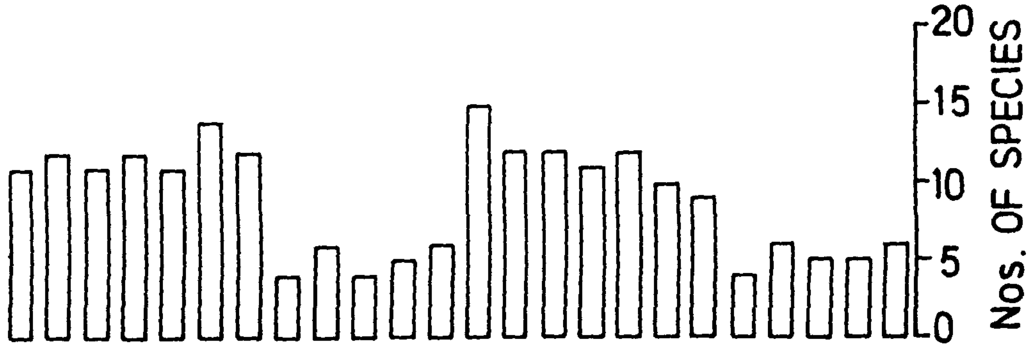

FIG. 29. NUMBERS OF ANOPHELES SPECIES OCCURING AT HARILIBHOGAN DURING THE PERIOD OF INVESTIGATION.

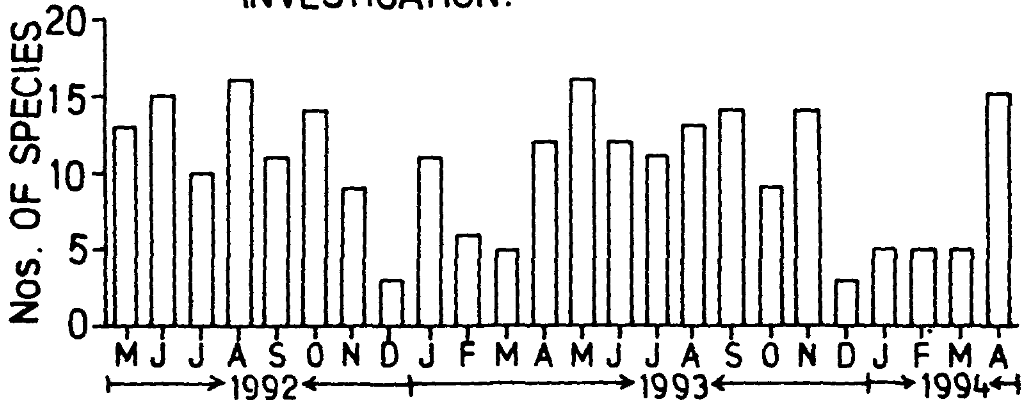

FIG. 30. NUMBERS OF ANOPHELES SPECIES OCCURING AT NORBONG DURING THE PERIOD OF INVESTIGATION.

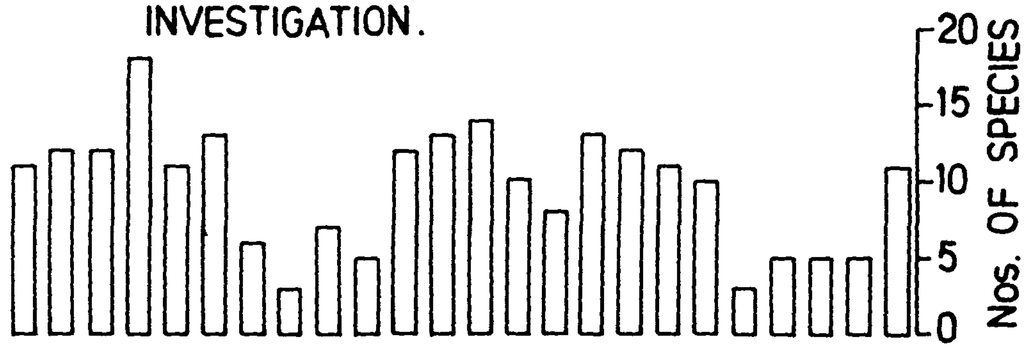

FIG. 31. NUMBERS OF ANOPHELES SPECIES OCCURING AT GARO-BASTI DURING THE PERIOD OF INVESTIGATION.

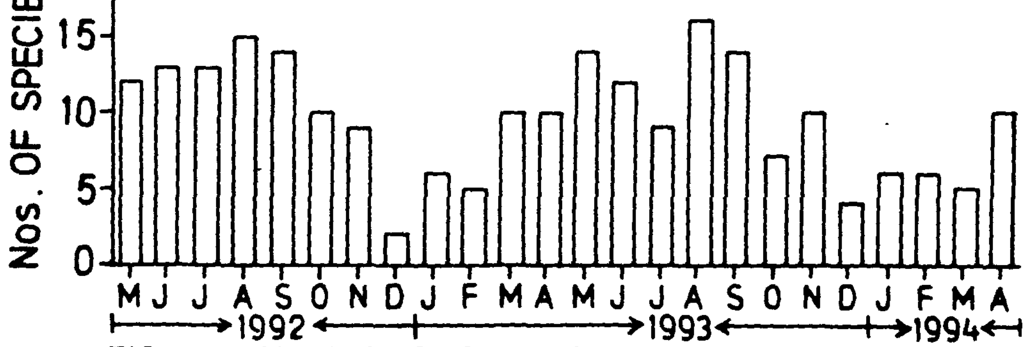

FIG. 32. NUMBERS OF ANOPHELES SPECIES OCCURING AT AMJOK DURING THE PERIOD OF INVESTIGATION.

The monthwise total Man-Hour Density also shows a similar pattern with wet season maxima and dry cool seasons minima in all the study area, exhibiting two peaks of abundance, viz., May and August in all the stations corresponding to the main rice plantation season. Considering the individual stations separately, the range of variation are Harilibhogan (54-3), Norbong (73-5), Garo-basti (59-3) and Amjok (60-4) respectively (Fig.34).

4) The total numbers of individual *Anopheles* species encountered showed a difference between stations. Harilibhogan had a total of 21 species occurring during the period of investigation. The total numbers of individual species shows a variation of which, the maximum are *Anopheles hyrcanus* 97(15.85%), *Anopheles phillipinensis* 96(15.68%) and the minimum number collected are *Anopheles subpictus* 1(0.16%) (Fig.35). At Norbong, the total numbers of *Anopheles* species occurring are 22, the maximum number of individual species collected at *Anopheles vagus* 114(16.76%), *Anopheles phillipinensis* 90(13.23%) and a minimum are of *Anopheles balabacensis* 1(0.15%) respectively (Fig.36). Garo-basti also showed the occurrence of 22 species and among the individual species collected the maximum are *Anopheles phillipinensis* 108(17.20%), *Anopheles maculatus* 71(11.30%) and the minimum are *Anopheles fluviatilis* 2(0.32%) and *Anopheles subpictus* 2(0.32%) (Fig.37). The largest composition of 23 species was

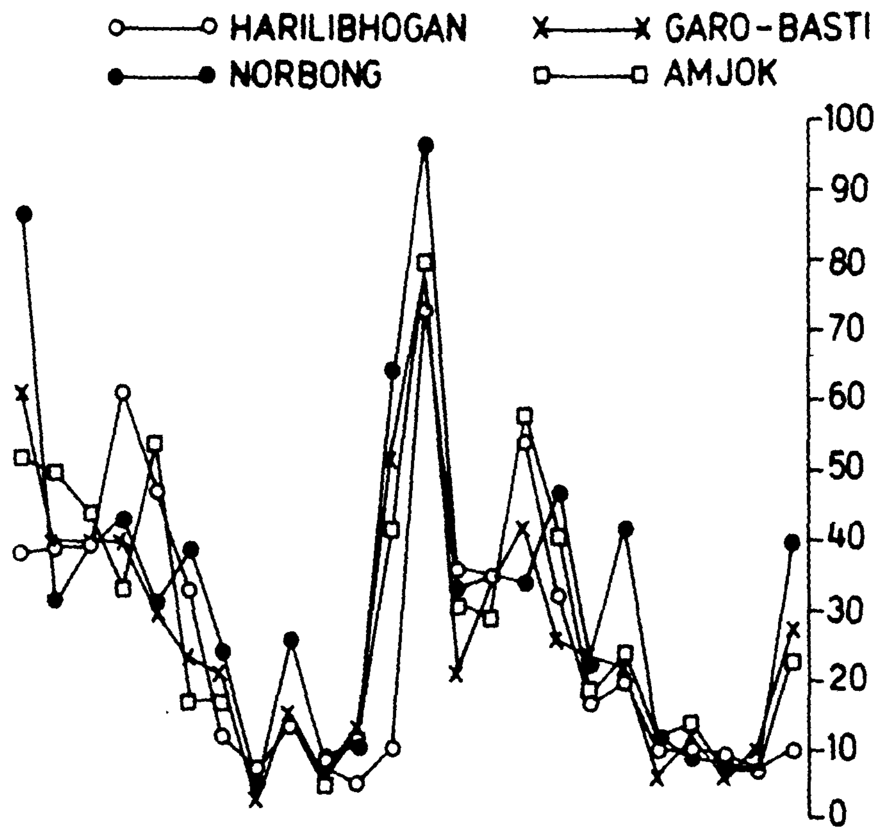

FIG. 33. TOTAL NUMBERS OF ANOPHELES SPECIES OF FOUR DIFFERENT STATIONS AT BYRNIHAT.

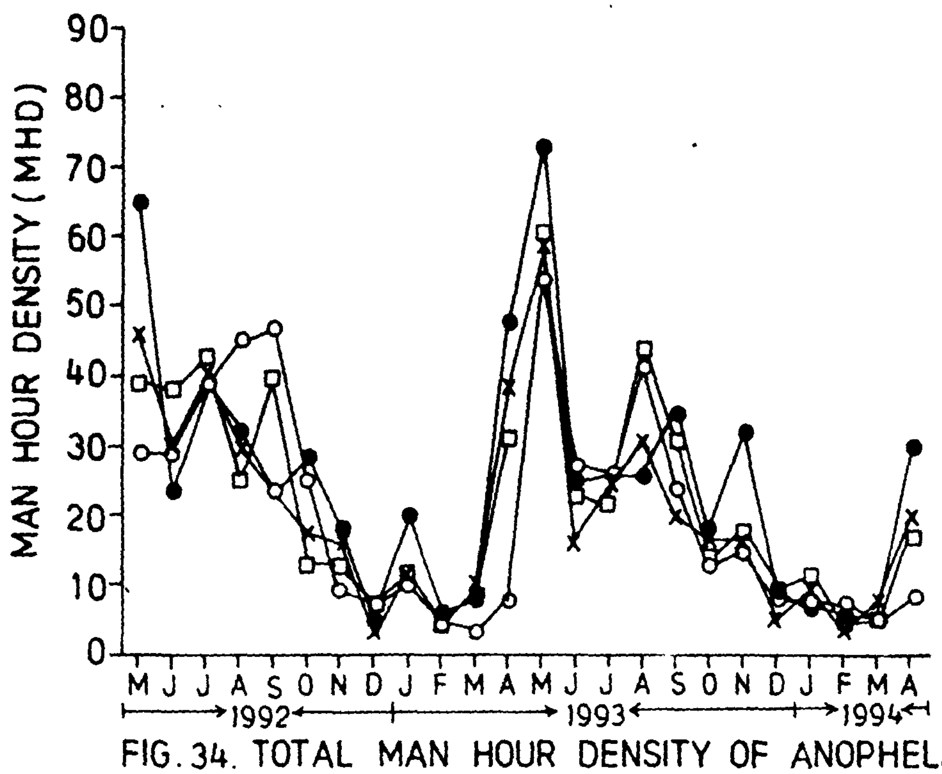

FIG. 34. TOTAL MAN HOUR DENSITY OF ANOPHELES SPECIES OF FOUR DIFFERENT STATIONS AT BYRNIHAT.

Code of *Anopheles* Species

1. *A. maculatus*
2. *A. karwari*
3. *A. aconitus*
4. *A. vagus*
5. *A. splendidus*
6. *A. hyrcanus*
7. *A. phillipinensis*
8. *A. jayporiensis*
9. *A. gigas*
10. *A. majidi*
11. *A. annularis*
12. *A. fluviatilis*
13. *A. aikeni*
14. *A. sundaicus*
15. *A. jamesi*
16. *A. kochi*
17. *A. balabacensis*
18. *A. stephensi*
19. *A. culicifacies*
20. *A. subpictus*
21. *A. tesselatus*
22. *A. barbitrostris*
23. *A. varuna*.

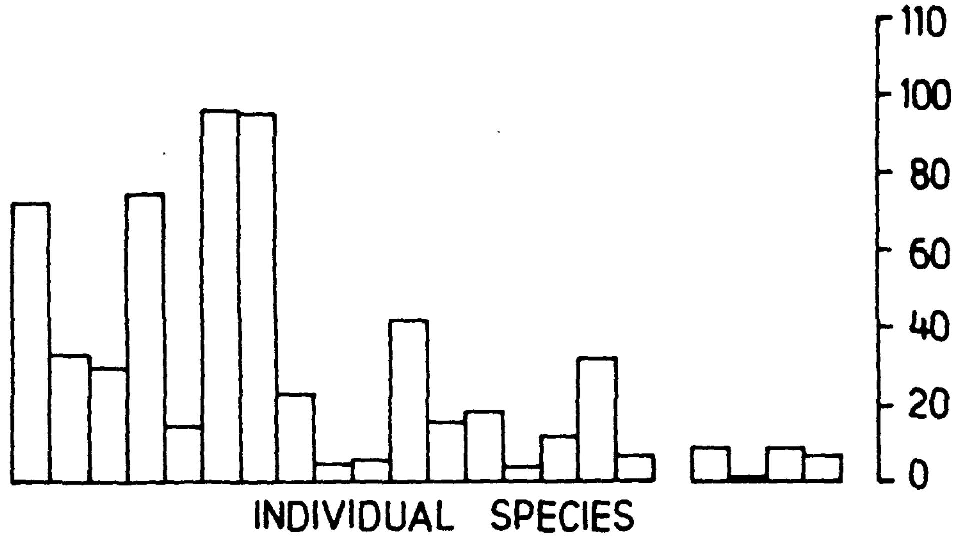

FIG. 35. TOTAL NUMBERS OF ANOPHELES SPECIES ENCOUNTERED AT HARILIBHOGAN DURING THE PERIOD OF INVESTIGATION.

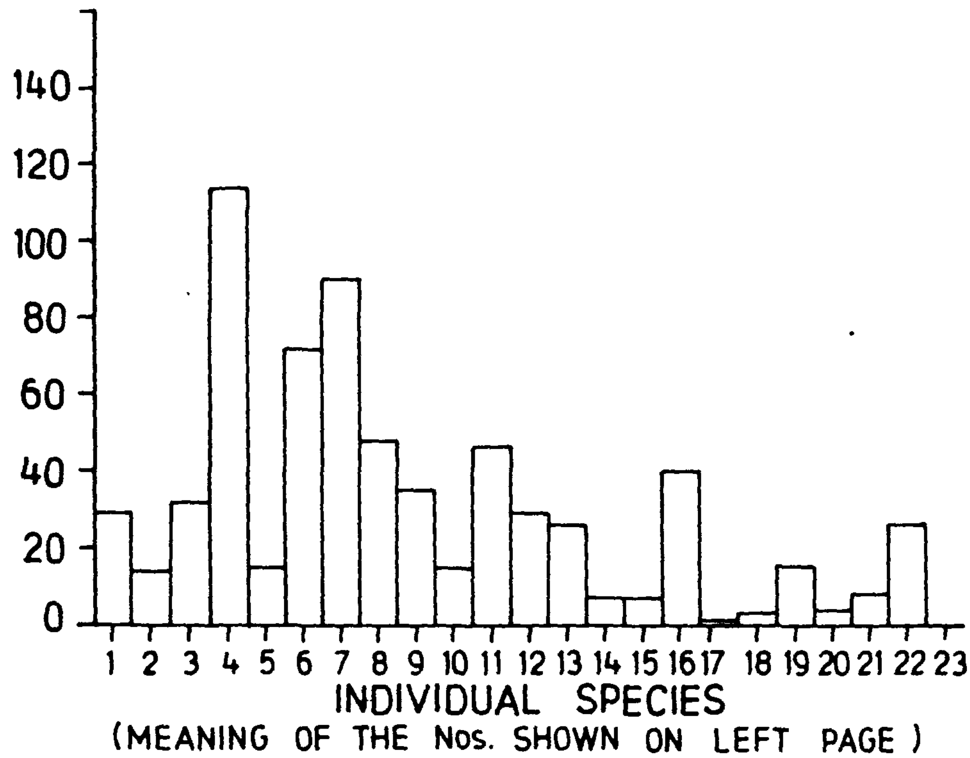

FIG. 36. TOTAL NUMBERS OF ANOPHELES SPECIES ENCOUNTERED AT NORBONG DURING THE PERIOD OF INVESTIGATION.

Code of *Anopheles* Species

1. *A. maculatus*
2. *A. karwari*
3. *A. aconitus*
4. *A. vagus*
5. *A. splendidus*
6. *A. hyrcanus*
7. *A. phillipinensis*
8. *A. jayporiensis*
9. *A. gigas*
10. *A. majidi*
11. *A. annularis*
12. *A. fluviatilis*
13. *A. aikeni*
14. *A. sundaicus*
15. *A. jamesi*
16. *A. kochi*
17. *A. balabacensis*
18. *A. stephensi*
19. *A. culicifacies*
20. *A. subpictus*
21. *A. tesselatus*
22. *A. barbitrostris*
23. *A. varuna.*

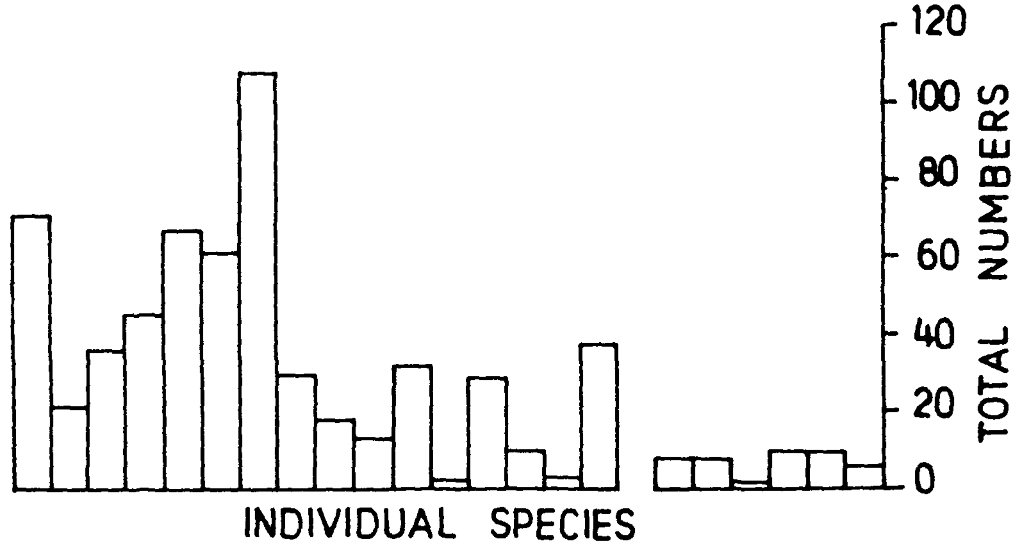

FIG.37. TOTAL NUBERS OF ANOPHELES SPECIES ENCOUNTERD AT GARO-BASTI DURING THE PERIOD OF INVESTIGATION.

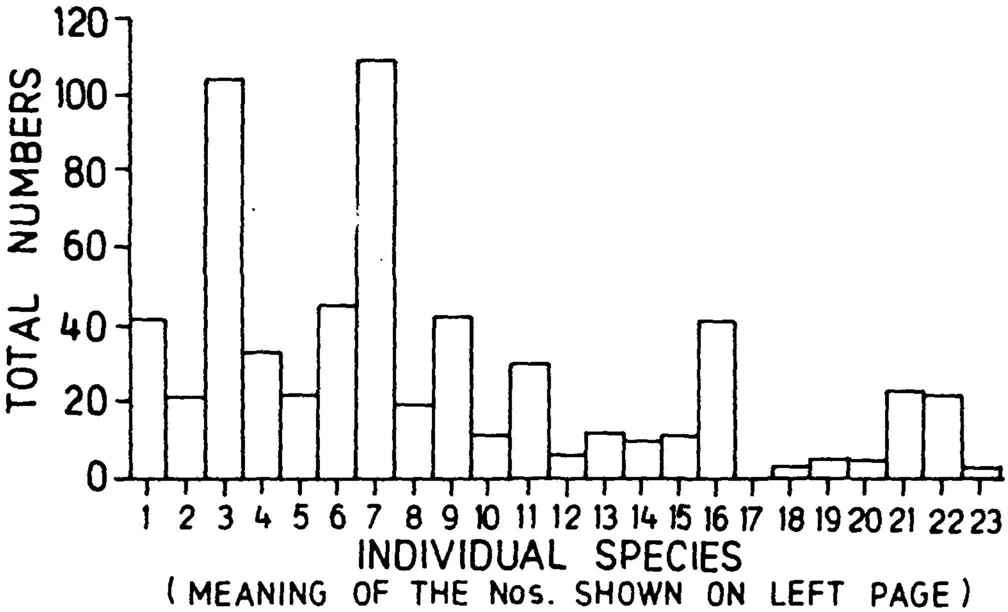

FIG.38. TOTAL NUMBERS OF ANOPHELS SPECIES ENCOUNTERED AT AMJOK DURING THE PERIOD OF INVESTIGATION.

encountered at Amjok of which *Anopheles phillipinensis* 109(17.36%), *Anopheles aconitus* 104(16.56%) formed the maximum and *Anopheles balabacensis* 1(0.16%) as the minimum of all the individual species encountered respectively (Fig.38). The Man-Hour Density (M.H.D.) of the individual species at every station explained a variation in numbers among the species and also form one station to another (Table-6). The range of variation at Harilibhogan are 3-0.03, Norbong 4.5-0.03, Garo-basti 3.4-0.06 and Amjok 3.6-0.09 respectively (Fig.39).

Statistical Analysis of *Anopheles* Adults Composition for Different Localities

<u>Coefficient Variation</u>

The correlation between the four stations is shown in Table-7. Garo-basti (40.0201) showed the highest variation, followed by Norbong (39.9896), Amjok (39.1984) and Harilibhogan (38.7091), the least variation among all the stations.

<u>Skewness</u>

It tells about the symmetry of the distribution. When the value is zero(o) - then is considered to be symmetrical, that means, normally distributed. And if the values is less or greater than zero(o), it means, that the distribution is

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4. *A. vagus*
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6. *A. hyrcanus*
7. *A. phillipinensis*
8. *A. jayporiensis*
9. *A. gigas*
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12. *A. fluviatilis*
13. *A. aikeni*
14. *A. sundaicus*
15. *A. jamesi*
16. *A. kochi*
17. *A. balabacensis*
18. *A. stephensi*
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20. *A. subpictus*
21. *A. tesselatus*
22. *A. barbitrostris*
23. *A. varuna.*

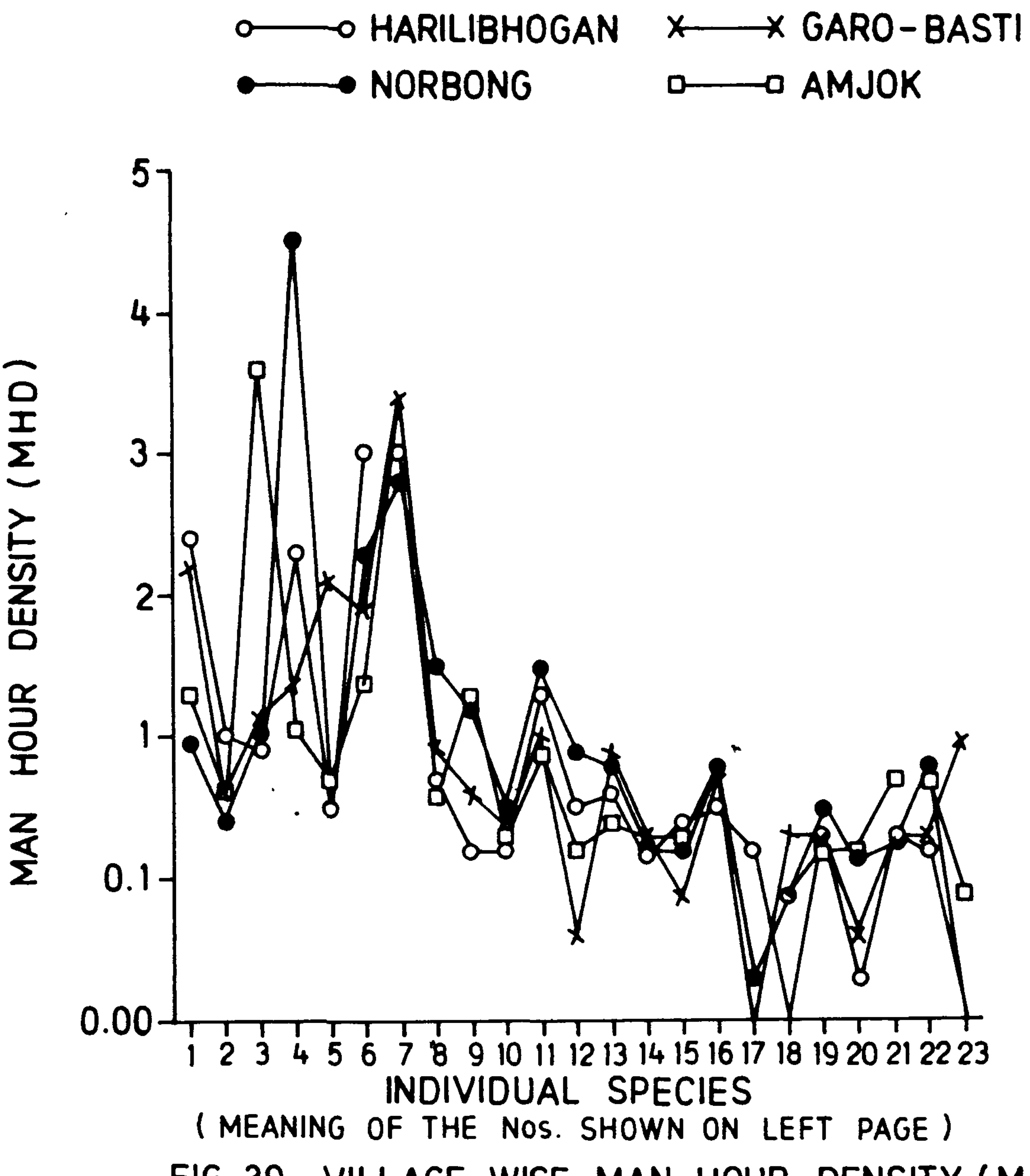

FIG. 39. VILLAGE WISE MAN HOUR DENSITY (MHD) OF ANOPHELES SPECIES OF FOUR DIFFERENT STATIONS AT BYRNIHAT AREA.

asymmetrical, that is, not normally or equally distributed. Skewness lies always between plus minus one(1) (± 1).

Table-7 shows that value at Harilibhogan (-0.5520) and Norbong (1.6172) indicate that the distribution is asymmetrical, that is, the distribution is not normally or equally distributed. Whereas, the value at Garo-basti (0.0557) and Amjok (0.0010) showed a somewhat symmetrical distribution or an almost normal distribution.

Kurtosis

It explained about the flatness or peakedness of the distribution. For a normal distribution, kurtosis should be '3', and hence for any distribution which has got kurtosis greater than '3' will be peaked more sharply than the normal curve and is known as Leptokurtic (that means narrow). While if the kurtosis is less than '3' the distribution is term as platykurtic (that is broad base).

From our Table-7, it can be noted that in all the stations, the value are found to be less than '3', which shows that the curve is a platykurtic type, that is, broadbase, and thereby explained that the species variation is quite high.

Table-7

Statistical Analysis of Adult *Anopheles* Species for Different Stations

Stations	Mean	Standard Deviation	Coefficient Variation	Skewness	Kurtosis
Harilibhogan	8.7917	3.4032	38.7091	−0.5520	0.4528
Norbong	10.2083	4.0823	39.9896	1.6172	1.8982
Garo-Basti	9.5417	3.8186	40.0201	0.0557	0.0213
Amjok	9.6250	3.7728	39.1984	0.0010	0.0001

DISCUSSION

The habits for any species of insects are neither homogeneous nor continuous. The importance of habitat structure upon the population dynamics of insects, to some extent, has been elucidated out by Elton (1949) and Southwood (1977). As pointed out by these authors, there are a number of environmental properties which in various ways make up the ecological factors of the local macrohabitat. To understand the ecology of Anopheles mosquitoes and the interpretation of data at population level in an ecosystem is often a complex task because of the multitude of both abiotic and biotic factors which control the population and the extent to which these factors operate differ considerably from one region to another. The method of shifting (Jhum) cultivation which is the predominant form of agriculture practice among the local people involves clear cutting of forest, followed by burning of the slash and burn cultivation on steep slopes, affects the amount of water passing through the sub-soil; dissolved substances and particulate matter being extensively lost through run-off, and also owing to blow off ash from the burnt site due to strong wind (Toky and Ramakrishnan, 1981, a&b). The uses of insecticides in the control of agricultural pest and other vector-borne diseases also generate a considerable impact on the environment. All these creates a direct instability on the environment as a whole and are the part of stresses to which biotic group of this region are usually subjected. The rate of metabolism of invertebrates is influenced by a wide variety of environmental stimuli (Nassar, 1977). It is the nature of these physiological responses to environmental factors which will determine the biological fitness of individuals of a population and which will ultimately define the distributional limits of the species. The collective environmental limits imposed by these responses determine the fundamental niche of an organism, while the interactions of environmental and biotic factors circumscribe the N-dimensional realized niche as defined by Hutchinson (1958).

The horizontal and longitudinal distribution among the organisms are closely related as most taxa select a micro habitat by optimizing the complex of local factors determining niche while being restricted to a physically distinct zone either by inability to tolerate extremes of one or a combination of those factors as they change with location. Examination of the physiological responses of individuals to specific environmental regimes allows the delineation of casual relationships underlying the distribution of a species (Fry, 1947).

Temperature and altitude difference are considered by many authors to account for most longitudinal and successional distribution patterns (Christopher, 1933; Sprules, 1947, Azeez, 1965; Bhat, 1975b; Nasar, 1977; Nagpal and Sharma, 1987). The general climatic condition of Byrnihat area located in the foothill (182 metres mean sea level) do show some uniqueness. The vegetation composition is being dominated by the broad leaf, *Artocarpus* species, *Saccharum* species, *Panicum* species, etc. It has been found that the pH is generally cyclical. Almost higher temperature values are noted throughout the year (Fig.3). This may be affected by the low altitudinal location of the study area (Hynes, 1970).

High rainfall and steep gradient in the course of the streams and river result in the periodic spates which also facilitate the filling up of the accessory habitat and ultimately form the potential breeding places for mosquitoes populations. The significant positive relationship between breeding group and rainfall is due to a chain of events as reported for *Aedes aegypti* (Tonn *et al.*, 1969, 1970). The steep relief of the upper area might have been expected to promote more rapid run-off than the gentle slopes of the low-land area, but this factor appeared to be encountered by the effects of vegetation-cover on the water retention capacity. Many workers (Hoover, 1944; Delfs, 1956; Rutten, 1958; Bullard, 1965; Binns, 1969; Gray and Edington, 1969; Bachkov, 1970; Likens *et al.*, 1970) have discussed the problems of increased run-off from areas where natural covers has been removed. The rainfall in the upper reaches consequently results rise in water level and increased in dilution of nutrient to an optimum level. This could be attributed to their significant positive relationship with mosquito larvae population (Covell and Baily, 1930; Gill, 1928, Yacob and Swaroop, 1941; Christopher, 1933).

In aquatic ecosystems, temperature is generally considered to be the major environmental properties which limit the distribution of invertebrate (George, 1969; Kinne, 1971). Variation in water temperature during and following rain are slight. The cooling effects of intense rain and an increased heat loss to wind in surface layer are quickly dissipated by turbulence. Elevation of the mean water temperature by 0.5-1.5°C because of surface run-off occurred on several occasions, when the shower was short and intense and followed a day of sunshine. The increases were transitory as losses to the relatively cooler air soon modified their effect (Bishop, 1973). The major factors affecting water temperature by the difference between the warming effects of radiation, absorption and the cooling effect of air (Ricker, 1934) and for tropical water body on particular, the type of substrate (lithological) and degree of exposure to direct sunlight (Geijskes, 1942). This could be attributed to the frequently observed higher water temperature than the air temperature in Garo-basti, Norbong and to some extent both at Harilibhogan and Amjok (Fig.3).

Each organism has a maximum and minimum environmental temperature between which life is possible but beyond which condition are lethal (Lehmkuhl, 1979). Even for individual species, these temperature limits are not absolutely fixed, since they may vary with different sexes, with different individuals, with different life-history stages, with different physiological states and in different parts of the geographical range. The aquatic stages of mosquitoes show an increasing sensitivity to both high and very low temperature (Muirhead-Thomson, 1940c). Eggs of *Anopheles lestris* survive the freezing of the water on which they laid (Otsuru and Ohmori, 1960). In Finnish Lapland, larvae of *Aedes punctor* are active at 0°C, and pupae are active at 1°C. Whereas at high temperature the early stages of *Aedes aegypti* shows variation in sensitivity to high temperature but in a less regular manner than in the species of *Anopheles culieifacies*, *Anopheles quadrimaculatus*, *Anopheles freeborni* and *Anopheles aztecus* (Pal, 1945; Bar-Zeev, 1957b). Larvae of *Anopheles quadrimaculatus* which had been in the 3rd instar less than 4 hours showed a somewhat greater resistance to high temperature than larvae which are within 24 hours of moulting to 4th instar (Barr, 1952). The larvae of *Anopheles gambiae* which live in exposed pools and puddles have a thermal death point on one hour exposure of 42°C, whereas the larvae of *Anopheles funestus* which live in water shaded by standing herbage have a thermal death point of 39°C (Haddow, 1943). Larval populations of *Anopheles quadrimaculatus* decline when surface temperature reach 38°C (Love and Welchel, 1957) and temperature of 40°C-43°C in the normal breeding sites of *Anopheles culicifacies* restrict this species in summer to wells and large tanks where the maximum temperature is 37°C (Pal, 1945). Generally, it appears that species of temperate region tend to be more sensitive to high temperature than tropical species (Takahashi, 1924; Wright, 1927; Muirhead Thomson, 1940C). In the present investigation, the water temperature range recorded is 12°-29°C at Harilibhogan, 13°-30°C at Norbong, 15°-31°C at Garo-basti, and 11°-27°C at Norbong (Fig.12). During the active breeding season, the temperature range is 22°-31°C, then in the cold season when temperature drop to a range of 11°-18°C. This could be an advance step by this population in order to adopt themselves to a newly invaded ecological set up. The rate of growth among the mosquitoes larvae was found to be more dependent on water temperature than much to other environmental factors (Wigglesworth, 1929; Marcovitch, 1960).

Each organism has a toleration range of pH terminated by a maximum and minimum, and possesses an optimum at some intermediate position (Welch, 1952). In a sense that every species has somewhere its extremes of tolerance of acidity and alkalinity (Prosser and Brown, 1961). The literature contains numerous positive contentions that pH is an important limiting factors for certain organism. In addition to the possible direct action of pH as a factor, it may also serve as an index of certain existing conditions in water. For example, the proper determination of pH may in addition give a measure of concentration of the ionised hydrogen, yield indirect information on the free carbon-dioxide, alkalinity, dissolved oxygen, organic loads, dissolved solid contents, etc. (Walch, 1952). In the case with mosquitoes larvae in natural water of varying pH shows that many species are able to live under both alkaline and acid conditions (MacGregor, 1929). *Anopheles culicifacies*, for example, has been recorded in the pH range of 5.4-9.8 (Senior-White, 1926, 1928b) and the tree-hole species *Anopheles plumbeus* and *Aedes geniculatus* from pH 4.4-9.3 (MacGregor, 1921; Keilin, 1932). Observations such as these and the knowledge that female mosquitoes are highly selective in nature in choice of oviposition site have prompted the suggestion that the distribution of larvae is controlled not by survival in suitable and extinction in unsuitable habitats but by the discrimination of the ovipositing female (Beklemishev and Mitrofanova, 1926; Beattie, 1932; Macan, 1961). The same conception is noted in the present study for the distribution of Anopheles larvae in general. The pH range recorded is between 5.8-6.8 at Harilibhogan, 6.2 to 7.8 at Norbong; 6.3

to 7.7 at Garo-basti and 5.9 to 7.2 at Amjok, is noted in their natural habitat (Fig.13). This reveal their leaning towards the acidic range of pH. Hynes (1974) reported that when water flows from non-calcareous rocks, such as slates, or granite, very little calcium is present in solution; the water is, therefore, soft and may be acidic. Acidity occurs particularly where the landscape is peaty because the water is quite unbuffered, the acids added from the peat are not neutralised and in physico-chemical term the pH is lowered. Walch (1952) also noted that ground water seepages and springs, at the base of the foot-hills had pH values as low as 5.0, so that partial neutralization of such contribution must occurs further. All these casual aspects could be attributed to the pH differences in Byrnihat water body.

The free carbon dioxide concentration which exhibits negative correlation with the *Anopheles* larvae population depends on the interaction of the various biotic and abiotic variables of the system. King and Heath (1967) have shown that the leching of newly fallen litter of deciduous trees has great effects in accelerating litter breakdown through increased palatability to the nearby soil organisms. They attributed this effect to the removal of polyphenols from the leaves by leaching. Kowal (1969) reported the importance of leaching and fungal inhibition of pine needle decomposition. Ricker (1957) explained that CO_2 concentration is also inter-dependent on lithology and soil type, rate of water flow, temperature, oxygen concentration, type of flora and fauna composition in the water body etc.

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Specific conductivity data indicated the ionic condition of the water body. Seasonal differences at each station is caused mainly by variation in the ionic composition of the precipitation and the diluting effects of large volumes of rain (Welch, 1952; Sioli, 1969 and Likens *et al.*, 1970). Slack and Feltz (1968) recorded higher conductivity and drop in pH as a result of increased leachate from fallen leaves. A similar situation is noted at the

Harilibhogan which also accounted for higher conductivity values than the remaining respective stations. During the breeding seasons it varies from 40.6 to 62.0 Mmho/cm in Harilibhogan, 30.8 to 50.6 Mmho/cm in Norbong, 40.6 to 57.0 Mmho/cm in Garo-basti and 40.1 to 50.4 Mmho/cm in Amjok (Fig.15). This higher requirement for conductivity in comparison to the rest of the year reflected through their significantly positive correlations with actively breeding populations.

The characteristic properties of the water having weak acid and weak bases have greater buffer action in preventing rapid changes in pH because of the fact that the initial ionization is low. Water containing large amounts of dissolved matters are most likely to show high buffer effects, while low buffer effect is to be expected in water very low in dissolved materials or rather organic colloides (Kleerekoper, 1955). This could be attributed to the higher alkalinity values at Norbong and Garo-basti (Fig.16). In the present investigation, the data on alkalinity shows considerable variable with time probably due to dilution during periods of high rainfall and high flow of water, as also reported by Hynes (1970) and Bishop (1973) for some other lotic system. Even though, as discussed earlier, many mosquitoes species are able to live under both alkaline and acid conditions (Senior-White, 1926; 1928b; MacGregor, 1929), but from investigation on this particular aspect, it shows that total alkalinity of the medium have a positive role in terms of Anopheles larvae population density.

The phosphate-phosphorus values reveals that the most free phosphate was quickly taken up by the expanded root of the aquatic macro-vegetation (Kemp, 1968) and by the aquatic macrophytes as well as algal group. In addition, it also reflect the allochthomous input (Andrews and Minshall, 1979). The role of phosphate-phosphorous in limnological study is well documented (Steward and Rohlieh, 1967; Vallenweider, 1968; Shanon and Brezonick, 1972; Golterman, 1975). Uptake of such a compound in mosquitoes larvae is faster and which occurs partly through the anal paillae and partly across the gut (Hessett and Jenkins, 1951). The malpighian tubes thus regulate the phosphorous balance of the mosquito by removing excess phosphorous from the blood and storing (Stich and Grell, 1955). The amount of phosphate and phosphorous in the natural water is comparatively less and since other organisms requires an adequate supply of phosphate and phosphorous it is generally regarded as a limiting factor (Shanon and Brezonick, 1972). The positive correlation of living organism populations and phosphate phosphorous could be the direct buffering mechanism by them to maintain a suitable pH range for the actively breeding population.

Ammonia is the principal excretory product of most organism and its mode of toxicity as well as concentration lethal to variety of organisms have been documented (Warren, 1962; Campell, 1973). It has been established that the distribution of aquatic fauna largely depends on the chemical properties of water. As external NH_3 concentration increases, the rate of diffusion outward from an animal decreases and toxicity ensures when tolerable body loads are exceeded. Consequently, the toxicity of ammonia to aquatic organisms is generally credited to the NH_3 molecule (Ellis, 1937; Wuhrmann and Workers, 1948; Downing and Merkens, 1955; Spotte, 1970; Hampson, 1976), despite the evidence that NH_4^+ adversely affects some physiological functions (Shaw, 1960; Maetz, 1972; Campbell, 1973). The chemistry of Ammonia in solution has been discussed by Whitefield (1974), Colt and Tchnobanoglous (1976). The proportion of total ammonia existing as NH_3 is dependent on temperature and ionic strength of the medium but primarily on the pH of the solution (Warren, 1962; Truessell, 1972; Skarheim, 1973; Whitefield, 1974). Calculation by these authors shows that the NH_3 increases as pH rises. The exact mechanism which brings about this detrimental effect of Ammonia on the Anopheles larvae is not known. Senior-White proved that, whatever the inhibitory effect may be, it does not operate by hindering the hatching of eggs. Beattie (1932) while discussing the results of her experiments of *Anopheles tarsimaculatus* has concluded that, if a pond is contaminated by urine, the female mosquito may avoid it because of an ammoniacal smell which may be perceptible to her. Beattie (1932) further contends that, from the results she obtained at Trinidad, the ammonia-nitrogen is considered to have some effect upon the oviposition of *Anopheles tarsimaculatus*, but no effect on the larval growth. This has also been previously shown by other workers (Beklemishev and Mitrofanova, 1926). In the present study, monthly data on ammonia-nitrogen and pH values also reveals similar conception.

High values of oxidisable organic matter noticed during winter and spring presumably due to reduced precipitation and water-logged condition which get diluted or rather washed-off with the commencement of rainy season. Seki *et al.* (1969) and Hynes (1970) reported that leachates from leaves contribute considerable concentrations of inorganic and organic materials to the water (10-25% of initial dry weight in the first three days). These dissolved fractions are particularly important in the impoverished waters found in tropical waters and there is growing evidence that these nutrients are rapidly incorporated either *de nevo* through physical phenomena or by microbial flocculation actively, into particulate organic components. Several authors consider that oxidisable organic matters form a part of an important constituent of mosquitoes larval food (Van Thiel, 1928; Hinman, 1932; Shipitsina, 1935) and this could be attributed to the significantly positive role on the distribution of Anopheles larvae, in the present study.

Environment plays a great role in determining the favourable condition for development of most mosquitoes communities (Christopher, 1933; Tonn *et al.*, 1969). All surface water like streams, rivulets, irrigated fields holding waters, wells accessory miscellaneous habitats, provide suitable breeding sites for mosquitoes communities. The general environmental conditions varies, however, in time as well as space; and the variations is reflected in the characteristic seasonal distribution of most mosquitoes communities of a given area. For the present study, a summary of the surveys of Anopheles larvae in the four stations of Byrnihat is given in Table-2. Both the potential larval habitats (rice-field and miscellaneous habitat) and those in which larvae were actually present are shown. The occurrence of larvae as seen, is almost throughout the year and their predominance may be due to wide range of larvae adaptability to physico-chemical characteristic of the breeding water (Sarkar *et al.*, 1978). But the changes in population density due to seasonal factors such as rainfall, humidity and temperature are also noticed. As such, the larvae abundance correspond during the month of May to October, which is the rainfall season, and the dry hot and cool period from November to April without or with minimum rainfall correspond to a period of very low larval population (Table-2). The study enable us to identify for each season the bulk of Anopheles larval population. During the periods of 24 months (May, 1992 to April, 1994), a total number of 3079 larvae were collected at Byrnihat area, of which the total number collected at Harilibhogan are 851 (27.65%), Norbong 766 (24.89%), Garo-basti 725(23.55%) and Amjok 730(23.91%). Even though, the analysis of collection for the four stations reveal no significant differences in both habitats (rice-field and miscellaneous habitats), however, throughout the year wide seasonal fluctuation are noticed. The data which have been averaged month-wise over all the stations individually revealed a marked seasonal variation of larvae occupying in both the habitats (rice-field and miscellaneous habitats). The conclusion drawn from the results was that there was marked changes in larval population which could be correlated with seasonal changes.

For twenty four consecutive months (May, 1992 to April, 1994), a monthly records of miscellaneous habitats was taken from all the individual study areas. Although there was no mark differences among all the stations, but changes in numbers of occupied and unoccupied structures was observed at different seasons. The numbers of miscellaneous habitats increases in the wet season (May to October) and decreases between the cool (November to February) and hot dry season (March to April), since the miscellaneous structures usually dried up during these seasons. The analysis of variance of miscellaneous habitats at Byrnihat area, over all the stations combine shows no interaction between villages and months, but a significant effect of months (Table-3). Rainfall form an important factor in larvae population size in area, where natural and artificial habitats (miscellaneous) are among the dominant breeding sources (McClelland, 1963). And almost throughout the year larvae population occurred in the same habitats those containing rain-water and these habitats were also more likely to contain organic debris and thus also form suitable habitat for other aquatic dwellers (Yasuno *et al.*, 1970).

The analysis of miscellaneous habitats for each of the four stations has also been done separately. The analysis of variance thus shows a significant interaction of both months and field in all the individual stations (Table-4). There is no doubt that the number of miscellaneous habitats (both occupied and unoccupied) varies from month to month in all the stations. Although the study showed a marked reduction in the month of November onward, it then exhibit a tendency to increase again in the month of May (Table-2). Thus, the reduction occurred at the beginning of the coal season and then the indication of recovery at the beginning of the wet season. Even though, as mentioned, the analysis of variance in all the individual stations shows a significant between months and fields, it would be appropriate to consider Harilibhogan separately, basing on the total number of miscellaneous habitats (Table-2). The proportions of the total available miscellaenous habitats investigated at Harilibhogan were remarkably large as compared to Norbong, Garo-basti and Amjok. The total percentage of miscellaneous habitats shows Norbong (24.35%), Garo-basti (23.55%) and Amjok (23.91%), whereas at Harilibhogan it contribute to (27.65%) of the total available miscellaneous habitats. However, most of these habitats at Harilibhogan, were relatively impermanent and were seldom occupied. The differences between Harilibhogan, from the other three stations (Norbong, Garo-basti and Amjok) with respect to the total numbers of miscellaneous habitats, were not due to the effect of weather but instead due to the ecological factor related to the number of miscellaneous structures present.

Environment plays a great role in determining the distribution of mosquitoes of an area. Among the environmental factors, climatic incorporating rainfall, temperature, and relative humidity are the decisive elements for mosquitoes growth. The daily rhythm of movement is controlled by the overall effect of the environmental cycle. The activities of an adult mosquitoes are of various sorts, such as dispersal, mating, feeding and egg laying (Rao, 1989). These behaviour is also believed as the net product of genetic factors and ecological factors. For example, while host selection and choice of breeding source appear to be governed by genetic factors, whereas density, longevity, feeding frequency etc. are dependent upon meteorological and climatological factors of a particular geographical location (Sadanand, 1989). These features vary in space and time for numerous species and within many broadly distributed species there are also local variations. For each species of mosquito, there are set of general environmental conditions most favourable for development. The variations in these conditions which are optimal will have effect on the population of that species. In a givren area the local distribution of a species is controlled by reaction to environmental differences among the available range of habitats. The general environment varies, however, in time as well as space; and this variation is reflected in the characteristic seasonal distribution of different species of mosquitoes of a given area.

The distribution of *Anopheles* adults at Byrnihat area was understandably higher during the wet season then during the dry season. The mean monthly maximum temperature of this area under investigation ranges from 14°C (January) to 29°C (June). The mean minimum temperature was lowest in January (11°C) and the highest in June (27°C). The area is under the influence of South-West monsoon and North-East monsoon. June and September were the months of heaviest rainfall and the periods from November to February was almost dry (Fig.3). During the course of investigation employing a total of 128 man hours, altogether 2587 adult *Anopheles* consisting of 23 species were collected. The maximum numbers among all the species collected are *Anopheles phillipinensis* 403(15.58%), *Anopheles vagus* 297(10.63%) and *Anopheles hyrcanus* 275(10.33%). Both human-dwelling (indoor) and cattle-dwelling (outdoor) collection were carried out concurrently at the same place. The number collected from the cattle-dwelling was nearly 80 times that in human-dwelling. Table-5 depict the month-wise total number of adult *Anopheles* species encountered during the periods of investigation. The total number collected was maximum during the wet season (May to October) which clearly indicates that the climatic factors, that is, temperature, rainfall, humidity, etc. of the rainy season favour the breeding of most species to a greater extent in comparison to the other seasons (Chandrahas *et al.*, 1979). The trend of the relative abundance of the adult *Anopheles* species also reveals that preferred abundance of the diversity vary from season to season, and the maximum of

20 species was observed on June, the minimum of 5 species in the month of December (Fig.23). The month-wise total population of *Anopheles* species also exhibits a seasonal fluctuation with the maximum at 280(10.32%) in the month of May and a minimum of 21(0.81%) and 22(0.85%) in the months of December and February (Fig.24).

To investigate in more details the possible differences within Byrnihat area, studies concurrent with those already described were made in the four stations (Harilibhogan, Norbong, Garo-basti, Amjok) at the individual levels. There is no evidence of greater differences from month to month, within the individual station with respect to climatic factors, the population also shows a general trend with maximum in wet season and minimum in cool and dry seasons. The differences between stations did not also seem closely related to the type of environment, but there are quite marked differences within individual station with respect to the total number collected and to some extent the types of species occurring. Month-wise analysis of the total number of species occurring at Harilibhogan varied from 15 types of species in the month of May to 4 types of species in the months of December and February (Fig.29). Norbong had a fluctuation range of 16 species in the months of May and August to 3 species in December (Fig.30). Whereas, at Garo-basti and Amjok, a maximum of 16 species was observed in the month of August and 3 species in the month of December (Figs.32,32). The total number of individual species encountered at the different stations during the period of investigation also did show marked differences. At Harilibhogan, out of the 21 species encountered, *Anopheles hyrcanus* 97(15.85%) and *Anopheles phillipinensis* 96(15.68%) form the largest population (Fig.35). At Norbong, from the 22 species encountered, *Anopheles vagus* 114(16.76%) and *Anopheles phillipinensis* 90(13.23%) form the maximum (Fig.36). At Garo-basti, *Anopheles phillipinensis* 108(17.20%) and *Anopheles maculatus* 71(11.30%) form the maximum of all the 22 species encountered (Fig.37). Whereas Amjok shows the present of 23 species (Fig.38) with *Anopheles phillipinensis* 109(17.36%) and *Anopheles aconitus* 104(16.56%) forming the largest dominant species. Thus, among the 23 species of *Anopheles* collected during the periods of investigation, the species not encountered are *Anopheles stephensi*, *Anopheles varuna* at Harilibhogan; *Anopheles varuna* at Norbong and *Anopheles balabacensis* at Garo-basti; whereas Amjok shows the existence of all the *Anopheles* species recorded. The month-wise total number of *Anopheles* species collectively at four different stations also shows a marked seasonal variation, with wet season maxima and the dry hot and cool seasons as minima, the peak end fall were also prominent. At the individual levels the stations -

Harilibhogan, Norbong, Garo-basti and Amjok, had a total population fluctuation range of 73-5, 97-5, 78-3 and 80-5 respectively (Fig.33). *Anopheles phillipinensis* known to be the principal vector of Byrnihat area (Rajagopal, 1976) and which form the maximum number among all the species encountered, is most abundance during the months of May to September, corresponding to the active malaria transmission time (Table-1). *Anopheles minimus* where it had long been recognised as the main vector of Byrnihat, was never collected, and is believed to have disappeared from the area (Rajagopal, 1976 and Kalra, 1978). The disappearance of *Anopheles minimus* is believed to be due to its susceptibility nature to residual insecticide D.D.T. (Betram, 1950).

Density denotes the number in which a species is encountered in specific time in a given area. Sir Ronald Ross himself mentioned that malaria transmission by *Anopheles* mosquitoes is a function of the number in which they occur. That means, malaria transmission cannot be possible if the *Anopheles* mosquitoes are lower in number per unit area then required. T. Ramachandra Rao (1984) in his book The Anophelines of India, further pointed out that even if a species has all the characteristic, which can make it a very efficient vector - being highly susceptible to infection, long-lived and biting man by preference - it can have no place in transmission in a locality if it does not occur in adequate number. In the present study at Byrnihat, the month-wise total Man Hour Density (MHD) at 62 (Fig.26). Whereas, the maximum species wise Man Hour Density (MHD) are of *Anopheles phillipinensis* (3.2) a malaria vector (Fig.27). The higher density in the wet season as seen, was due to the favourable environmental conditions and subsequently also the increase in the breeding. Thus, explained that transmission season, corresponds to the period of higher density of the vector (Russell and Rao, 1942).

The species-wise Man Hour Density which has also been worked out, explained the variation in number among the species and also from station to station (Table-6). The total Man Hour collection was highest at Norbong (21.25). Next in order was at Garo-basti (19.63); Amjok (19.63) and the least at Harilibhogan (19.13). The range of Man Hour Density variation at Harilibhogan are 3-0.03; Norbong 4.5-0.03, Garo-basti 3.4-0.06 and Amjok 3.6-0.09 respectively. The monthly Man Hour Density at Harilibhogan shows the highest (50.4) in the month of May 1993 and lowest (0.3) in the month of March 1993. Norbong shows the highest (73.29) in the month of May 1993 and lowest (0.5) in the month of December 1993. Garo-basti reveals the highest (50.91) in the month of May 1993 and lowest (0.5) in the month of December 1993. Amjok exhibit the highest (60.1) in the month of May, 1993 and lowest (0.4) in the month of February, 1993

(Fig.34). Though as previously mentioned, the trend in all the stations shows the highest population wet season and lowest in the hot dry and cool seasons, variation is often seen considering the data at the individual levels, with respect to their total population and the species-wise occurrence and abundance. Garo-basti and Amjok, despite their similarity with respect to the total population (Table-6), differences occurs in the total number of species present; which is 22 in Garo-basti and 23 in Amjok, also the total population of their common species varies. These variations within an area as pointed out by T. Ramachandra Rao (1984) in his book <u>The Anophelines of India</u> arise dur to various factors like human population, infiltration and migration, then also the availability and extent of suitable breeding places, the dispersal, degree of infiltration and exodus etc. which are influenced by biological and physical factors, varies from locality to locality, greatly effects the distribution patterns and adaptability of a species. Menon and Rajagopalan (1980) also referred that absent of close proximity of breeding places to habitations, plus the lack of any sizeable number of poultry, cattle and other animal greatly effect the density of the mosquitoes. The differences in these factors as seen in the present study, must have also resulted in the variations of the Man Hour Density among the individual stations.

The *Anopheles* species population in all the stations showed a decrease from November but recovered again by May. Examination of the total number of *Anopheles* species occurring in the individual stations also suggested that neither area is more affected by weather conditions than the other. Hence, differences of the species composition in almost all the stations were due neither to any obvious ecological factors related to the type of habitat but the significant effect of months.

The analyses of Coefficient Variation that has been worked out in all the four stations, revealed that Garo-basti (40.0201) has more or less better species composition in respect to the three other stations - Harilibhogan (38.7091); Norbong (39.9896); and Amjok (39.1984). The least species composition among all the stations was Harilibhogan (Table-7).

From the data statistical analyses for skewness shows that the values of the individual stations was Harilibhogan (-0.5520); Norbong (1.6172); Garo-basti (0.0557) and Amjok (0.0010), thereby revealing a difference among all the stations. Therefore, on comparing the values obtained we can emphasize that the species distribution at Harilibhogan and Norbong is not equally or evenly distributed, but Garo-basti and Amjok indicates that the *Anopheles* species distribution as evenly or normally distributed (Table-7).

The statistical analyses of Kurtosis of *Anopheles* species for the individual station shows that the month-wise species variation in almost all the stations exhibit a platykurtic type of curve, that is, less than 3" [Harilihogan (0.4528); Norbong (1.8982); Garo-basti (0.0213) and Amjok (0.0001)], thus explaining that the species variation is quite high (Table-7).

Considering all the data together, it would be reasonable to conclude that there was marked reduction of *Anopheles* species population during the hot dry and cool seasons, and the magnitude and timing of the decline vary slightly from place to place. From our study we can also say that among all the environmental factors, rainfall form an important factor, where the natural and artificial temporary miscellaneous habitats also form the main breeding sources, apart from the rice-field. The results, therefore, fully support the conclusion reached by McClelland (1963) that the rainfall is an important factor in adult population size in area where natural water containers or temporary artificial habitats are the dominant breeding sources. The data also clearly showed that local environmental changes, as well as climatic changes influenced the composition, distribution and variation of the different *Anopheles* species occurring in the area.

Part-II

ULTRASTRUCTURAL STUDIES

INTRODUCTION

Integument is one of the primary organ systems of an animal, which is truly remarkable, complex organ of diverse structure and function. The generalized structure of insect integument can be divided into three parts : one cellular layer, the epidermis; two non-cellular layers, the basement membrane; and the cuticle. Insects cuticle which form the uppermost (surface) part of the integument can be seen to consists of the major different layers - (i) a comparatively thick endocuticle; (ii) a moderately thin exocuticle and (iii) a very thin epicuticle. Endocuticle and exocuticle are often referred together as procuticle. Epicuticle despite its comparative thinness, is exceedingly complex and possesses characteristics that make it can extremely important layer of the cuticle.

The term cuticle may be defined as the material secreted onto or deposited on the outer surface of the epidermal cells, which solidifies there to form the exoskeleton. Presence among the epidermal cell are dermal glands which also play in the secretion of cuticle through the integumentary ducts.

Cuticle is undifferentiated remnants of the ectoderm, and as a consequence, its cells preserve in a high degree the potentiality of transportation, secretion, excretion, and sensitivity, while further more, they retain a large capacity for development, as known in the many specialized organs that have been evolved in the integument, as well as the adaptive function to protection and locomotion.

The cuticle overlying all the sensory organs and respiratory surfaces are adequately permeable to the appropriate molecules. It must bear the brunt of all external things and form of energy that touches upon; it must be able to receive impressions of changes in the environment to which it responds. Further it is the principal agent of the motor mechanism.

The reaction of an insect to the environment, that is, its adjustment to the external changes by movement or other form of responses activated from within, is dependent on two accessory conditions : (i) the outer surface of the animal must be in part at least "sensitive" to environment changes; (ii) the sensitive areas must have connection with the motor mechanism. Responses of animal towards its environmental changes is through the special receptors which constitute the "sensible", which set off a chain of events that ultimately result in the nerve impulse towards the internal environment (the nervous system). The energy sensed by insects in the form of various mechanical changes falls under the general heading of mechano-reception; the mutual attraction and repulsion form of energy is referred to as chemoreception; and in other instances, the energy stimulating a given sense organ in form of electromagnetic waves (or photons), as, for example, light and heat energy are considered under the headings photorecption and thermoreception, respectively.

The first step in understanding the behaviour of a given species is a thorough examination of the sensory physiology of the species. Insects are provided with a great variety of external sense organs having characteristic structural differences in both the cuticular and cellular parts. It is very difficult, however, to isolate the various types of organs for the experimental purposes, and for this reason, we can, in most cases, only form an opinion as to their probable function based on a study of their structure; the structure is often so widely different from that of any organs of known function in other animals that many insects sense organs cannot yet be satisfactorily identified as receptors for any particular group of stimuli. The sense organs of insects, therefore, are generally classified on purely anatomical basis. The receptor complex is formed of the cuticula, the sense cell or group of sense cells, and the associated chitinogenous cells, called sensillum.

Basing on the anatomical differences various types of sensilla may take the form of a spine, a scale, or a minute peg, and it may be sunken into a pit or deep cavity of the integument. The different types of sensilla known among the insect species are (i) *Sensilla trichodea*, (ii) *Sensilla basiconica*, (iii) *Sensilla chaetica*, (iv) *Sensilla ampulacea*, (v) *Sensilla coeloconica*, (vi) *Sensilla placoidea*,

(vii) *Sensilla campaniformia*, (viii) *Sensilla clariformium*, (ix) Sensory peg, (x) Pit sensilla, (xi) Hair-plate sensilla, etc.

Sensilla trichodia is a typical setiform variety, which occurs in most numerous and varied form. The general identifiable differences in morphology is believed to correspond to different sensory function. Briefly, *Sensilla trichodia* types and sub-types have been supposed to be receptive to odors and are distinguished as contact chemosensilla. Many of them, however, functions as Mechano-receptors.

Sensilla basiconica is a small peglike or conical structure and occurs on all parts of the body. The possible chemoreception function of organs of this kind is suggested by thinness and apparent permeability of the cuticular wall of the external process, and by the presence of a large vacuole surrounding the fascicle of sense cell processes.

Sensilla chaetica are merely trichoid sensilla in which the external process is bristle-like or spine-like rather than typical setiform. They are tactile in function and according to Dethier (1963) the mechanosensitive hairs fall into two groups : (i) Velocity sensitive, and (ii) Pressure sensitive.

Sensilla coeloconica are peg organs sunken into depressions of the body wall. This sensilla is further differentiated into two types : (i) Large sensilla coeloconica and (ii) Small sensilla coeloconica. Large sensilla coeloconica consist of short pegs (about 5 mm in length) in the floor of a pit, the sides of which close partly over the peg tip. Although no electrophysiological evidence exists, it is believed to be probably olfactory in function.

Small sensilla coelonica is further sub-divided into two or three so called "small sensilla coeloconica." The morphological features are consistent with the "no pore, inflexible socket" hygro-/thermosensilla. Although much variation in response has been found, such sensilla often have two cells that respond to changes in humidity, one cell being inhibited, and a third cell that responds positively to decreasing temperature (i.e. a "cold" cell).

Sensilla placoidea are oval or eliptical plate surrounded by a narrow ring of membrane and are also distinguished as plate organs. They closely resemble that of sensilla basiconica having multiple sense cells. Sensilla placoidea are generally regarded as olfactory in function.

Sensilla ampullaceae are peg organs in deep pits of the body wall. Their morphological similarity to small sensilla coeloconica suggests that the sensilla ampullaceae are "no pore sensilla with inflexible socket. It is believed to be sensitive to thermal and moisture stimuli.

Sensilla campaniformia externally appears, in some cases, as small dome-like papillae, slightly convex and are usually less than 25-microns in diameter. They may sometimes take the form of minute discs slightly sunken into the body wall, resembling in surface view vacant hair follicles. However, they are usually distinguished from the circular hair sockets by a more elliptical or oval form. Earlier, the function of the organ is a subject on which there is some difference of opinion, but McIndoo (1914, 1915) has given much experimental reason for believing that the organs are receptors of odor stimuli.

Hair-plate sensilla are composition of groups of trichoid sensilla which are present in the joint regions of insect legs, antenna, neck, wings and abdomen. Hair-plate sensilla function as propireceptors or position detectors. These receptors provide the insect with continuous information as to the position of the various body parts and the tensions of the various muscles.

Capitate pegs sensilla are thin-walled "multiporous pitted" or "MPP" sensilla. Morphological evidence build a strong circumstantial case suggesting its involvement in CO_2-detection.

Grooved pegs sensilla are thick-walled, and sculpted "Multiporous grooved" or "MPG" sensilla. Physiological evidence as provided by many authors shows that it is olfactory in function and respond to vapours of ammonica, acetone, and water (by excitation) and of acetic acid and anisole (by inhibition).

The detailed structural aspects made on the sensory organs of the insects in the optical microscope, did not give any satisfactory result due to some drawbacks, like low resolving power and depth of the field. In optical microscope these receptors (sense organs) can only be detected like small hairs, and it was also been generally believed that these receptors viz. chemoreceptors nerve ending were covered by a specialized cuticle, through which the stimulating substance was able to pass. It was not until the electron microscope came into use and also adequate methods of preparing material to be examined with it has been developed that clear picture regarding the structure of the insects sense organs were understood for the first time (Slifer *et al.*, 1970). In these present days, with the help of electron microscopy, the detail structure of the sensory organs, like the shape and size could be differentiated, of which each type has got a specific name and function. As for example, the structure of sensilla companiformia could now be ascertained, such as, the round omni-directional and the oval preferential type. And it is now known that the omni-directional type, of the receptor functions in all direction, whereas in case of preferential type, the receptive action is on one direction only. However, during the past 30 years special method for identifying the pores of the sense organs with a solution of crystal violet applied externally on the surface of the insect (Slifer, 1960) and examination of the material in a medium with suitable refractive index, and the uses of silver-stained section have also permitted the identification of the structural aspects of the sense organs even with light microscope. But the problem again lies with the low resolving power of the optical microscope thereby making the use of these methods to larger structures only.

Though the basic principle and function of the scanning electron microscope and optical microscope are similar, the main difference between the two, i.e. in the illuminating beam. In an optical microscope the source of illumination is light, whereas in scanning electron microscope the illumination is by electron. Electrons are preferred owing to their much smaller, wave-length (0.06 A°) in comparison to that of light (2,000 to 7,500 A°), and it is known that smaller the wave-length of the illuminating beam, the greater is the resolving power.

Studies on insect sense organs that began with Hodgson *et al.* (1955) and thereafter continued by others viz. Hocking (1960); Slifer (1960, 1961, 1962); Schneider (1963, 1964); Hocking (1964); Boeckh *et al.* (1965); Bullock *et al.* (1965);

Wigglesworth (1965); Deither (1966); Haskell (1966); Schoonhoven *et al.* (1967); Schoonhoven (1967); Deither *et al.* (1968); Edman *et al.* (1968); Corbiere-Tricthane (1971); Arbogast *et al.* (1972); Mercer *et al.* (1973); Chu-Wang *et al.* (1975); Zacharuk (1985); Kennedy (1986); Allan *et al.* (1987); Ameismeier *et al.* (1988); Dey (1988, 1991); Dey and Dkhar (1992), have all helped us to understand the physiological behaviour of the insects.

Further, the electron microscopic studies on such structure, which started extensively among the insects during the early seventies onwards, have provided abundance evidence confirming the conclusion derived from the study of their structure. The information concerning the structure of the sense organs obtained with this modern technique is now available for quite a number of insect species, as seen in works done by various workers, such as, Rowley *et al.* (1972); McIver (1975); Barlin *et al.* (1981); Itoh *et al.* (1984); McIver *et al.* (1975, 1978, 1984, 1985); Lee *et al.* (1985); Bogner *et al.* (1986); Murlis (1986); Sutcliff *et al.* (1987); Tichy (1987); etc. It is now, therefore, possible to state for many species with a high degree of confidence, as could not be done prior to the usage of this instrument.

The sense organs or receptors play a vital role in the life of the insects. Basically, the function of any sense organ is to receive some form of energy (stimulus) from the environment and subsequently set off a chain of events that ultimately result in a nerve impulse (Dethier, 1963). Description of the physiological study of behaviour and control, consists in the analysis of the movements of the whole organism, into a series of reflexes or observed correlation between stimulus or response. Behaviour is determined, or at least influenced at every step, by the stimuli to which the sense organs are subjected, and the functions of the sense organs and their powers of discrimination are discovered largely by observations on behaviour. Therefore, it is not possible to separate these completely. The various types of sensilla differ from one another on the function they perform. *Sensilla trichodia*, *Sensilla placodea*, *Sensilla basiconica*, *Sensilla campaniformia* etc. are present in different quantities on the body and appendages of the insects. The number and arrangement also vary from species to species. In other words these sensilla are also known as chemoreceptors, photoreceptors, mechanoreceptors, depending on their respective function. The body and appendages of an insect are covered with innumerable hairs, and it is difficult to touch the animal without bending some of these hairs, which will lead to the response and alertness of the animal. That is why, they are considered to be very intelligent due to their sensitiveness and their fast response to any stimuli applied to them. Snodgrass (1935) stressed that the sense organs are often poetically said to be the "windows of the soul".

The multifaceted manifestations of nature to which insects respond through sense organs include :

1) The energy of moving masses or molecules of matter, and the energy of contact with stationery matter, giving the sensation of touch (tactile sense).

2) Vibrations of matter that give the sensation of sound (auditory sense).

3) Vibrations that give the sensation of heat or of changes in temperature (thermal sense).

4) Substances in a chemically active state (ionization) that give the sensation of taste (gustatory sense).

5) The impalpable state of matter that gives the sensation of small (olfactory sense).

6) Electromagnetic vibrations of certain magnitudes giving the sensation of light (visual sense).

7) Gravity, for which there is no equivalent in consciousness (static or geotropic sense). Complexity of sensory system, however, is determined by the behaviour of an insect. Those insects which lead a comparatively simpler life are found to contain less-developed cuticular sensory system. In our present study, from the behavioural analysis it was found that mosquitoes show an array of complex behaviour in terms of its nutrition, locomotion, reproduction

etc. Hence it was thought a detailed study of sensory system with Electron Microscope would throw some light in understanding its behaviour.

The life of a female mosquito is governed, in large part, by its orientation responses to stimuli from important resources, such as, resting sites, oviposition sites, nectar sources, mates and blood hosts. Odors emanating from these (with probable exception of mate) are known to be important orienting stimuli for the female mosquito. All these are contributed by the presence of cuticular sensory receptors used to detect odor, heat and humidity. The cuticular parts of the various kinds of mosquito sense organs differ conspicuously and may take the form of hairs, pegs, pines, papillae, loops, plates, etc. This, together with their position on or near the surface of the body wall where they are accessible for study, provides the primary data for a system of classification of these sense organs. This, in fact, is not a new approach and has been studied by previous workers (Brett, 1938; Muirhead-Thomson, 1940; Kennedy, 1940; Rao, 1947; Brown, 1951; Roth *et al.*, 1952; Bellamy *et al.*, 1952; Hocking, 1953; Sippel *et al.*, 1953; Haddow, 1954; Smart *et al.*, 1956; Kalmus *et al.*, 1960; Kellogg *et al.*, 1962; Ismail, 1962; Steward *et al.*, 1963; Hocking, 1964; Haskell, 1966; Acree *et al.*, 1968; Wood *et al.*, 1968; Klassen, 1968; Kellog, 1970; McIver, 1970, 1971), in which the sensory structure are separated into classes on the basis of their cuticular component. However, new information gained with electron microscope along with physiological and behavioural studies of attractants and other directing influences in the mosquito's life now necessitates changes in the system used previously. References to literature on such aspects are found in works done by Omar *et al.* (1971); McIver (1972, 1973, 1974, 1982, 1984); Schreck *et al.* (1972); Elizarov *et al.* (1972); Nayar *et al.* (1973); Davis *et al.* (1972, 1975, 1976); Service (1974); Boo *et al.* (1975, 1976, 1980); Davis (1977); Magnarelli (1979); Gillet (1979); Browne *et al.* (1981); Bidlingmayer *et al.* (1981); Bently *et al.* (1982, 1989); La Salle (1982); Jaenson (1985); Allan (1987); Sutcliff (1987, 1994); Bowen (1991), etc. Although with advance application of morphological techniques and cataloging of the sensory complements is now achieved, less comprehensive and no useful information is available on sensillar morphology. Sutcliff (1994) stated that very little new information on mosquito sensory morphology has been forthcoming in the last 10 years. It is, therefore, still important that proper morphological descriptions of specific sensillar types continue to be made to support electrophysiological and behavioural studies of attractants.

In line with brief statement of the scope, the main objective of the present study regarding the morphological descriptions, is to review our present knowledge of the sensory organs of individual mosquito species, and to evaluate and compare with those of other insect species. The presently study was undertaken on the "ultra-structural studies of cuticle surface and the associated sensory system" of the sensory organs involving two known mosquitoes species - *Anopheles vagus* and *Anopheles annularis*.

MATERIALS AND METHODS

The mosquitoes - *Anopheles annularis* (female) and *Anopheles vagus* (female) were collected from Byrnihat areas during the year 1992-1994. The body parts - Head, thorax, abdomen, leg and wings were excised after killing the insect by exposing them to benzene vapour. Altogether six of the individual species were used for the study. They were then secured horizontally to the brass stub (10 mm diameter x 30 mm high) with the help of double coated adhesive tape, connected via a patch of silver paint to ensure charge conduction. Samples were directly mounted on the stub, since they were hard cuticular structures, which need no preparatory techniques (Wolley and Vossbrinck, 1977). Care was taken to avoid trapped air bubble. A thin conductive coating of gold was applied to the sample using a JFC 1100 (Jeol) ion sputter, establishing a relatively low vacuum (10^{-3} torr) in the sputtering chamber. The coated samples were observed with a JSM-35 CF (Jeol) scanning electron microscope operated at 15 KV. Tilt control was fixed at 0° for setting the specimen stage in a horizontal position. WD selector was used to set the working distance (WD) to 15 mm.

RESULTS

Anopheles annularis - In *A. annularis*, the sensilla which could be detected are sensilla trichodea, sensilla chaetica, sensory peg, spinules, hair-plates, basiconica and campaniformia.

Sensilla trichodea - Eleven types of sensilla trichodea were detected in various locations on the body of the species. The different types of the sensilla varied in their length, breadth, diameter and also on their surface feature. Some of these sensilla had other special characteristic apart from those mentioned.

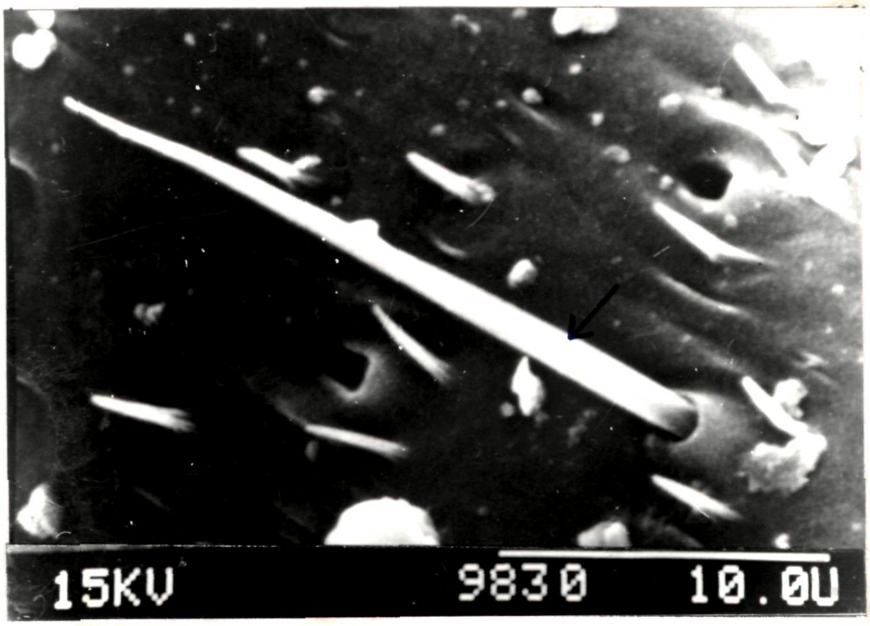
<u>Sensilla trichodea type-I</u> : The shaft is slightly tapered and longitudinally grooved. This sensilla is located on the femur of meta-thoracic leg, meso-thoracic leg and pro-thoracic leg (Figs.40, 41, 42). These sensilla range in length from 18.33 m to 52 m, the width at the base vary from 1.28 m to 2 m, while that at the tip from 0.25 m to 0.83 m. The sensilla is socketted and the diameter of the socket vary between 2.5 m to 3.5 m (Table-8a).

<u>Sensilla trichodea type-II</u> : The shaft is tapered and has faint longitudinal grooved. These sensilla is located on the tibia of the meta-, meso- and pro-thoracic legs. The sensilla occurs over the entire area of the segment (Figs.43,44,45). The sensilla trichodea type-II range from

Fig.40 Scanning Electron Micrograph (S.E.M.) picture of the femur of meta-thoracic leg of *Anopheles annularis* showing the sensilla trichodea type-I x 3600 µn.

Fig.41 SEM picture of the femur of meso-thoracic leg of *A. annularis* showing the sensilla trichodea type-I x 1100 µn.

Fig.42 SEM picture of the femur of pre-thoracic leg of *A. annularis* showing the sensilla trichodea type-I x 1800 µn.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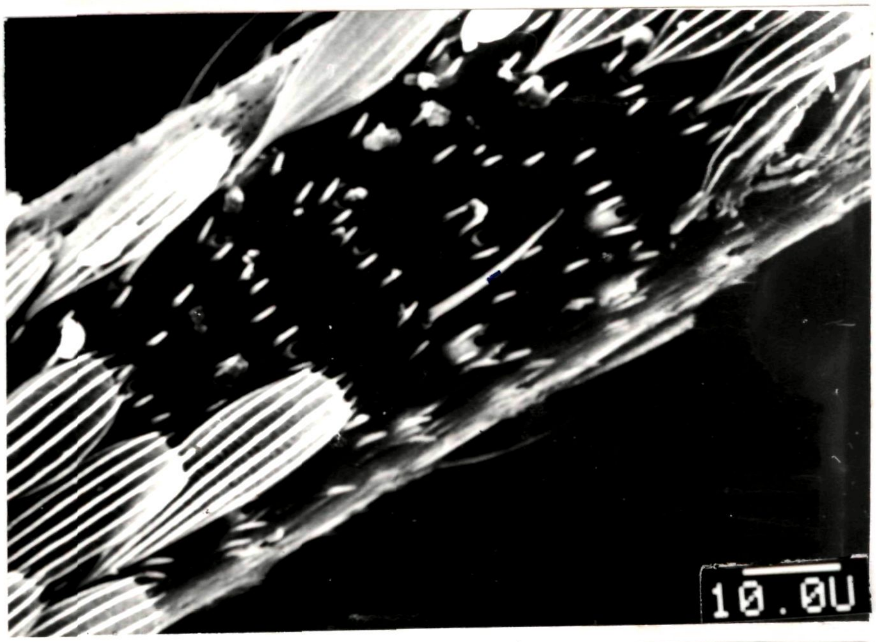
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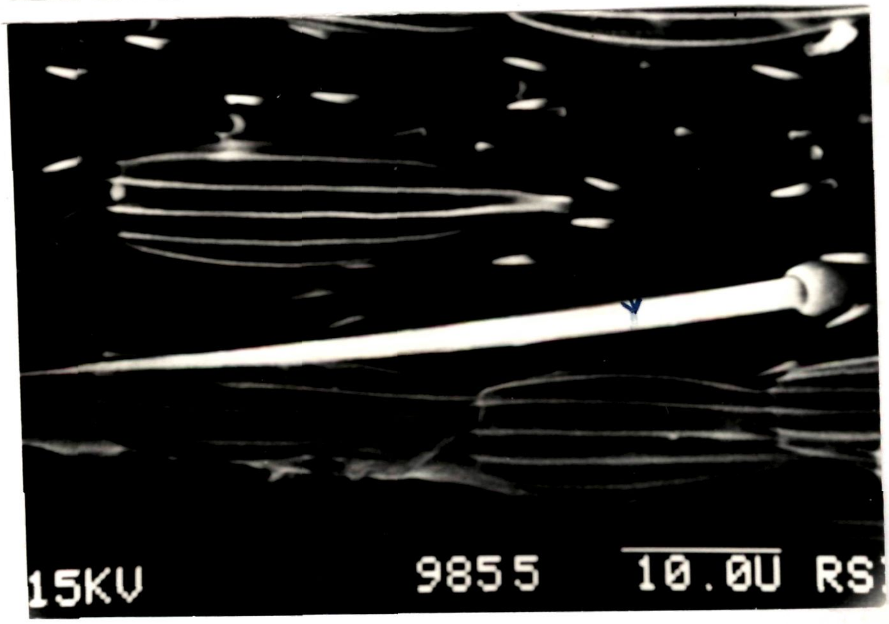
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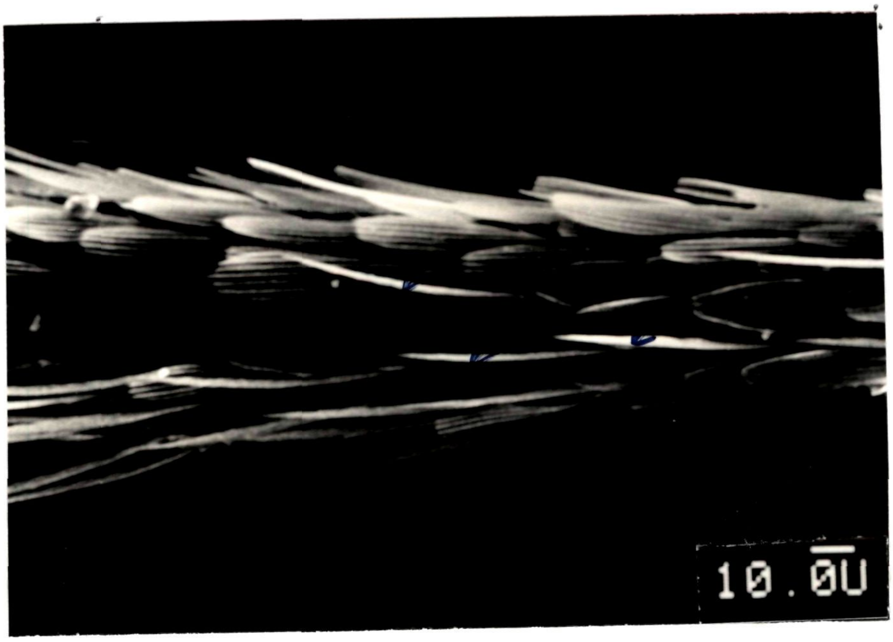
Fig.43 SEM picture of the tibia of meta-thoracic leg of *A. annularis* showing the sensilla trichodea type-II x 480 µn.

Fig.44 SEM picture of tibia of meso-thoracic leg of *A. annularis* showing the sensilla trichodea type-II x 1600 µn.

Fig.45 SEM picture of tibia of pro-thoracic leg of *A. annularis* showing the sensilla trichodea type-II x 1000 µn.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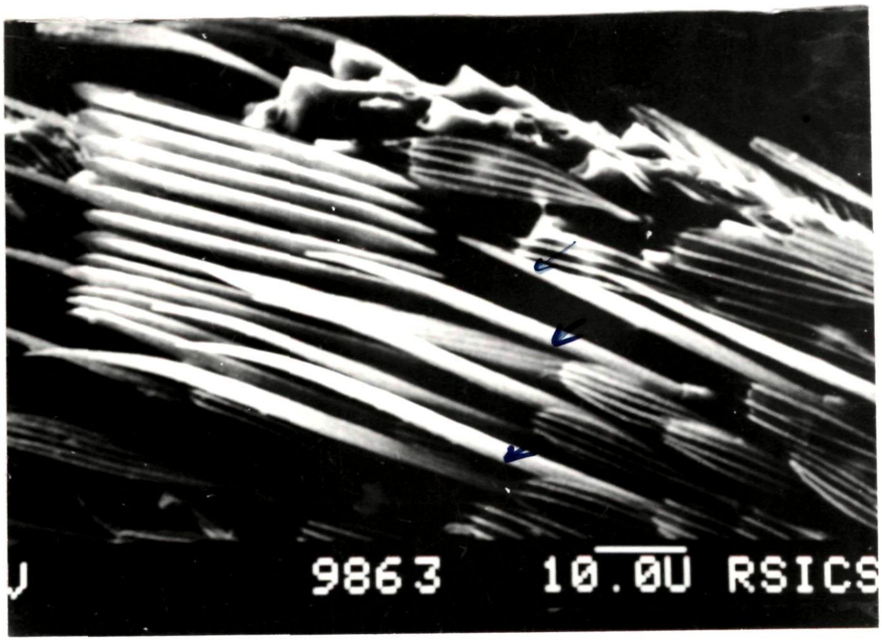
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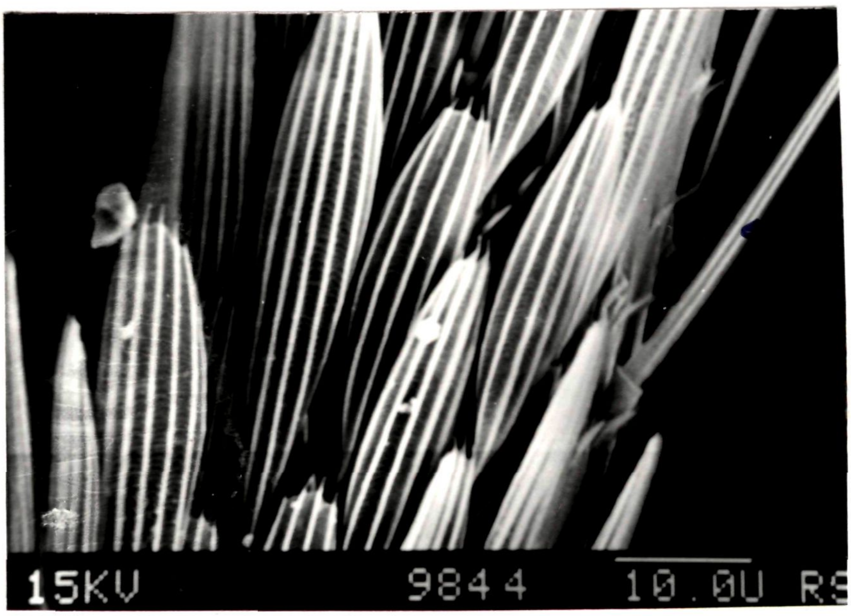
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Table-8(a)

Distribution Pattern and Dimension of Different Types of Sensilla Trichodea of *Anopheles annularis*

Sensilla	Location	Breadth			Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
		Length (µ)	Base (µ)	Tip (µ)				
Type-I	1 Femur of Meta-thoracic Leg	22.50	1.28	0.25	2.56	Longitudinal Grooves	$2/mm^2$	
Type-I	2 Femur of Meso-	18.93	1.67	0.83	2.50	Longitudinal Grooves	$1/mm^2$	
Type-I	3 Femur of Pro-thoracic Leg	52	2	0.50	3.50	Longitudinal Grooves	$2/mm^2$	
Type-II	1 Tibia of Meta-thoracic Leg	86	2	1	3.30	Longitudinal Grooves	$19/mm^2$	
Type-II	2 Tibia of Meso-thoracic Leg	43.33	2.35	0.28	4.12	Longitudinal Grooves	$2/mm^2$	
Type-II	3 Tibia of Pro-thoracic Leg	3.33	1.33	0.66	3.63	Longitudinal Grooves	$17/mm^2$	
Type-III	1 First Tarsal Segment of Metathoracic Leg	62.31	6.00	0.77	3.85	Longitudinal Grooves	$2/mm^2$	Tip Portion Slightly Curved
Type-IV	1 Tarsal Segment of Meso-thoracic Leg	52.86	4.29	1.43	5.71	Longitudinal Grooves	$4/mm^2$	Almost a Blunt Tip
Type-V	1 Third Tarsal Segment of Pro-thoracic Leg	13.75	1.75	3.14	3.75	Longitudinal Grooves	$6/mm^2$	Tip Portion divided into five parts
Type-VI	1 Dorsal Surface of the Head	184	4	1	9.31	Longitudinal Grooves	$3/mm^2$	Socket Round and Double Chambered

Sensilla	Location	Length (µ)	Breadth: Base (µ)	Breadth: Tip (µ)	Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
Type-VII	1 Dorsal Surface of the Abdomen	78.46	2.31	0.38	5.38	Smooth	2/mm^2	Socket Round
Type-VIII	1 Wing Surface	23.71	1.42	0.57		Longitudinal Grooves	1/mm^2	Non-Socketted and Rough Edges
Type-VIII	2 Fifth Tarsal Segment of pro-thoracic leg		1.3	0.11		Longitudinal Grooves	1/mm^2	Non-Socketted and Rough Edges
Type-IX	1 First Tarsal Segment of Pro-thoracic Leg	28.21	2.82	0.26	5.13	Longitudinal Grooves	4/mm^2	
Type-X	1 Probosis (TIP)	22.73 to 23.64	1.36 to 1.82	0.91	1.82 to 2.73	Longitudinal Grooves	Innumerable	
Type-XI	1 Base of the Probosis	42.5 to 145	2.5 to 3.75	0.63 to 1.25	5 to 7.5	Longitudinal Grooves	Innumerable	
Type-XI	2 Tip of the Palpi	60 to 61.67	3.33	0.21 to 1.67	5	Longitudinal Grooves	Innumerable	
Type-XI	3 Surface View of Antenna	77.78 to 88.89	2.22	0.56	4.35	Longitudinal Grooves	Innumerable	

33.33 m to 86 m in length, the width at the base is between 1.33 m to 2.35 m, and that at the tip from 0.28 m to 1 m. These sensilla are socketted and having a diameter varying between 3.33 m to 4.12 m (Table-8a).

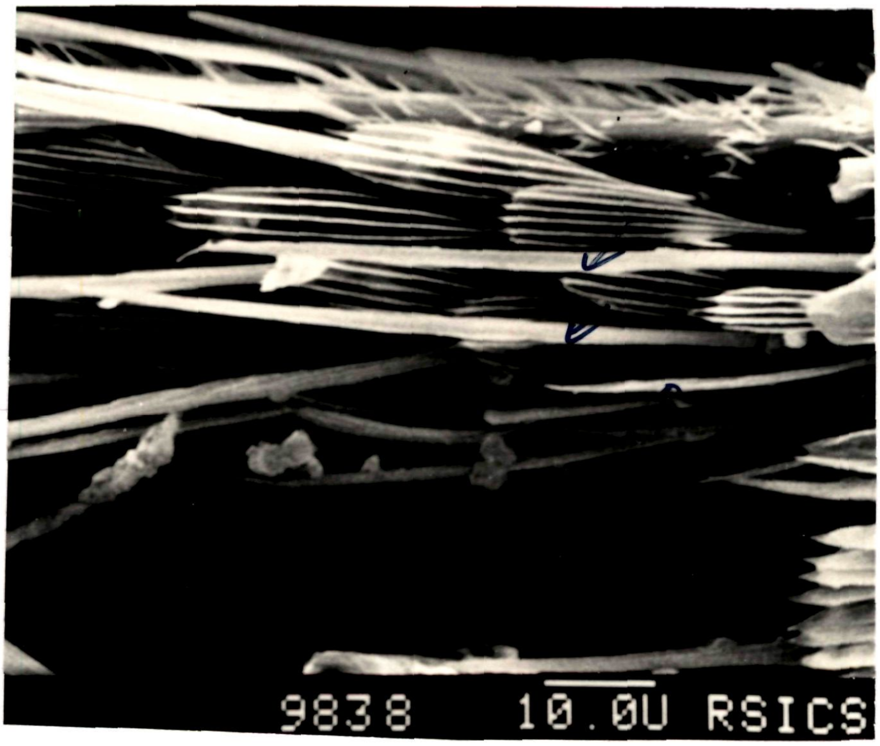
<u>Sensilla trichodea type-III</u> : The shaft is tapered and has a faint longitudinal grooved, and the distal end of the shaft is slightly curved. This sensilla is located only on the first tarsal segment of the meta-thoracic leg (Fig.46). The sensilla is approximately 62.31 m in length, the width at the base is 6 m and that at the tip is 0.77 m. These sensilla are socketted and the diameter is approximately 3.85 m (Table-8a).

<u>Sensilla trihodea type-IV</u> : The shaft is almost blunt at the tip, and has a faint longitudinal grooved. These sensilla is located only on the tarsal segment of meso-thoracic leg (Fig.47). The sensilla has the approximate length of 52.86 m, whereas the width at the base is 4.29 m and that at the tip is 1.43 m. These sensilla are socketted and have a diameter of around 5.71 m (Table-8a).

<u>Sensilla trichodea type-V</u> : The shaft has longitudinal groove and the broad tip portion is divided into five parts. These sensilla are located only on the third tarsal segment of the pro-thoracic leg (Figs.48a,b,c). The approximate

Fig.46 SEM picture of tarsus of meta-thoracic leg of *A. annularis* showing sensilla trichodea type-III x 1200 µm.

Fig.47 SEM picture of tarsus of meso-thoracic leg of *A. annularis* showing sensilla trichodea type-IV x 660 µm.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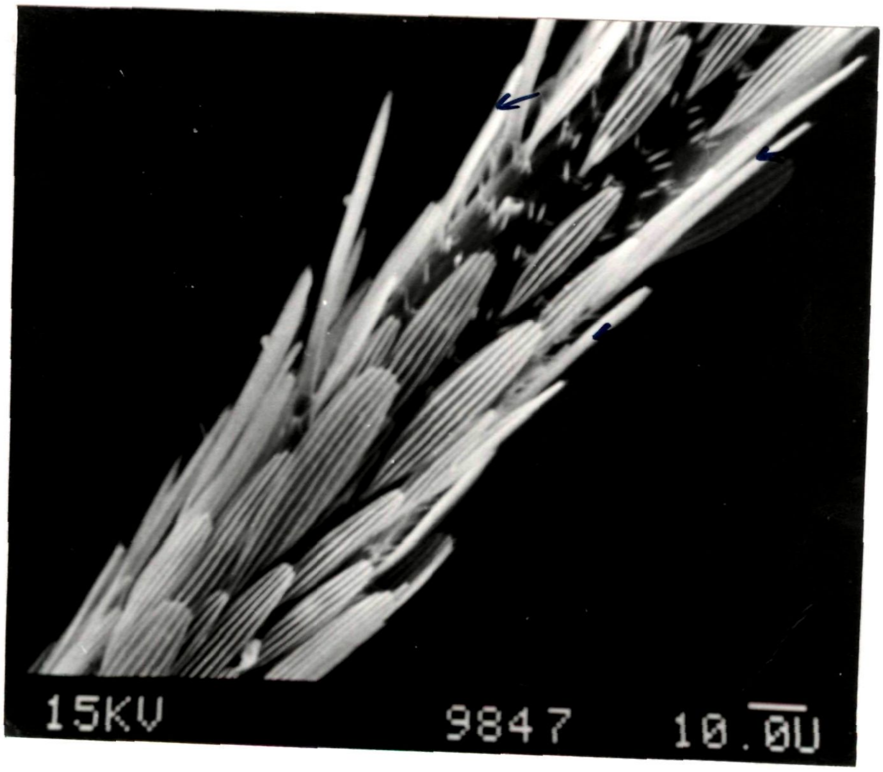
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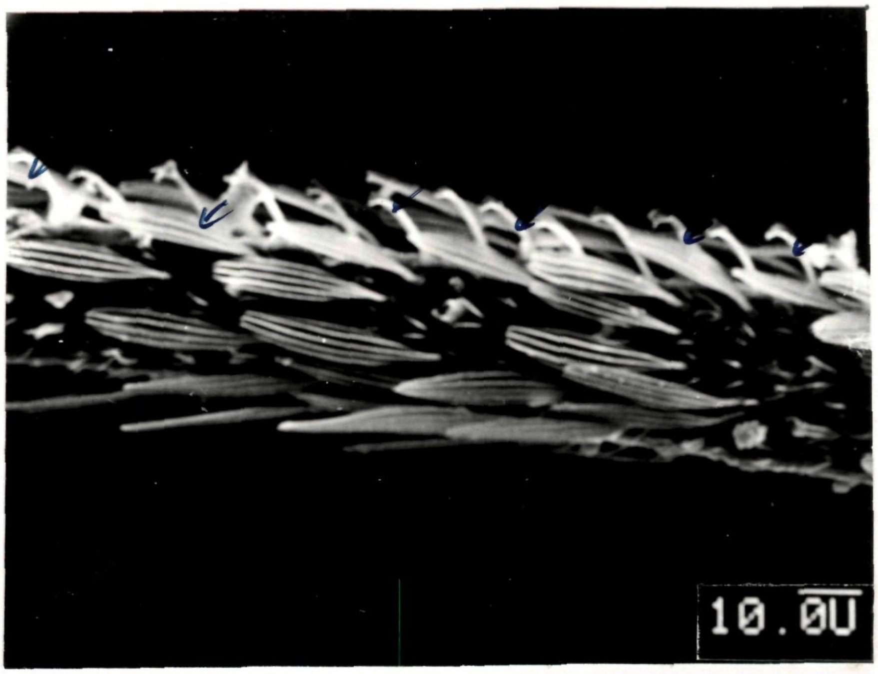
Fig.48(a) SEM picture of the third tarsal segment of pro-thoracic leg of *A. annularis* showing sensilla trichodea type-V x 720 µn.

Fig.48(b) SEM picture of the third tarsal segment of the pro-thoracic leg of *A. annularis* showing the tip of the sensilla trichodea type-V x 6600 µn.

Fig.48(c) SEM picture of the third tarsal segment of the pro-thoracic leg of *A. annularis* showing the base of the sensilla trichodea type-V x 7200 µn.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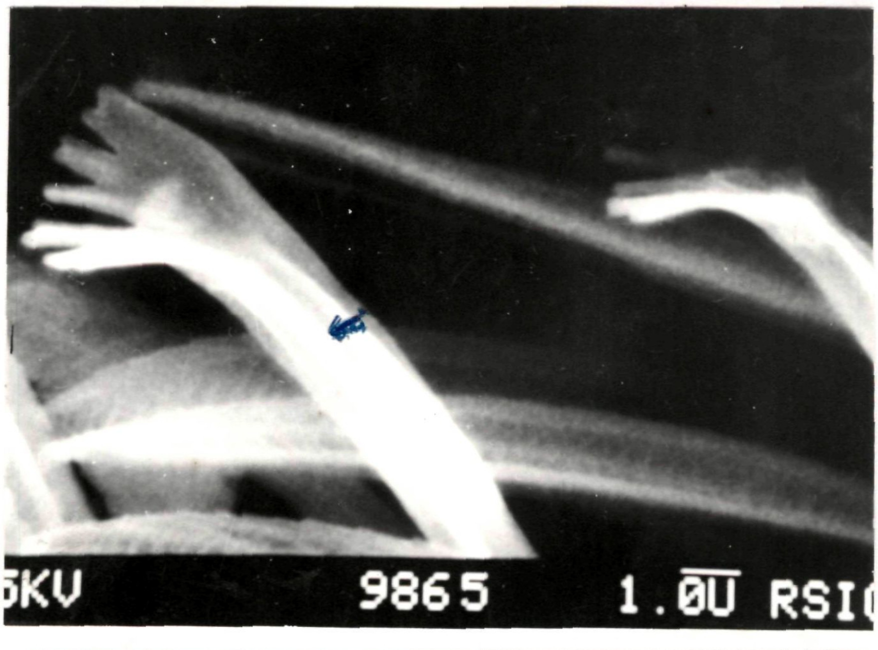
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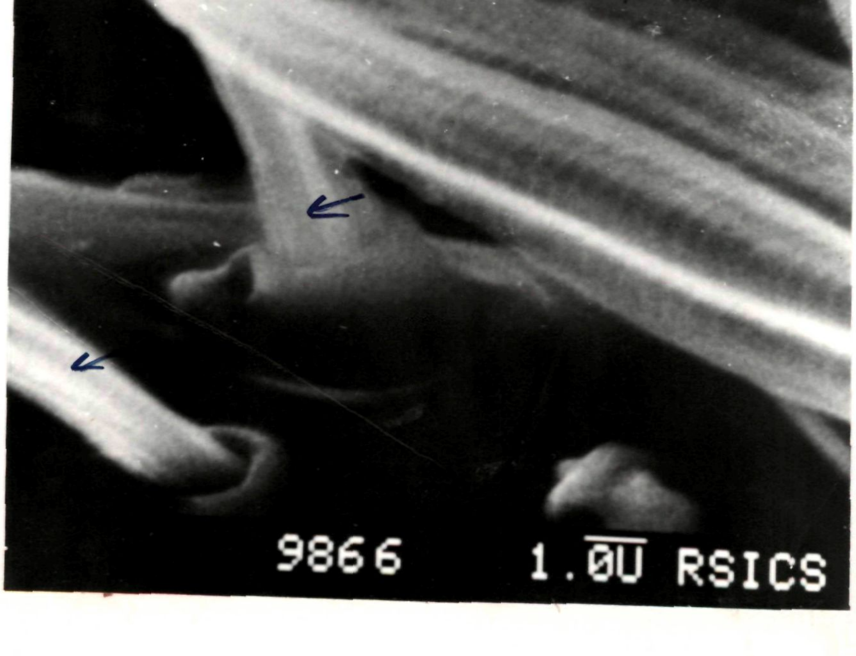
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length is 13.75 m, the width at the base is 1.75 m, and that at the tip is 3.75 m (Table-8a).

Sensilla trihodea type-VI : The shaft is tapered and has a distinct longitudinal groove. These sensilla are located on the anterior most tip of the dorsal surface of the head (Figs.49a,b,c). These sensilla have an approximate length of 10.84 m, the width at the base is 4 m, whereas that at the tip is 1 m. The sensilla is socketted having a double-chambered and with a diameter of 9.31 m (Table-8a).

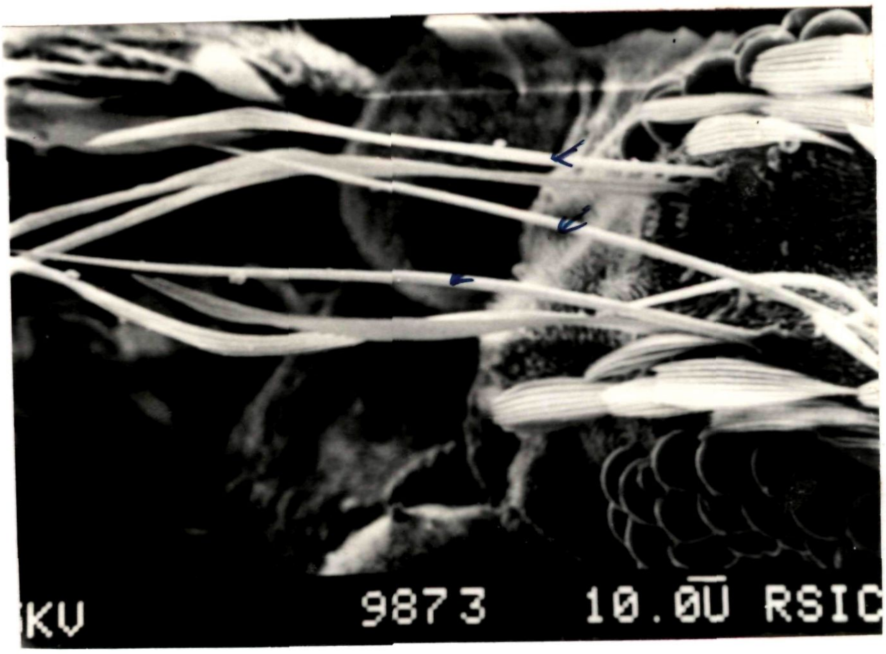
Sensilla trichodea type-VII : The shaft is tapered having a smooth surface. These sensilla are located on the dorsal surface of the abdomen (Figs.50a,b,c). These sensilla are approximately 78.46 m in length, the width at the base is 2.31 m and that at the tip is 0.38 m. The sensila are also socketted having a diameter of 5.38 m (Table-8a).

Sensilla trichodea type-VIII : The shaft is tapered, rough edges, almost round tip and width indistinct socket. The surface feature of the sensilla bear longitudinal groove. The sensilla are localised on the surface of the wing and fifth tarsal segment of pro-thoracic leg (Figs.51a,b,c). The length of the sensilla is 23.71 m, the width at the base between 1.3 m to 1.42 m and that at the tip between 0.11 m to 0.57 (Table-8a).

Fig.49(a) SEM picture of the anterior most tip of the head (Dorsal) of *A. annularis* showing the sensilla trichodea type-VI × 440 µn.

Fig.49(b) SEM picture of the anterior most tip of the head (Dorsal) of *A. annularis* showing the base of the sensilla trichodea type-VI × 2600 µn.

Fig.49(c) SEM picture of the anterior most tip of the head (Dorsal) of *A. annularis* showing the surface feature of the sensilla trichodea type-VI × 8600 µn.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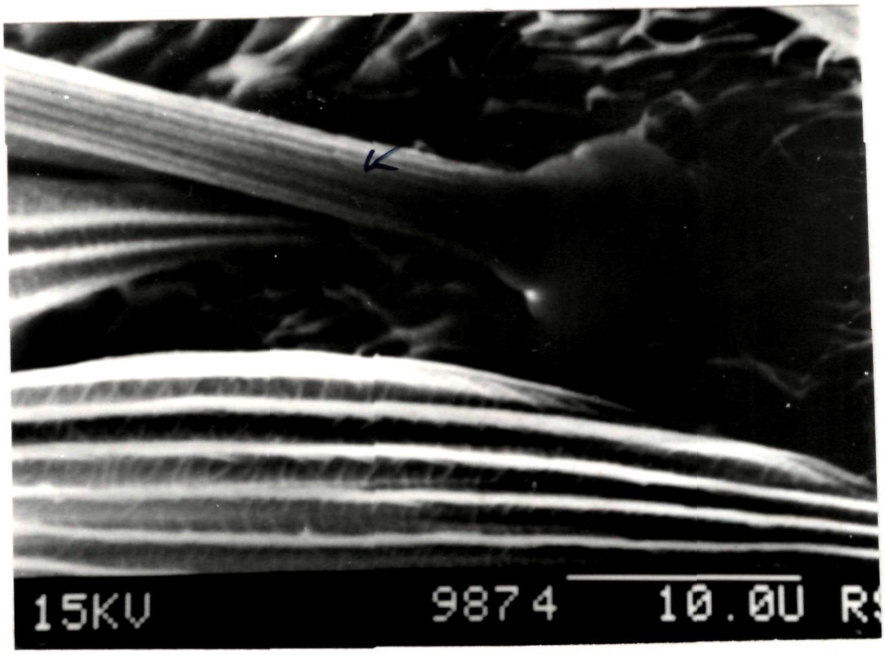
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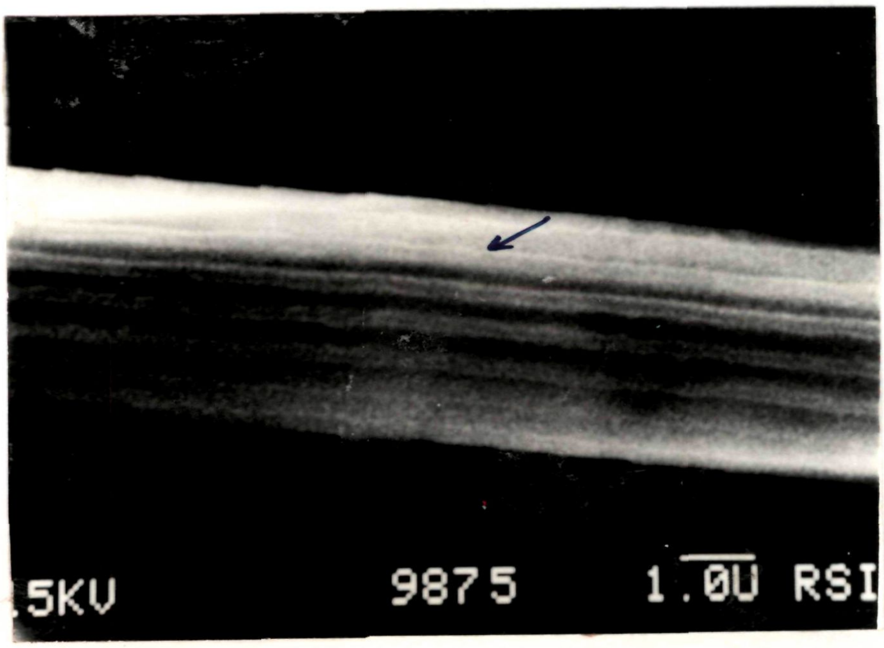
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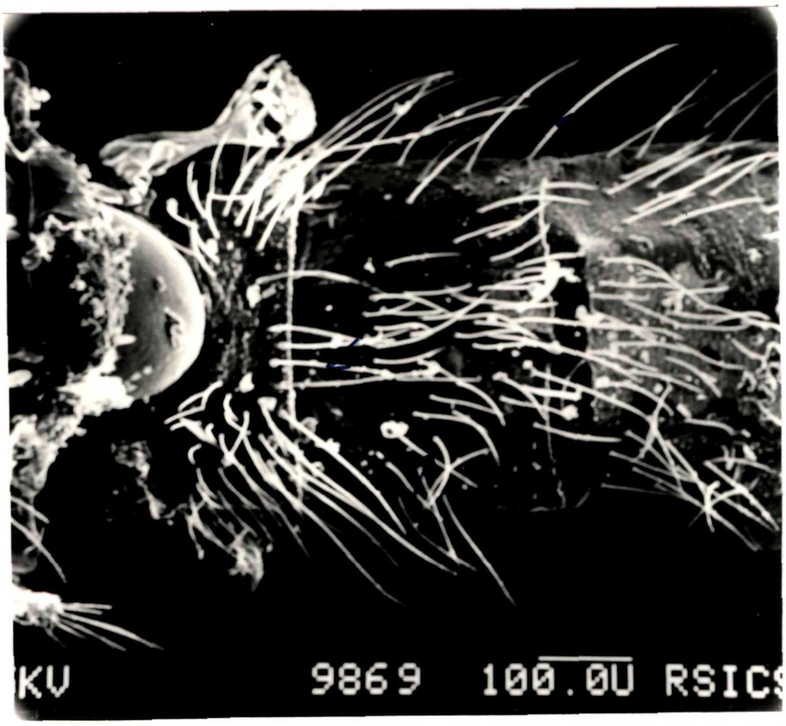
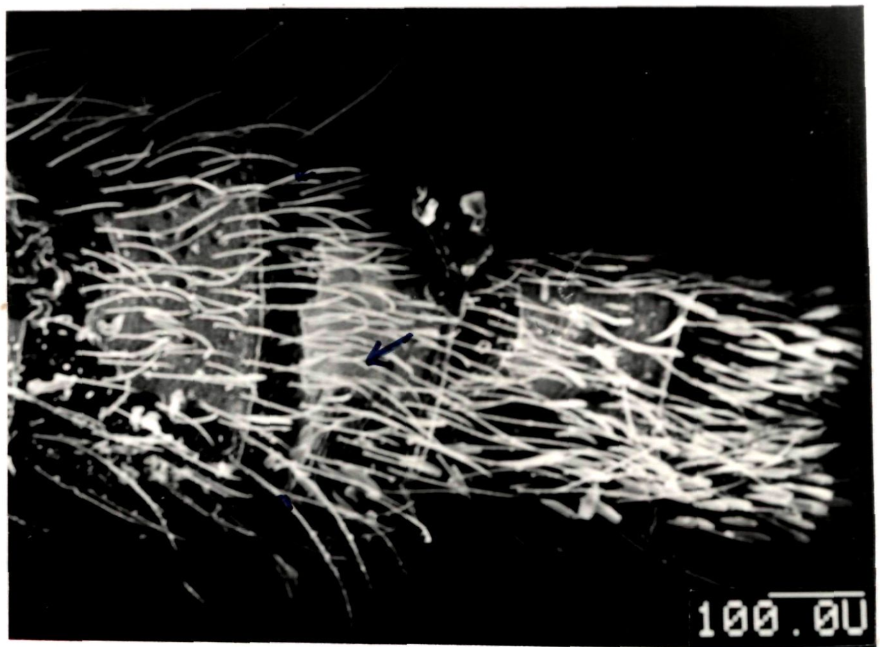
Fig.50(a) SEM picture of the 1st, 2nd and 3rd abdominal segments (Dorsal) of *A. annularis* showing the sensilla trichodea type-VII x 120 µn.

Fig.50(b) SEM picture of the 4th, 5th, 6th and 7th abdominal segments (Dorsal) of *A. annularis* showing the sensilla trichodea type-VII x 110 µn.

Fig.50(c) SEM picture of the sensilla trichodea type-VII located on the dorsal surface of the abdomen of *A. annularis* x 1200 µn.


KV
9869 100.0U RSICS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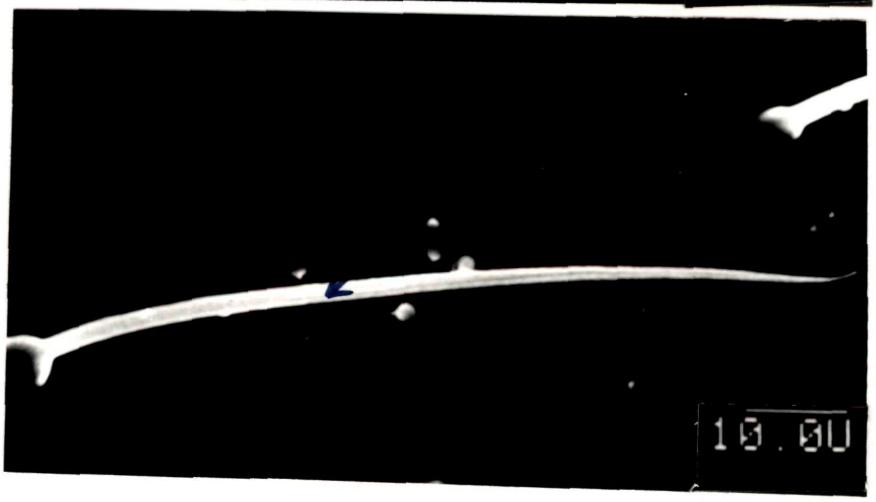
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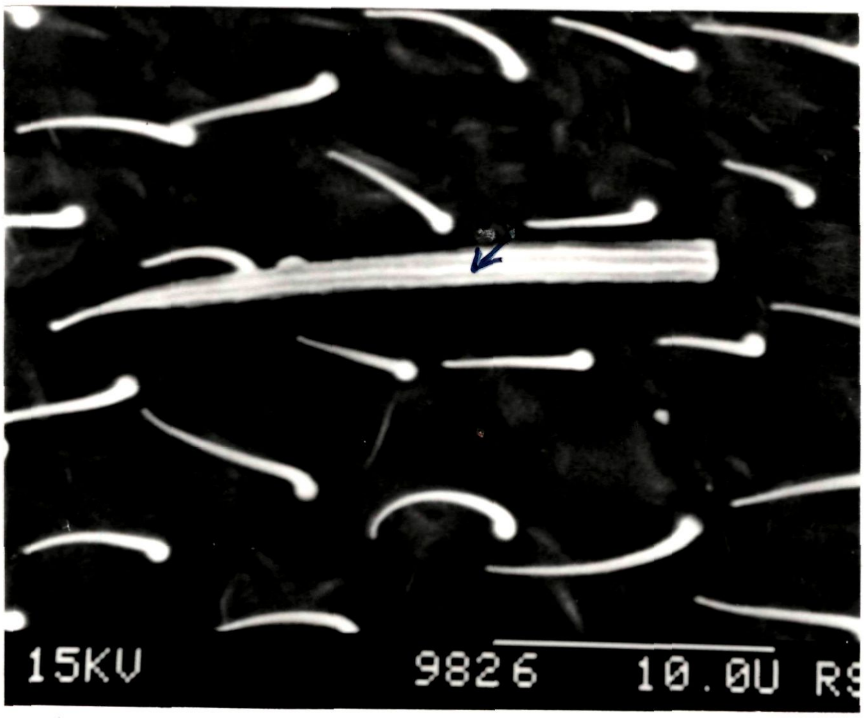
Fig.51(a) SEM picture of the wing surfaces of *A. annularis* showing the sensilla trichodea type-VIII × 3200 µn.

Fig.51(b) SEM picture of the posterior region of the sensilla trichodea type-VIII located on the wing surface of *A. annularis* × 1500 µn.

Fig.51(c) SEM picture of the fifth tarsal segment of prothoracic leg of *A. annularis* showing the sensilla trichodea type-VIII × 8600 µn.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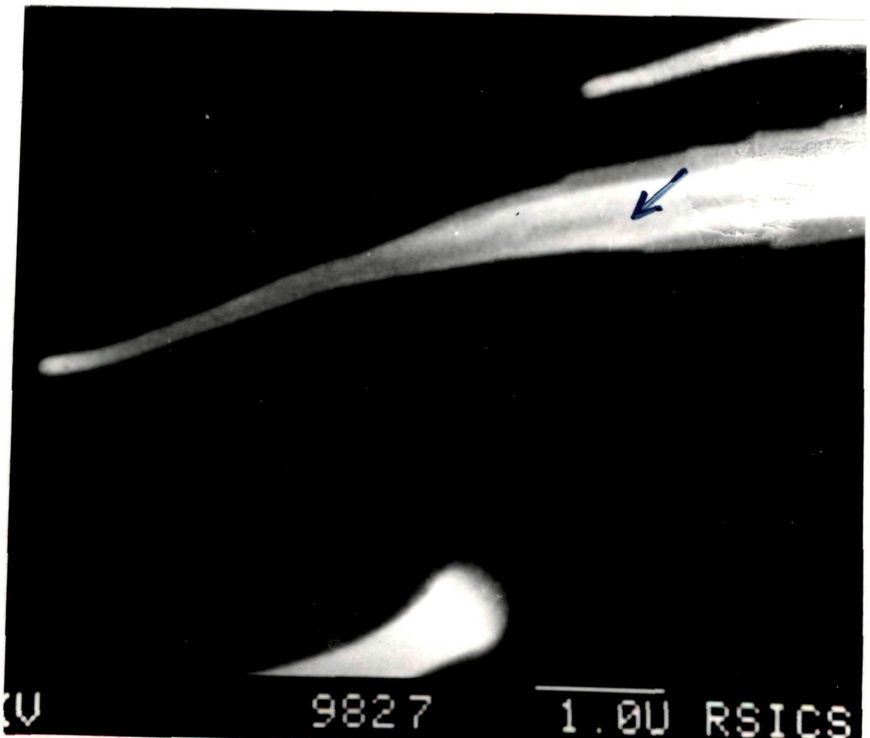
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1.0U

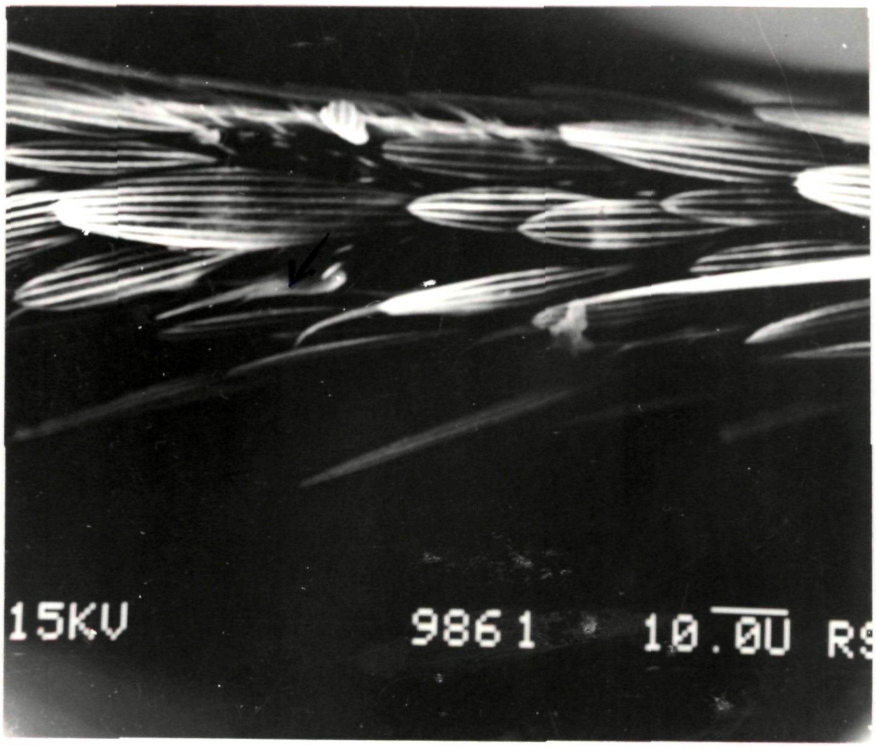
Sensilla trichodea type-IX : These sensilla bears a peculiar characteristic, having a swollen base and the anterior part is divided into three parts, and each bearing a pointed tip. The surface feature of the sensilla reveal a faint longitudinal groove (Figs.52a,b). The sensilla is located on the first tarsal segment of pro-thoracic leg. The length of the sensilla is approximately 28.21 m, the width at the base is 2.82 m and that at the tip is 0.26 m. The sensilla is socketted and with a diameter of 5.13 m (Table-8a).

Sensilla trichodea type-X : The shaft is tapered, having longitudinal groove and with rough edges. These sensilla are localised only on the anterior most tip of the probosis. The appearance of these sensilla is curved towards the distal point (Fig.53). The length range from 22.73 m to 23.64 m, the width at the base also vary between 1.36 m to 1.82 m and that at the tip is approximately 0.91 m. The diameter of the sensilla varies between 1.82 m to 2.73 m (Table-8a).

Sensilla trichodea type-XI : The shaft is tapered, slendered, having longitudinal groove and with rough edges. These sensilla are located on the base of probosis, general surface of the palpi and antenna (Figs.54a,b,c,d). These sensilla vary in length at 42.5 m to 145 m, the width at the

Fig.52(a) SEM picture of the first tarsal segment of prothoracic leg of *A. annularis* showing the sensilla trichodea type-IX x 860 µn.

Fig.52(b) SEM picture of the first tarsal segment of prothoracic leg of *A. annularis* showing the enlarge view of the sensilla trichodea type-IX x 3600 µn.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9861
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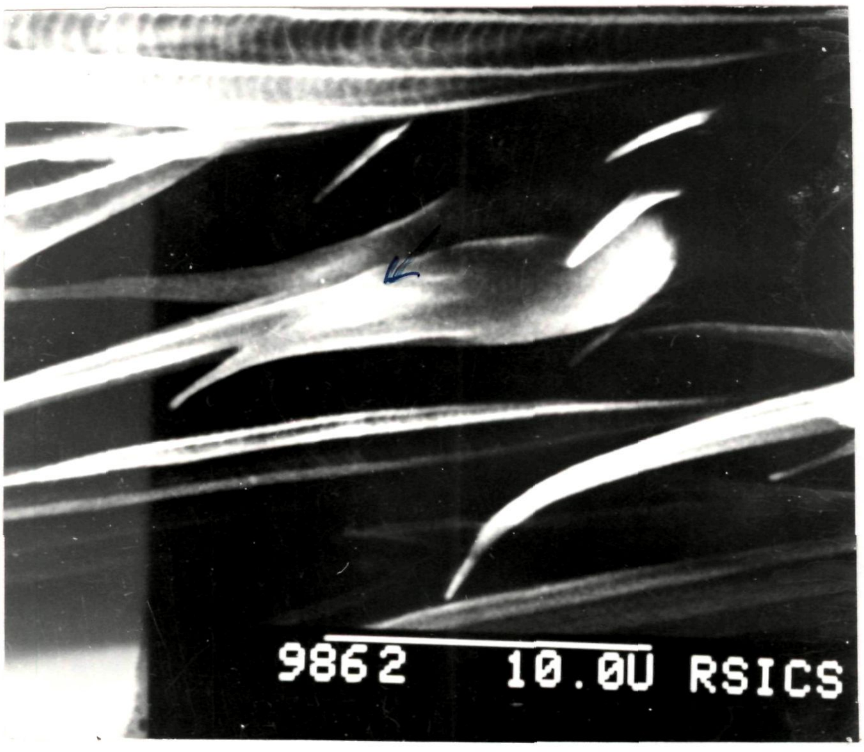
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Fig.53 SEM picture of the tip of the probosis of *A. annularis* showing the sensilla trichodea type-X x 1200 µm.

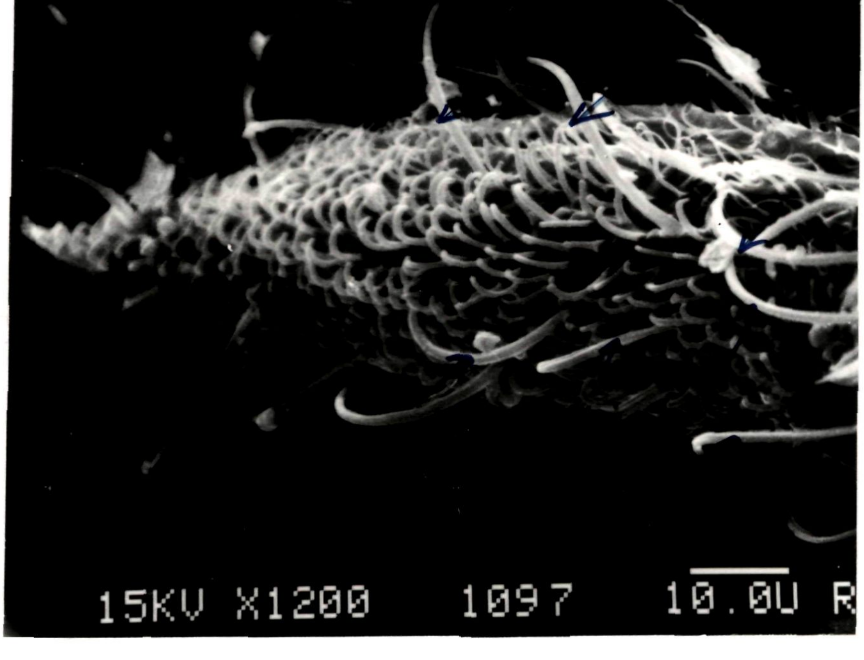
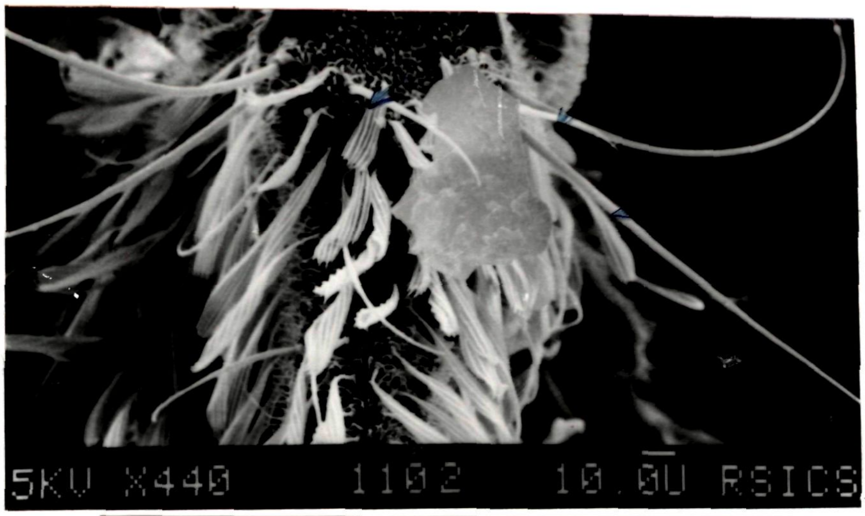

15KV X1200 1097 10.0U R

Fig.54(a) SEM picture of the base of the probosis of *A. annularis* showing the sensilla trichodea type-XI x 440 µn.

Fig.54(b) SEM picture of the tip of the palpi of *A. annualaris* showing the sensilla trichodea type-XI x 660 µn.

Fig.54(c) SEM picture of the general surface of the palpi of *A. annularis* showing the sensilla trichodea type-XI x 1200 µn.

Fig.54(d) SEM picture of the tip of the antenna of *A. annularis* showing the sensilla trichodea type-XI x 1000 µn.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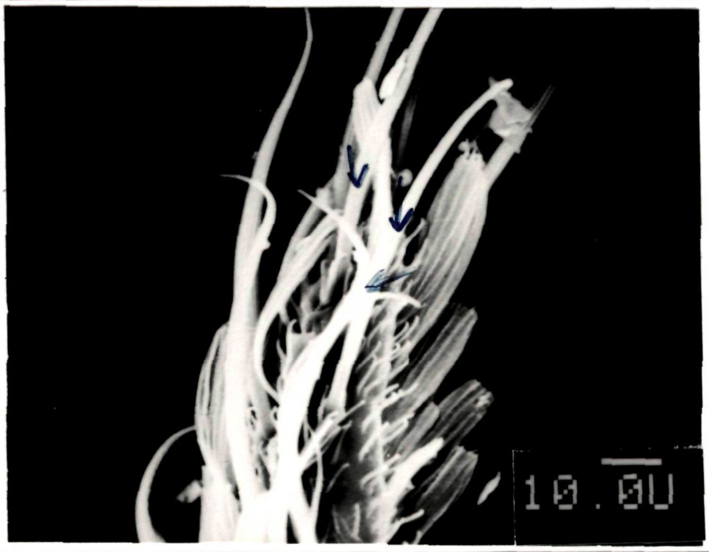
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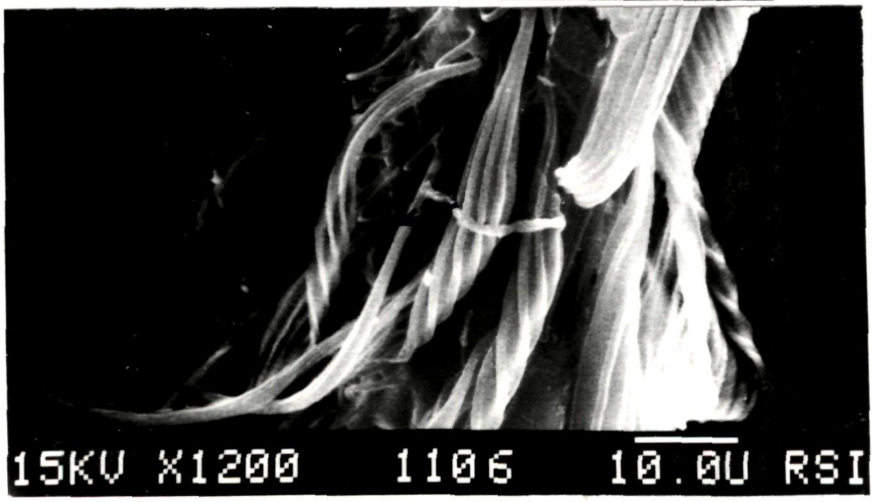
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10.0U

base between 2.5 m to 3.75 m and that at the tip is 0.63 m to 1.25 m. The diameter of the socket range from 5 m to 7.5 m, as seen on the base of probosis. That of the palpi, the length range between 60 m to 61.67 m. The width at the base at 3.33 m and that at the tip range from 0.21 m to 1.67 m. The diameter of the socket is approximately 5 m; whereas in the case of antennal surface, the length shows a variation of 77.78 m to 88.89 m. The width at the base is 2.22 m and that of the tip at 0.56 m. The diameter of the socket is approximately 4.35 m (Table-8a).

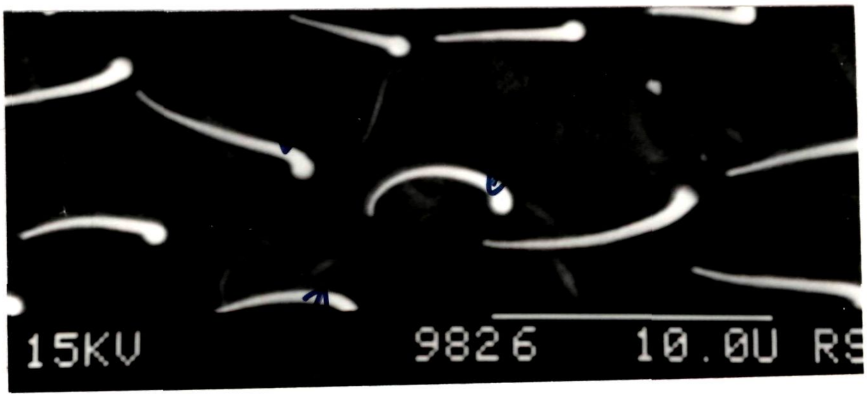
Sensilla chaetica : There are six types of sensilla chaetica present in the different locations on the body surface of the *Anopheles annularis*.

Sensilla chaetica type-I : The shaft is slightly tapered, smooth and almost curved at the anterior region. These sensilla is located on the wing surface, coxa/trochontar of meso-thoracic leg and base of the femur of pro-thoracic leg (Figs.55a,b,c). These sensilla chaetica type-I range from 1.67 m to 6.82 m in length, the width at the base range from 0.45 m to 0.91 m and that at the tip between 0.23 m to 0.45 m. The sensilla is socketted, round shaped, and having a diameter ranging from 0.48 m to 1.14 m (Table-8b).

Fig.55(a) SEM picture on the wing surface of *A. annularis* showing the sensilla chaetica type-I x 3200 µn.

Fig.55(b) SEM picture of the coxa/trochontar of meso-thoracic *A. annularis* showing the sensilla chaetica type-I x 2000 µn.

Fig.55(c) SEM picture of the base of the femur of pro-thoracic leg of *A. annularis* showing the sensilla chaetica type-I x 2200 µn.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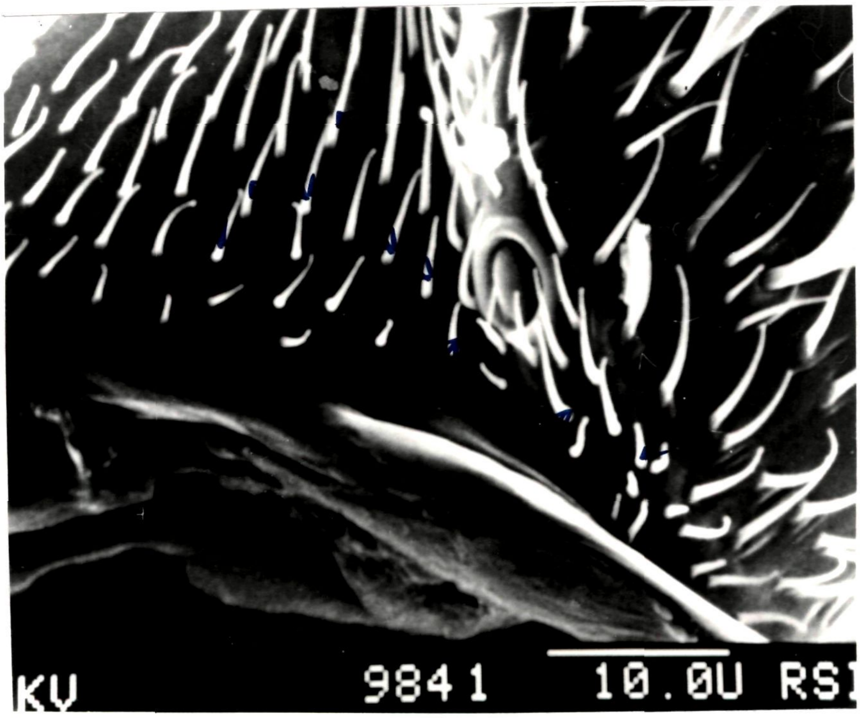
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9841
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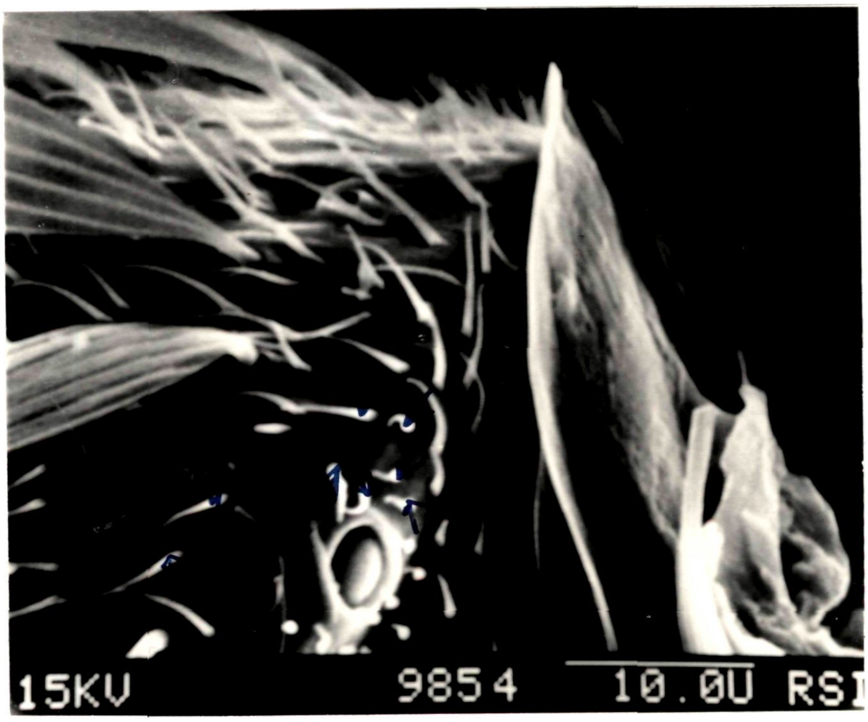

15KV
9854
10.0U RSI

Table-8(b)

Distribution Pattern and Dimension of Different Types of Sensilla Chaetica of *Anopheles annularis*

Sensilla	Location	Breadth			Diameter of the Socket (μ)	Surface Feature	Total Number	Any other special Characteristic
		Length (μ)	Base (μ)	Tip (μ)				
Type-I	1 Wing Surface	6.29	0.57	0.29	1.14	Smooth	Innumerable	Socket Round
Type-I	2 Coxa/Trochontar of Meso-thoracic Leg	1.82 to 6.82	0.45 to 0.91	0.23 to 0.45	0.48 to 0.95	Smooth	Innumerable	Socket Round
Type-I	3 Trochontar/ Femur joint of Meso-thoracic Leg	1.67	0.63	0.42	0.83	Smooth	Innumerable	Socket Round
Type-II	1 Trochontar of Meta-Thoracic Leg	7.89	0.91	0.26		Smooth	Innumerable	Pointed Tip and Non-socketted
Type-III	1 Femur of Meta-thoracic Leg	2.05 to 1.79	0.51 to 0.77	0.39		Smooth	Innumerable	Blunt-Tip and Non-Socketted
Type-III	2 Femur of Meso-thoracic Leg	7.27	0.91	0.45		Smooth	Innumerable	Blunt-Tip and Non-Socketted
Type-III	3 Tarsal Segment of Meso-thoracic Leg	7.	0.6	0.2		Smooth	Innumerable	Blunt-Tip and Non-Socketted
Type-III	4 Goxa of Pro-thoracic Leg	4.58	0.42	0.21		Smooth	Innumerable	Blunt-Tip and Non-Socketted
Type-III	5 Trochontar/ Femur Joint of Pro-thoracic Leg	6.25	0.83	0.42		Smooth	Innumerable	Blunt-Tip and Non-Socketted
Type-IV	1 Dorsal Surface of the Head	4.71	0.59	1	0.63	Smooth	Innumerable	Socket-Round and Tip Pointed
Type-IV	2 Dorsal Surface of the Abdomen	2.5 to 5	0.5	0.13 to 0.25	0.51 to 0.77	Smooth	Innumerable	Socket-Round and Tip Pointed
Type-IV	3 Dorsal Surface of the Thorax	1.25 to 5	0.5	0.25 to 0.37	0.5 to 0.75	Smooth	Innumerable	Socket-Round and Tip Pointed

Sensilla	Location	Breadth			Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
		Length (µ)	Base (µ)	Tip (µ)				
Type-V	1 Tibia/Tarsal Joint of Meta-thoracic Leg					Smooth	Innumerable	Base and Tip Portion Pointed
	2 Tibia/Tarsal Joint of Pro-thoracic Leg	39.09	0.45	0.45		Smooth	Innumerable	Base and Tip Portion Pointed
Type-VI	1 Surface view of Probosis	6.5	1	0.5		Smooth	Innumerable	Curved-Shaft with Blunt-Tip
Type-VI	2 Surface view of Palpi	4.55 to 8.18	0.91	0.45 to 0.91		Smooth	Innumerable	Curved-Shaft with Blunt-Tip
Type-VI	3 Surface view of Antenna	11.75	1.74	0.87		Smooth	Innumerable	Curved-Shaft with Blunt-Tip

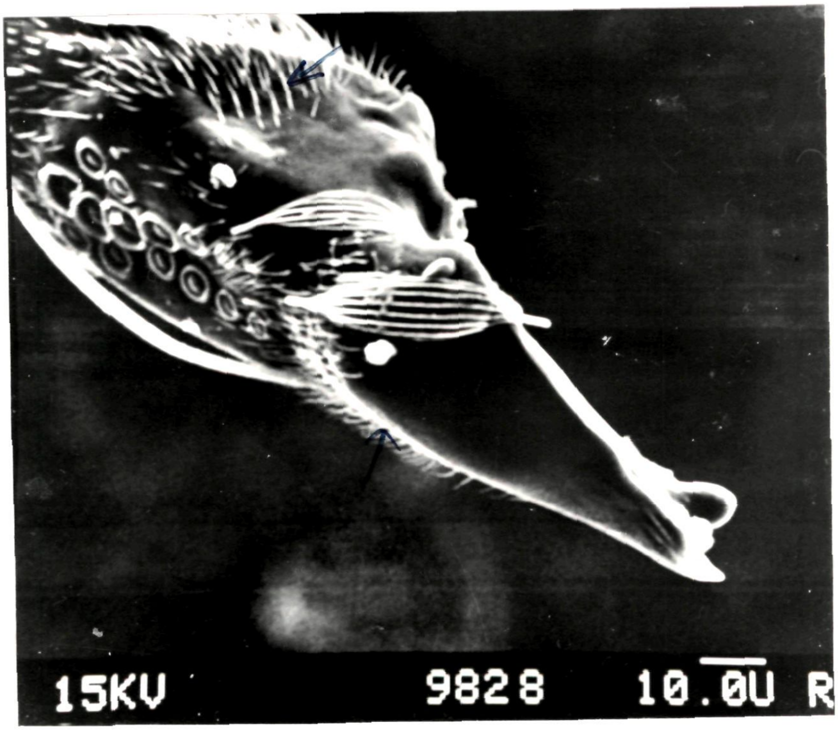
Sensilla chaetica type-II : The shaft is pointed, smooth and non-socketted. These sensilla is located on the trochontar of meta-thoracic leg (Figs.56a,b). The length of these sensilla is approximately 7.89 m, the width at the base is 0.91 m and that at the tip is 0.26 m (Table-8b).

Sensilla chaetica type-III : These are among the most innumerable sensilla and are located on the femur of meta-thoracic leg, femur of meso-thoracic, tarsal segment of meso-thoracic leg, coxa of pro-thoracic leg and trochontar/femur of pro-thoracic leg (Figs.57a,b,c,d,c). These sensilla is non-socketted, smooth and with a blunt tip. The sensilla chaetica type-III located on the femur of meta-thoracic leg shows a variation of 1.79 m to 2.05 m, the width at the base range from 0.51 m to 0.77 and that at the tip is approximately at 0.39 m (Table-8b), whereas, the sensilla located on the femur of meso-thoracic leg, tarsal segment of meso-thoracic leg, coxa of pro-thoracic leg and trochontar/femur joint of pro-thoracic leg range in length between 4.58 m to 7.27 m, the width at the base range from 0.42 m to 0.91 m and that at the tip from 0.2 m to 0.45 m (Table-8b).

Sensilla chaetica type-IV : The shaft is tapered, smooth and socketted. These sensilla re located on the dorsal surface of the head, thorax, and abdomen

Fig.56(a) SEM picture of the coxa/trochontar of meta-thoracic leg of *A. annularis* showing the sensilla chaetica type-II x 780 µn.

Fig.56(b) SEM picture of the trochontar of meta-thoracic leg of *A. annularis* showing the sensilla chaetica type-II x 2000 µn.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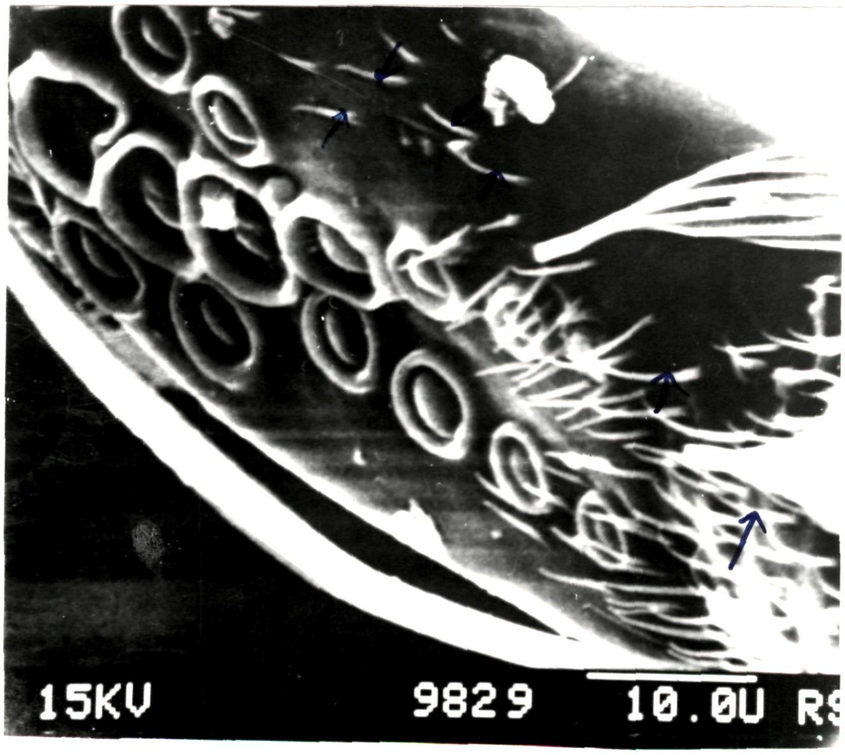

15KV
9829
10.0U RS

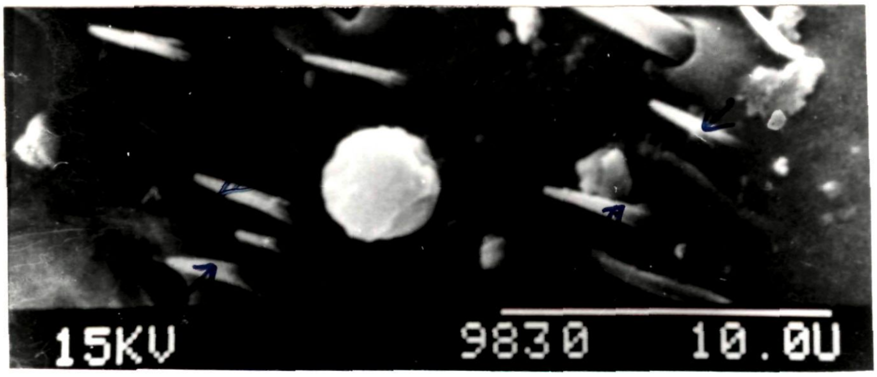
Fig.57(a) SEM picture of the femur of meta-thoracic leg of *A. annularis* showing the sensilla chaetica type-III x 3600 µn.

Fig.57(b) SEM picture of the femur of meso-thoracic leg of *A. annularis* showing the sensilla chaetica type-III x 1100 µn.

Fig.57(c) SEM picture of the tarsus of meso-thoracic leg of *A. annularis* showing the sensilla chaetica type-III x 660 µn.

Fig.57(d) SEM picture of coxa of pro-thoracic leg of *A. annularis* showing the sensilla chaetica type-III x 2200 µn.

Fig.57(e) SEM picture of trochontar/femur joint of pro-thoracic leg of *A. annularis* showing the sensilla chaetica type-III x 2200 µn.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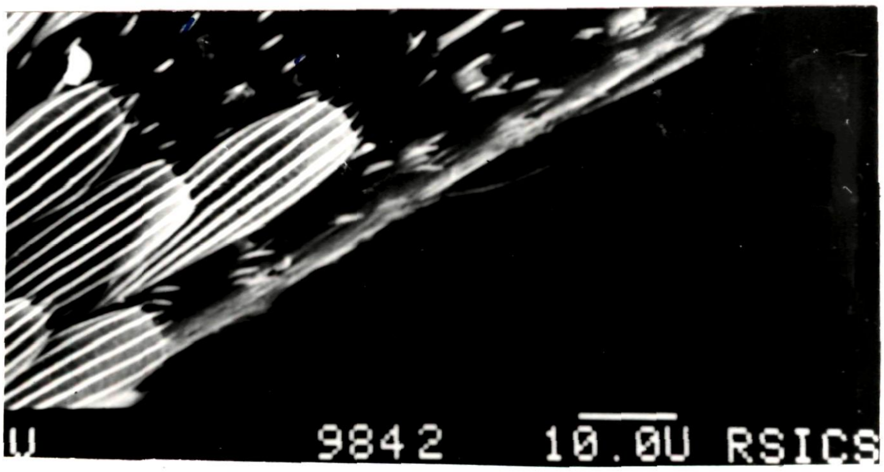
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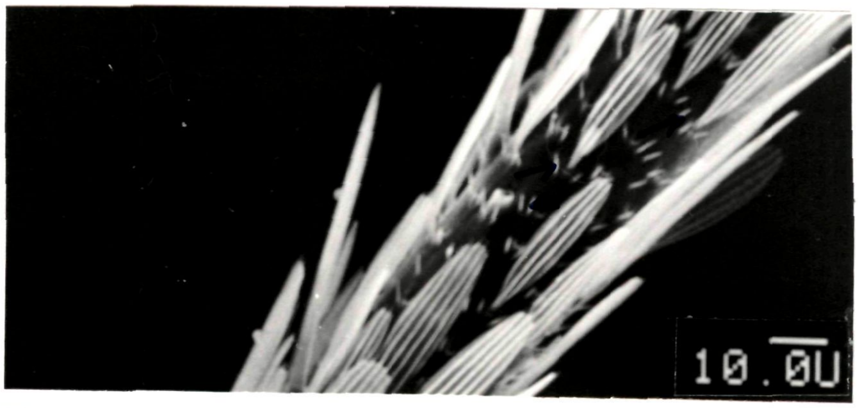
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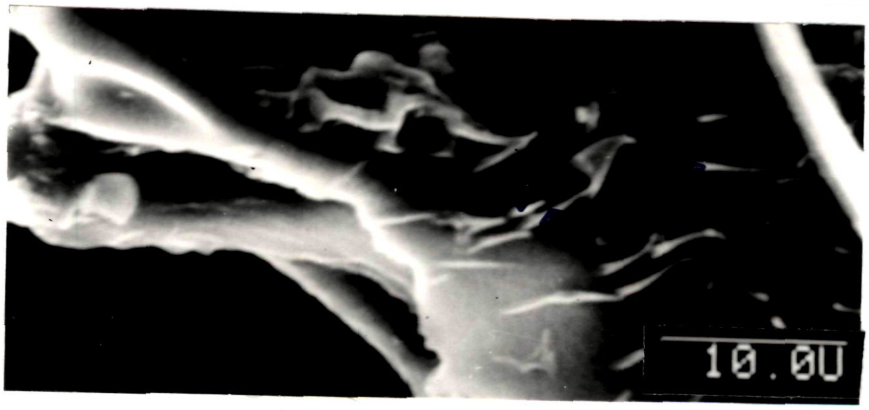
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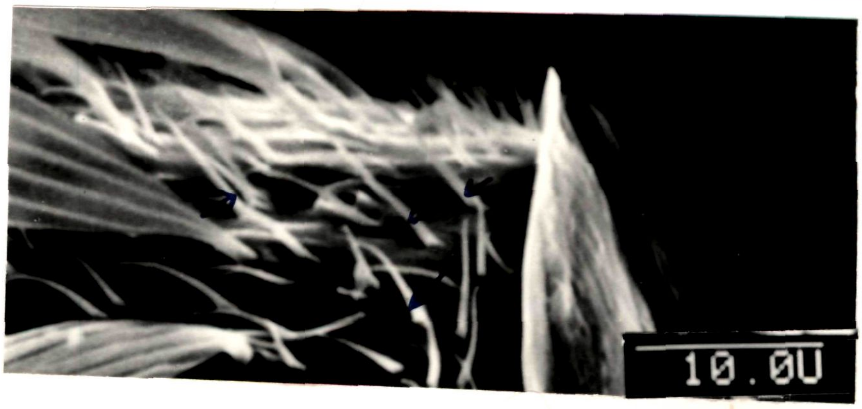
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(Figs.58a,b,c). The sensilla range from 1.25 m to 5 m in length, width at the base vary from 0.5 m to 0.6 m and that at the tip between 0.13 m to 1 m. The diameter of the socket range from 0.5 m to 0.77 m (Table-8b).

Sensilla chaetica type-V : The shaft is smooth, non-socketted and with the tip and base portion pointed. These sensilla are located on the tibia/tarsal joint of the meta- and pro-thoracic leg (Figs.59a,b). The sensilla has an approximate length of 39.09 m, the width at the base at 0.45 m and that at the tip at approximately 0.45 m (Table-8b).

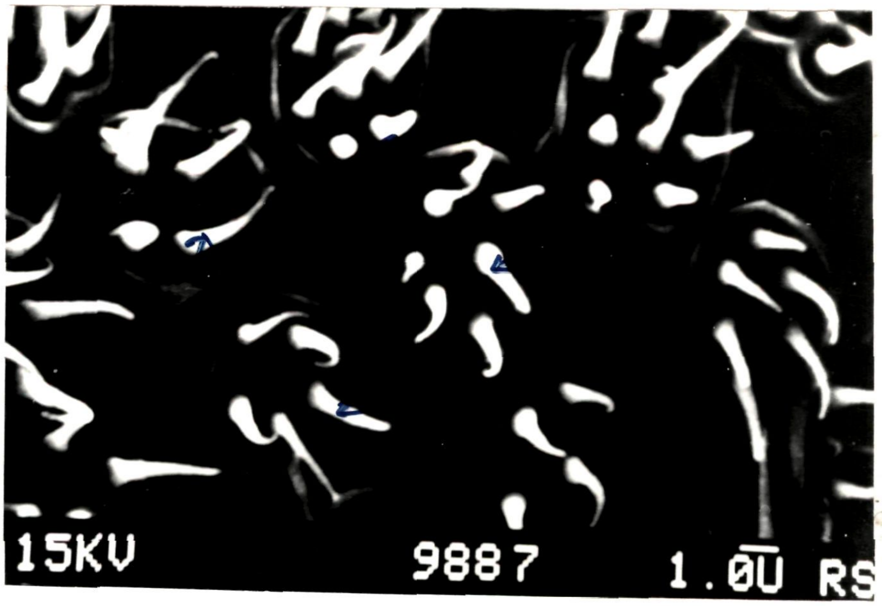
Sensilla chaetica type-VI : The shaft is smooth, curved, non-socketted and with a blunt tip. The sensilla is located on the surface of the probosis, palpi and antenna (Figs.60a,b,c). The sensilla chaetica type-VI range from 4.55 m to 11.75 m in length, the width at the base range from 0.91 m to 1.74 m and that at the tip between 0.45 m to 0.91 m (Table-8b).

Sensory Peg : The shaft is blunt at the tip, non-socketted and surface feature are smooth. These sensilla is innumerable in occurrence and is localised only on the femur of meta-thoracic leg (Fig.61). The length of the sensilla range at 5.33 m, the width at the base is approximately 0.5 m and that at the tip is around 0.17 m (Table-8c).

Fig.58(a) SEM picture of the dorsal surface of the head of *A. annularis* showing the sensilla chaetica type-IV x 4400 µn.

Fig.58(b) SEM picture of the dorsal surface of the thorax of *A. annularis* showing the sensilla chaetica type-IV x 4400 µn.

Fig.58(c) SEM picture of the dorsal surface of the abdomen of *A. annularis* showing the sensilla chaetica type-IV x 3200 µn.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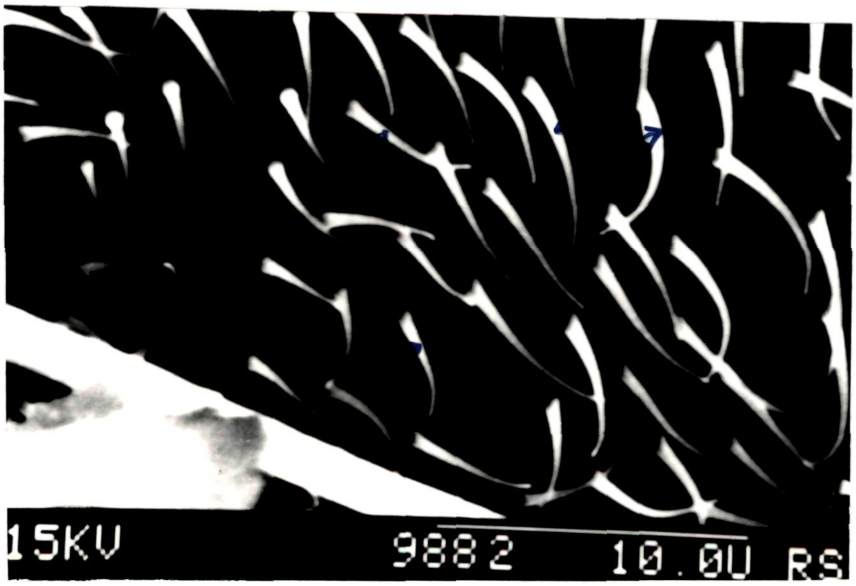
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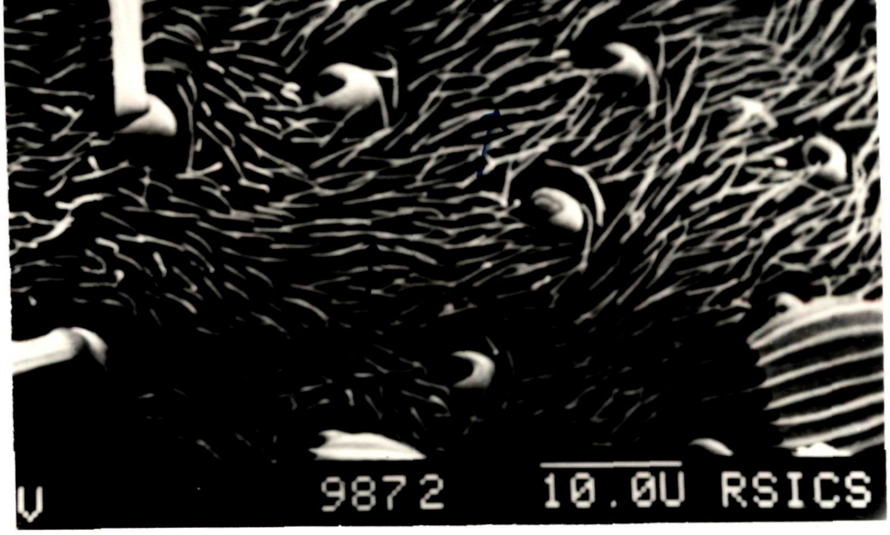
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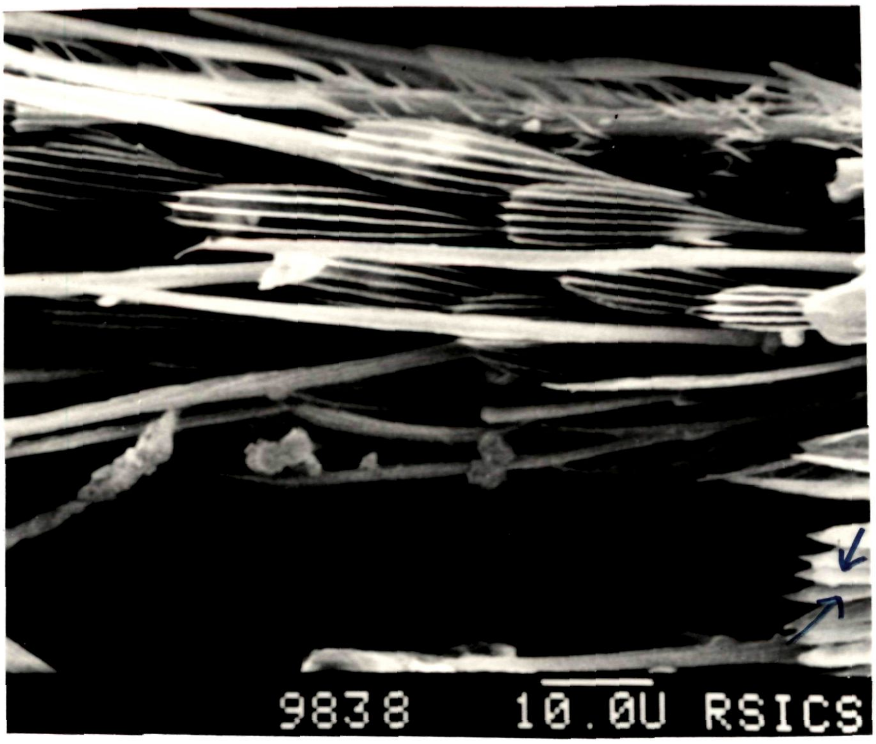
Fig.59(a) SEM picture of the tibia/tarsal joint of metathoracic leg of *A. annularis* showing the sensilla chaetica type-V × 1200 µn.

Fig.59(b) SEM picture of the tibia/tarsal joint of prothoracic leg of *A. annularis* showing the sensilla chaetica type-V × 1000 µn.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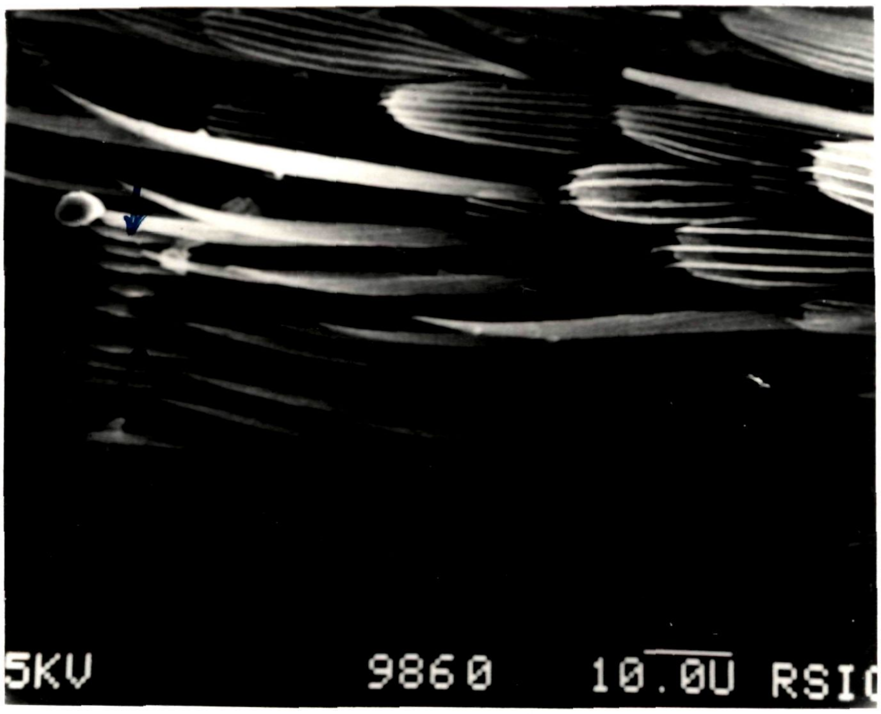
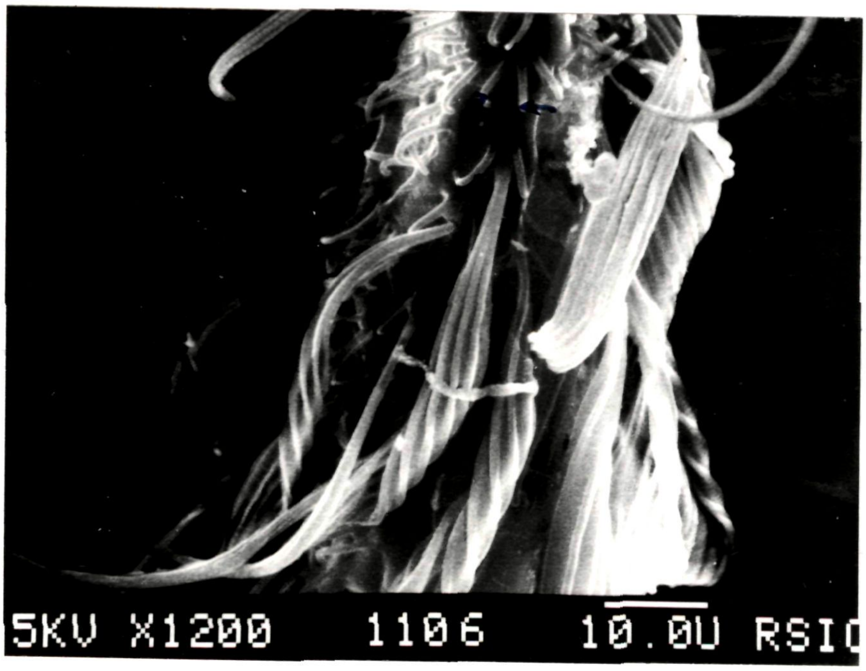

5KV 9860 10.0U RSIC

Fig.60(a) SEM picture of the probosis of *A. annularis* showing the sensilla chaetica type-VI × 1200 µn.

Fig.60(b) SEM picture of the palpi of *A. annularis* showing the sensilla chaetica type-VI × 1200 µn.

Fig.60(c) SEM picture of the palpi of *A. annularis* showing the sensilla chaetica type-VI × 2000 µn.


15KV X1200 1097 10.0U R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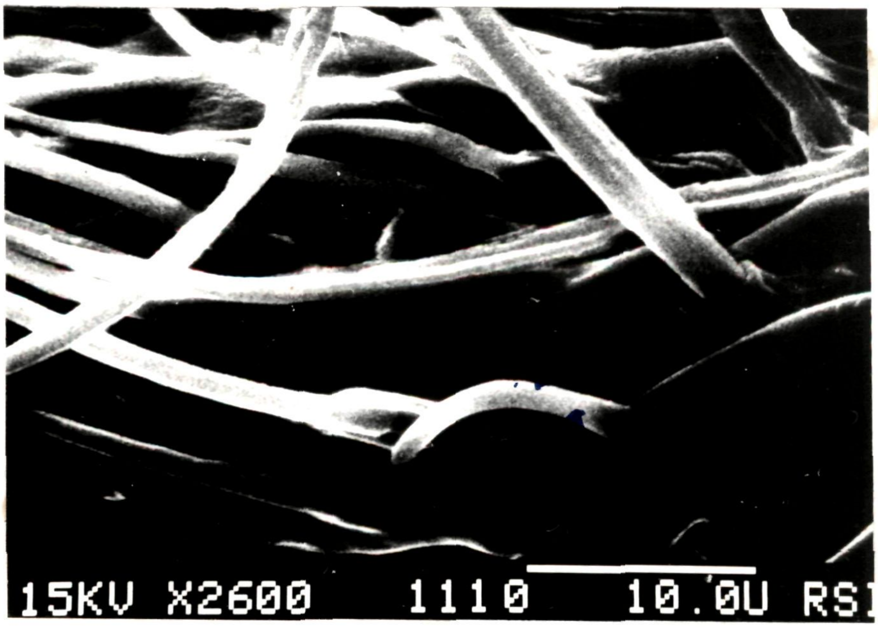
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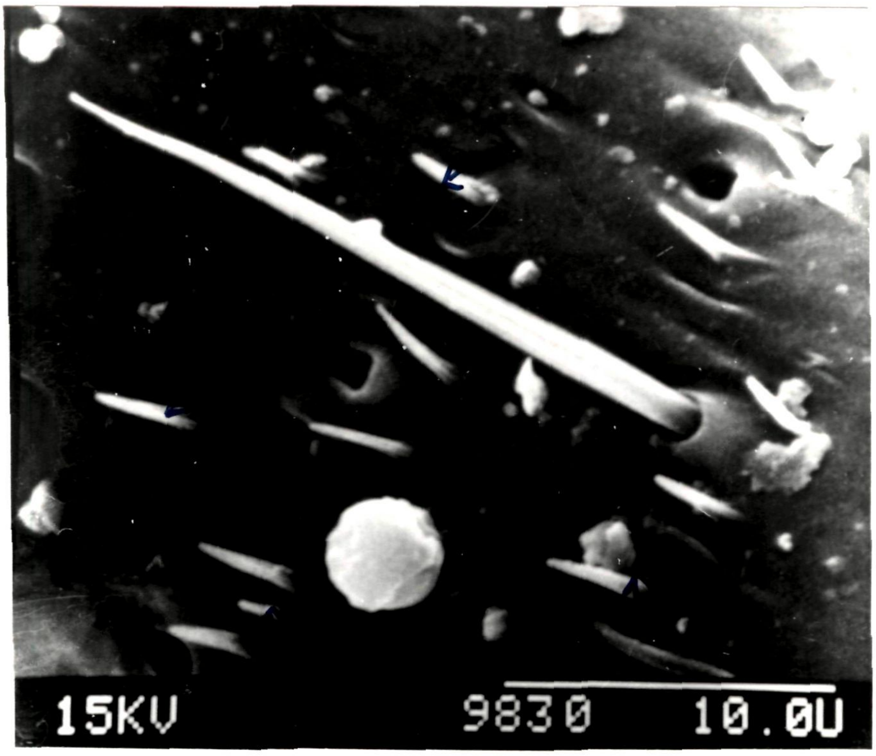
Fig.61 SEM picture of the femur of meta-thoracic leg of *A. annularis* showing the sensory peg x 3600 µn.

Fig.62 SEM picture of the femur of meta-thoracic leg of *A. annularis* showing the spinules type-I x 3600 µn.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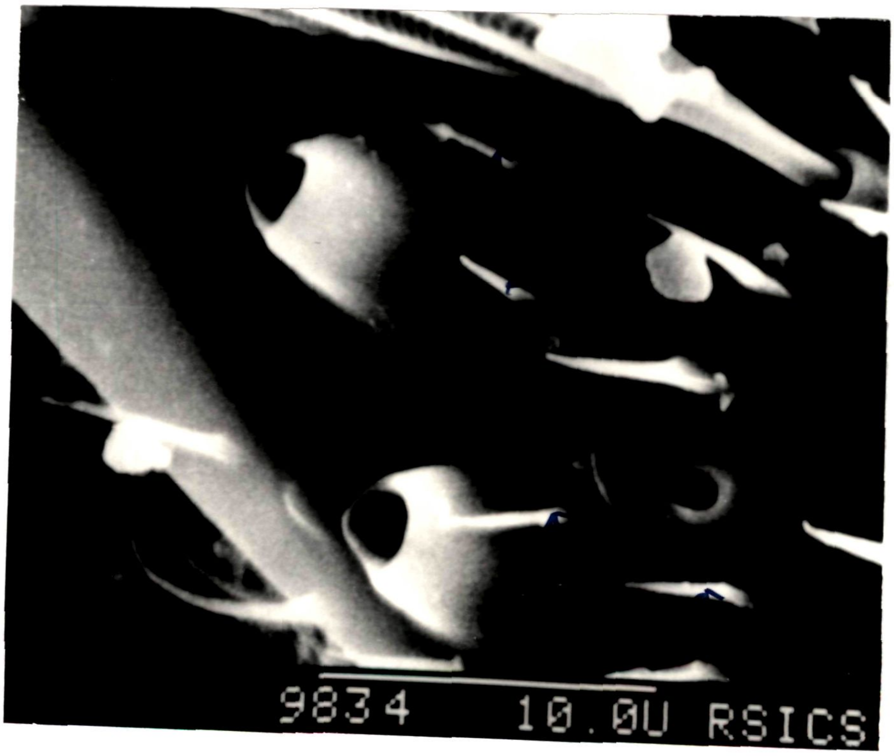
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RSICS

Table-8(c)
Distribution Pattern and Dimension of Different Types of Sensilla of *Anopheles annularis*

Sensilla	Location	Breadth Length (μ)	Base (μ)	Tip (μ)	Diameter of the Socket (μ)	Surface Feature	Total Number	Any other special Characteristic
Sensory Peg	1 Femur of the Meta-thoracic Leg	5.33	0.5	0.17		Smooth	Innumerable	
SPINULES Type-I	1 Femur of the of Meta-thoracic Leg	5	0.5	0.13		Smooth	6/mm^2	
Type-II	1 Femur of the Femur of Meso-thoracic Leg	3.33	0.83	0.41		Smooth	Innumerable	
Type-II	2 Femur of the Meta-Thoracic Leg	3.5	0.5	0.25		Smooth	Innumerable	
Type-III	1 Base of the Claws of Meso-thoracic Leg	27.89	1.18	0.26		Smooth	3/mm^2	Occurs in Cluster
HAIR PLATE Type-I	1 Base of the Claws of Pro-thoracic Leg	10 to 17.5	0.5 to 1	0.25 to 0.63		Smooth	9/mm^2	Tip of the Shaft Curved and Non-socketted
Type-II	1 Ventro/ Lateral view of thorax	62.5 to 135	1.25 to 2.5	0.63 to 1.25	2.5 to 7.5	Smooth	Innumerable	
BASICONICA	1 Surface of the Antenna	11.30	1.74	0.43	2.61	Smooth	Innumerable	

Sensilla	Location	Diameter(μ)	Total Number	Any other special characteristic
CAMPANIFORMIA	1 Trochontar of Meta-Thoracic Leg	7.89	6/mm^2	
CAMPANIFORMIA	2 Coxa/Trochontarr of Meso-Thoracic Leg	4.55	1/mm^2	
CAMPANIFORMIA	3 Base of the Femur of Pro-thoracic Leg	4.58	1/mm^2	

Spinules : Three types of spinules have been detected on the different locations of the body. The sub-types are recognised on the basis of their length, width at the base and tip, surface feature and also the diameter of the socket.

Spinules type-I : The smooth, tapered hair shaft points distally and is distinctly curved in the distal 1/3 to 1/2 of its length. The sensilla is located on the femur of meta-thoracic leg (Fig.62). The length of this sensilla is approximately 5 m, the width at the base at 0.5 m and that at the tip is 0.13 m (Table-8c).

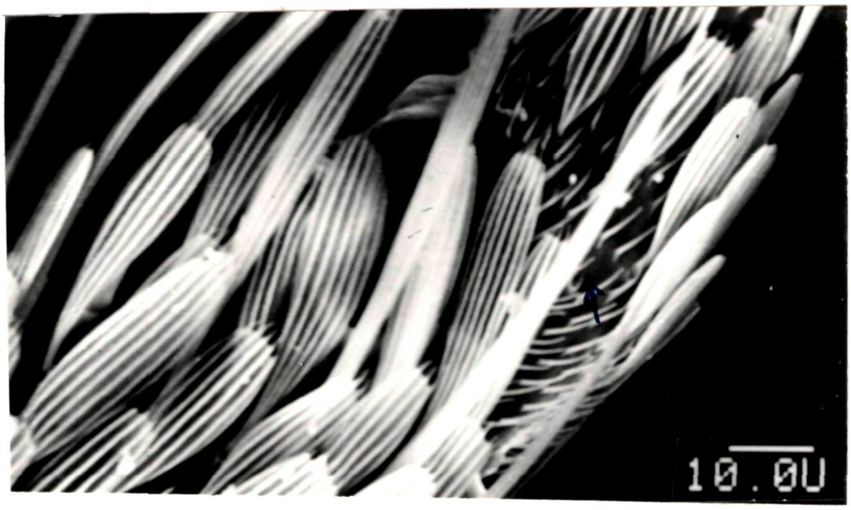
Spinules type-II : The shaft is slightly tapered, non-socketted and surface feature smooth. The spinules type-II is located on the femur of meso- and pro-thoracic leg (Figs.63a,b). These sensilla range from 3.33 m to 3.5 m in length, the width at the base vary between 0.5 m to 0.83 m and that at the tip is 0.25 m to 0.41 m (Table-8c).

Spinules type-III : These sensilla occur in cluster and originating from the same base. The shaft are smooth and non-socketted. These sensilla are located only at the base of the claws of meso-thoracic leg (Fig.64). The sensilla are about 27.89 m in length, the width at the base is about 1.18 m and that at the tip is approximately 0.26 m (Table-8c).

Fig.63(a) SEM picture of the femur of meso-thoracic leg of *A. annularis* showing the spinules type-II x 1000 µn.

Fig.63(b) SEM picture of the femur of pro-thoracic leg of *A. annularis* showing the spinules type-II x 1800 µn.

Fig.64 SEM picture of base of the claws of meso-thoracic leg of *A. annularis* showing the spinules type-III x 1600 µn.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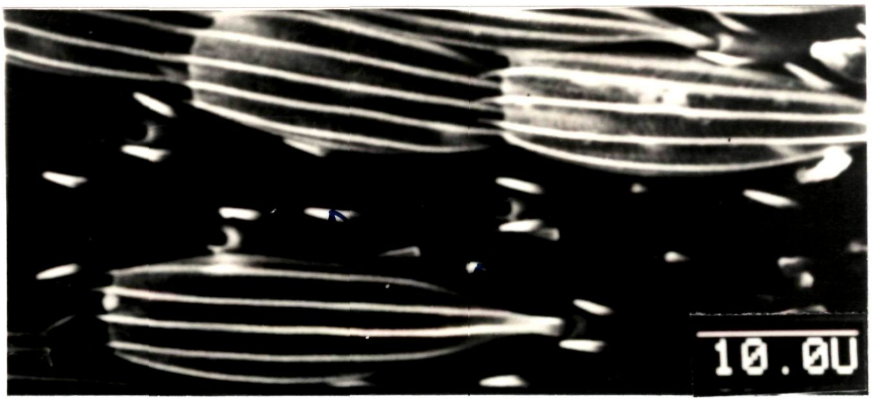
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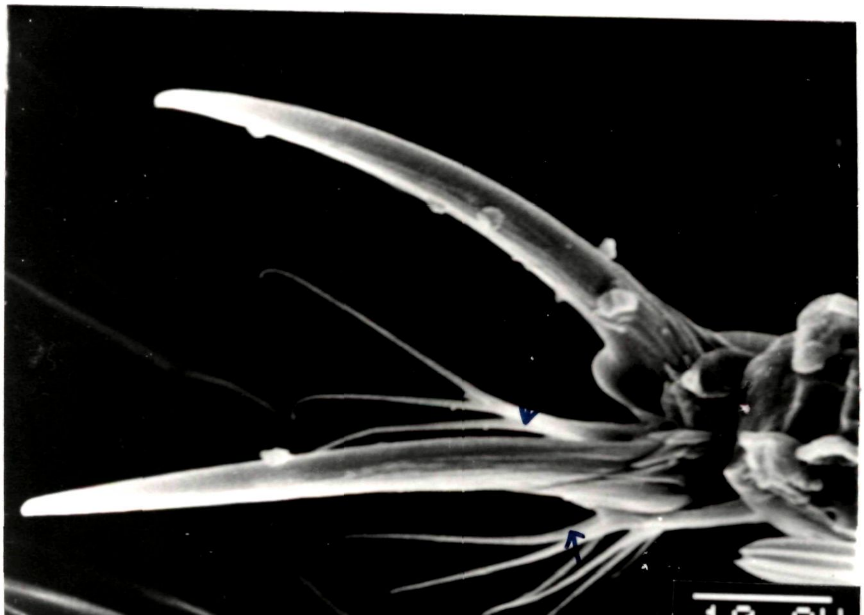
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Hair plate : These sensilla have been detected at the base of the claws of pro-thoracic leg and the ventro/lateral region of the thorax. The sensilla are of two types (Type-I,II) and are differentiated basing on the length, breadth as well as the shape, size and diameter of the socket.

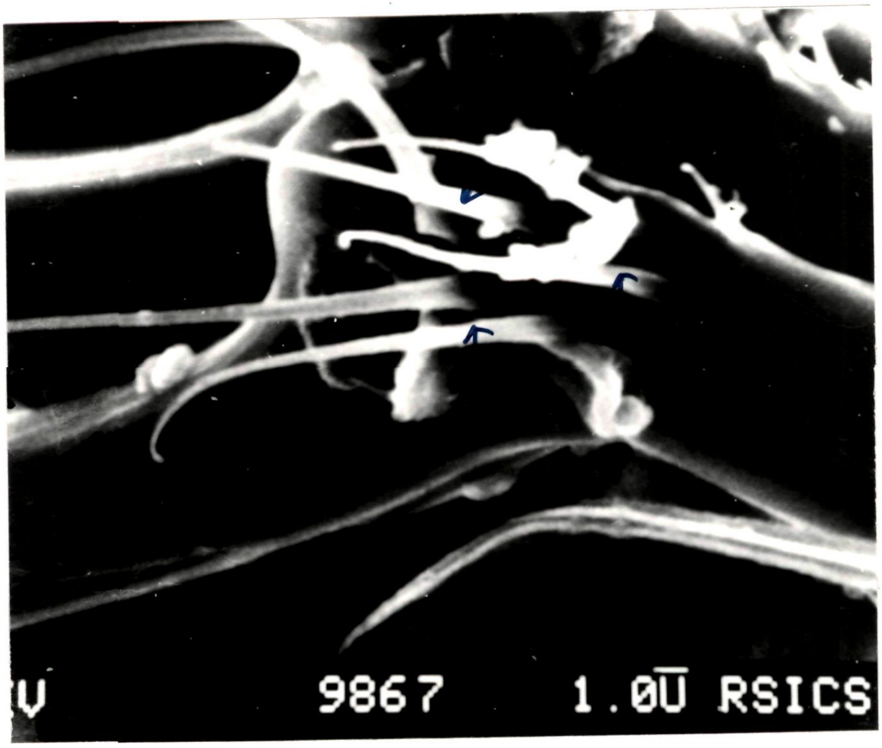
Hair plate type-I : The smooth, tapered hair shaft point distally and is distinctly curved towards the tip of its length. The tip portion of the shaft is round and the socket are absent. This sensilla is located only on the base of the claws of pro-thoracic leg (Fig.65). These sensilla range in length from 10 m to 17.5 m, the width at the base range from 0.5 m to 1 m and that at the tip from 0.25 m to 0.63 m (Table-8c).

Hair plate type-II : The shaft is tapered, smooth and originating from a distinct socket. these sensilla are localized only on the ventral/lateral region of the thorax (Fig.66). The sensilla range from 62.5 m to 135 m in length, and having the basal diameter between 1.25 m to 2.5 m, whereas that at the tip between 0.63 m to 1.25 m. The diameter of the socket vary between 2.5 m to 7.5 m (Table-8c).

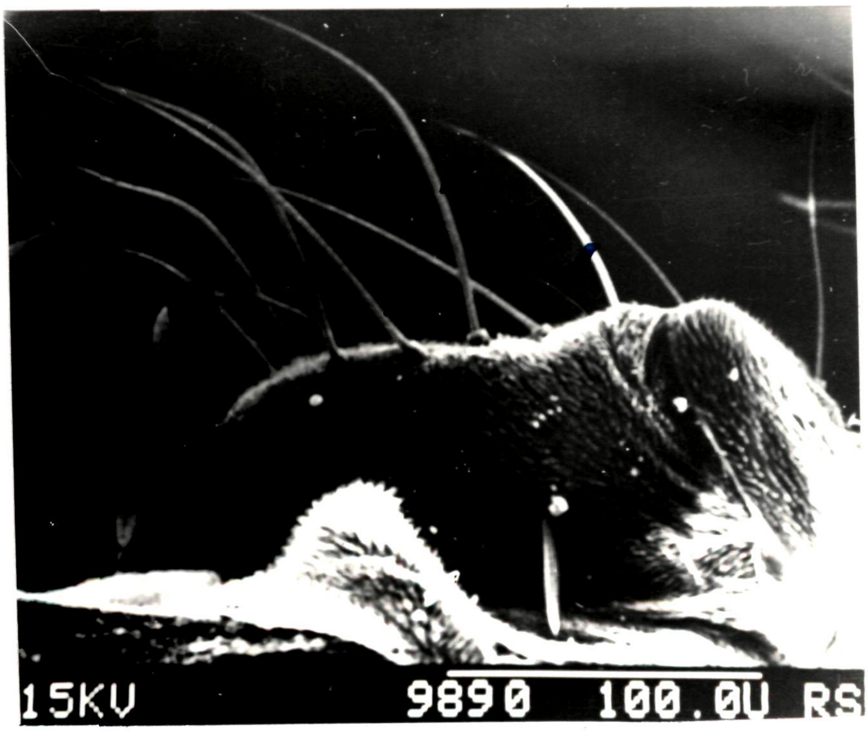
Basiconica type-I : The shaft is tapered, smooth and socketted. These sensilla is located on the surface of the antenna (Fig.67). The length of the sensilla range from

Fig.65 SEM picture of base of the claws of pro-thoracic leg of *A. annularis* showing the hair-plate type-I x 4000 µn.

Fig.66 SEM picture of ventral/lateral region of thorax of *A. annularis* showing the hair-plate type II x 360 µn.



V
9867
1.0U RSICS



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100.0U RS

11.30 m to 17.39 m, the width at the base vary between 1.30 m to 17.39 m, the width at the base vary between 1.30 m to 1.74 m and that at the tip is approximately 0.48 m. The sensilla are socketted having its diameter ranging between 2.17 m to 2.61 m (Table-8c).

Campaniformia : This sensory receptor is somewhat circular in shape and is located on trochontar of meta-thoracic leg, coxa/trochontar of meso-thoracic leg and at the base of the femur of pro-thoracic legs (Figs.68a,b,c). The diameter of the sensory receptor range from 4.55 m to 7.89 m (Table-8c).

Anopheles vagus : In *A. vagus* the sensilla that could be detected are sensilla trichodea, sensilla chaetica, spinules, hair-plate, basiconica, cuticular-pit and campaniformia.

Sensilla trichodea : There are five types (Type-I, III, IX, XII, XIII) of sensilla trichodea detected in various locations on the body surfaces of the species. The sub-types are differentiated basing on the length, breadth, diameter of the socket, surface feature and also other special characteristic.

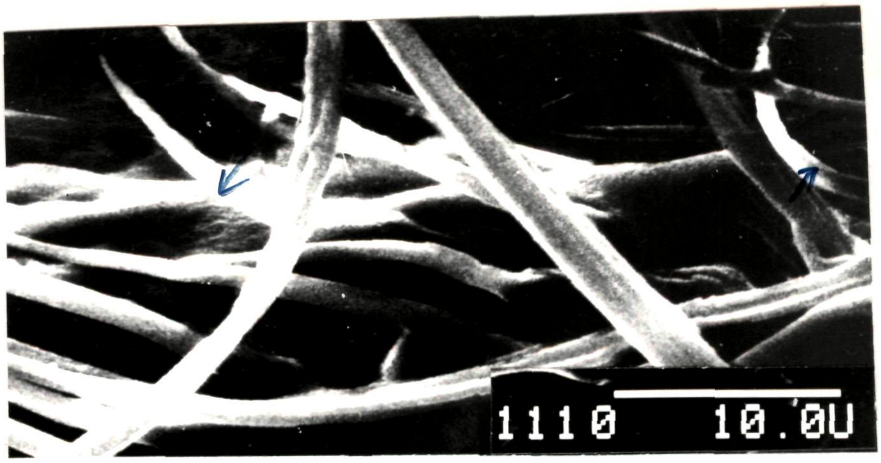
Sensilla trichodea type-I : The shaft is tapered and has prominent longitudinal groove. The sensilla point

Fig.67 SEM picture of the surface of antenna of *A. annularis* showing the sensilla basiconica type-I x 2600 µn.

Fig.68(a) SEM picture of coxa/trochontar of the meta-thoracic leg of *A. annularis* showing the sensilla/receptor campaniformia x 2000 µn.

Fig.68(b) SEM picture of coxa/trochontar of the meso-thoracic leg of *A. annularis* showing the sensilla/receptor campaniformia x 2000 µn.

Fig.68(c) SEM picture of base of the femur of pro-thoracic leg of *A. annularis* showing sensilla/receptor campaniformia x 1100 µn.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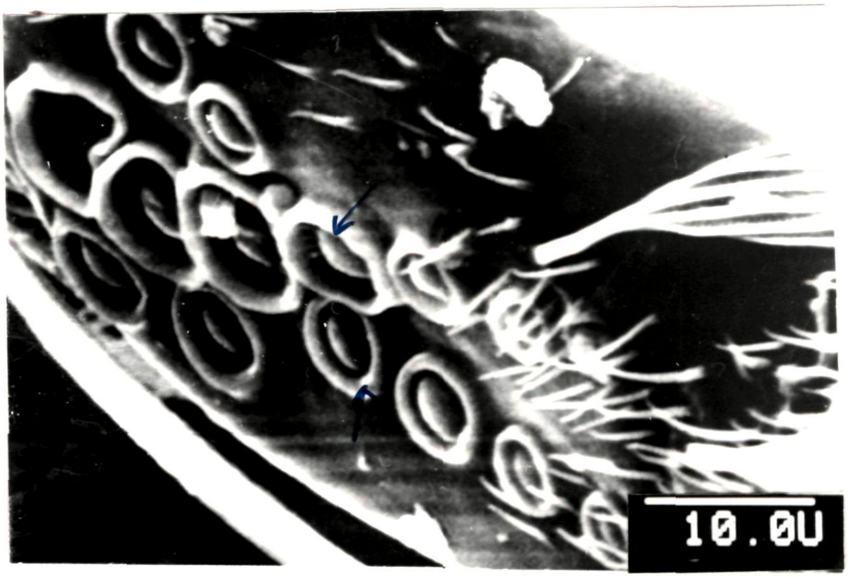
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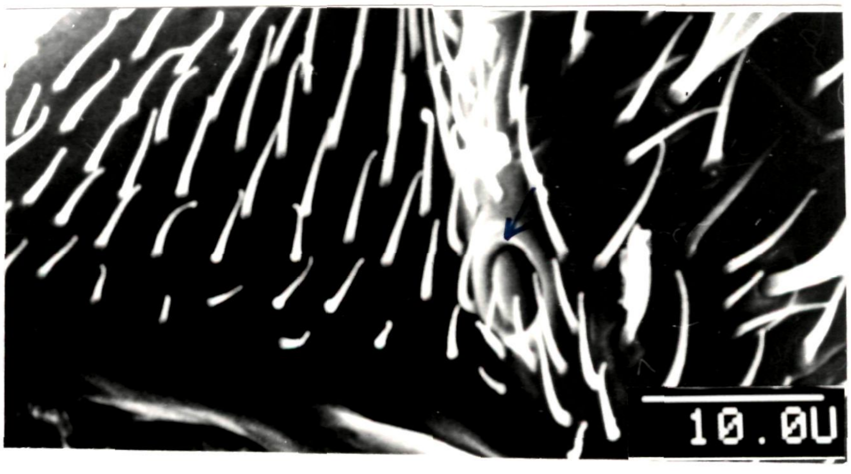
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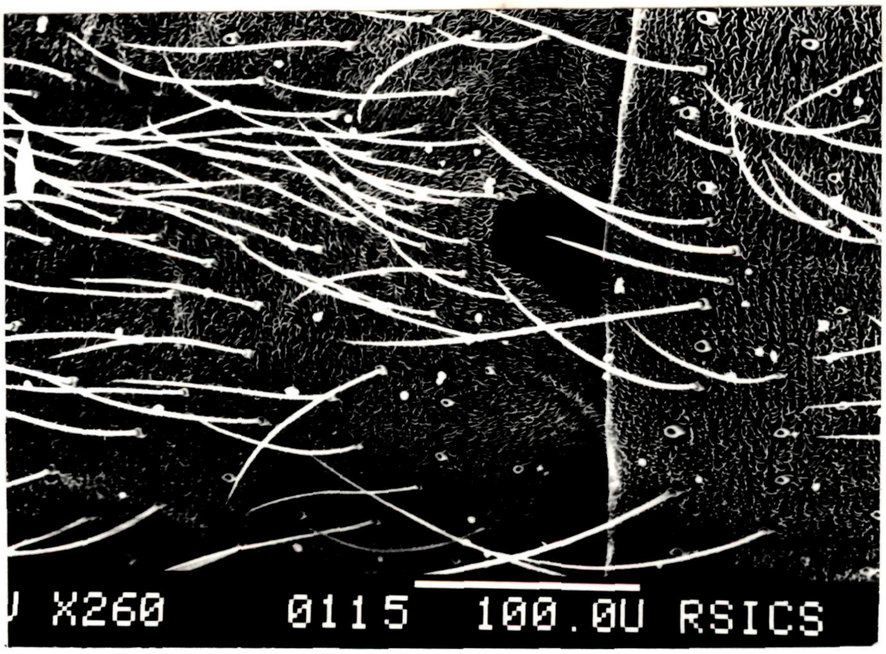
distally and outwardly and somewhat curved toward the distal end. These sensilla trichodea type-I are among the most numerous sensilla under this sub-types, and located on the dorsal/ventral surface of the abdomen (Figs.69a,b,c), on the surface of the antenna (Figs.70a,b), on the surface of palpi (Fig.71), coxa of meta-thoracic leg (Fig.72), trochontar of meta-thoracic leg (Fig.73), femur/tibia joint of meta-thoracic leg (Fig.74), tibia of meta-thoracic leg (Fig.75), tarsus segment of meta-thoracic leg (Fig.76), coxa/trochontar of meso-thoracic leg (Fig.77), tibia of meso-thoracic leg (Fig.78), tarsus of meso-thoracic leg (Fig.79), coxa/ trochontar of pro-thoracic leg (Fig.80), and the tip of the last tarsal segment of pro-thoracic leg (Fig.81). The sensilla located on the dorsal/ventral surface of the abdomen and on the surfaces of the antenna are among the largest under this sub-type, the length of which, range from 109 m to 194 m, the width at the base range from 1.67 m to 2.87 m and that at the tip between 0.56 m to 0.83 m. The sensilla are socketted having a diameter of 2.86 m to 5.71 m (Table-9a). Whereas, those sensilla located on other regions as mentioned above, has its length ranging from 50 m to 91.67 m. The width at the base range from 1.43 m to 3.75 m and that at the tip it range from 0.16 m to 2.5 m (Table-9a).

Fig.69(a) SEM picture of the surface of abdomen (Dorsal) of *Anopheles vagus* showing the sensilla trichodea type-I x 260 µn.

Fig.69(b) SEM picture of the surface of abdomen (ventral) of *A. vagus* showing the sensilla trichodea type-I x 320 µn.

Fig.69(c) SEM picture of the base of sensilla trichodea type-I located on the dorsal/ventral surface of the abdomen of *A. vagus* x 3600 µn.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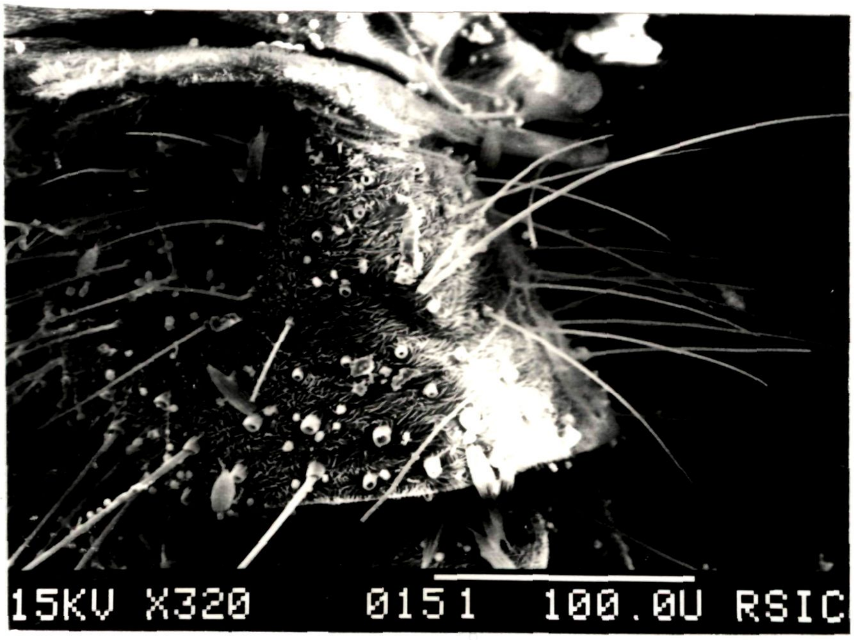
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15KV X4400 0113 1.0U

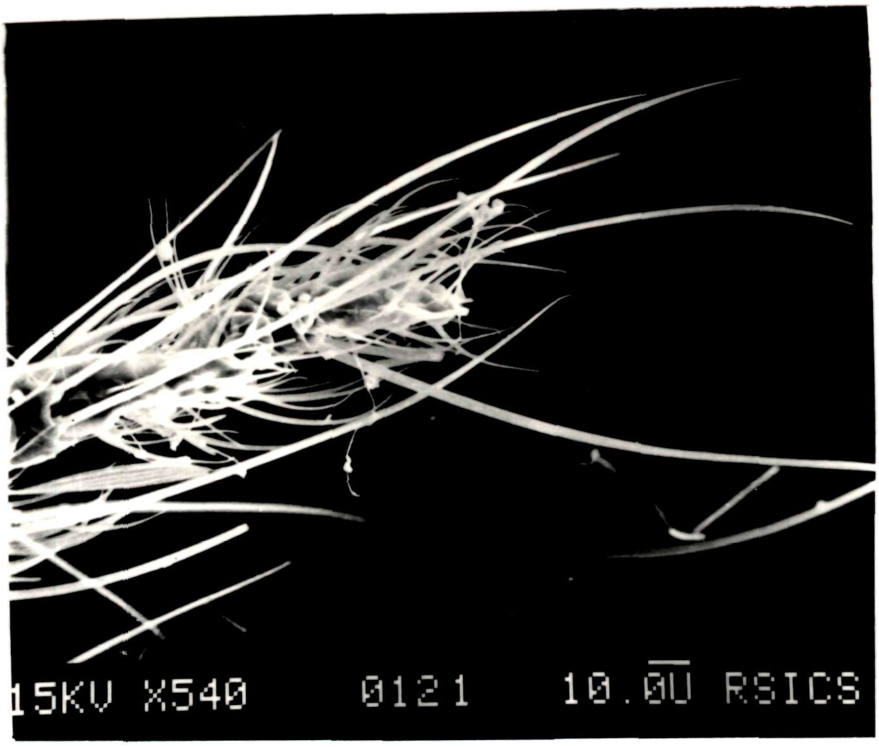
Table-9(a)
Distribution Pattern and Dimension of Different Types of Sensilla Trichodea of *Anopheles vagus*

Sensilla	Location	Breadth Length (μ)	Base (μ)	Tip (μ)	Diameter of the Socket (μ)	Surface Feature	Total Number	Any other special Characteristic
Type-I	1 Dorsal and Ventral view of Abdomen	108.57 to 194.3	2.86 to 2.87	0.72	2.86 to 5.71	Longitudinal Groove	Innumerable	
Type-I	2 Tip Portion of Antenna	81.67 to 173.3	1.67	0.56 to 0.83	4.29	Longitudinal Groove	Innumerable	
Type-I	3 Surface view of Palpi	57.14	2.14	1.43	4.29	Longitudinal Groove	$2/mm^2$	
Type-I	4 Coxa of Meta-thoracic Leg	52.5 to 81.25	2.5	1.25	3.75	Longitudinal Groove	$2/mm^2$	
Type-I	5 Trochontarr of Meta-thoracic Leg	51.43 to 71.43	1.43 to 2.86	0.36	2.86 to 4.29	Longitudinal Groove	Innumerable	
Type-I	6 Femur/Tibia Joint of Meta-thoracic Leg	91.67	3.33	2.5	6.67	Longitudinal Groove	Innumerable	
Type-I	7 Tibia of Meta-thoracic Leg	50	2.5	0.16	4.29	Longitudinal Groove	$3/mm^2$	
Type-I	8 Tarsal Segment of Meta-thoracic Leg	50	3.33	1.67	3.64	Longitudinal Groove	$1/mm^2$	
Type-I	9 Coxa/Trochontar of Meso-thoracic Leg	67	3	0.25	5	Longitudinal Groove	$6/mm^2$	
Type-I	10 Tibia of Meso-thoracic Leg	75	3.75	1.25	5	Longitudinal Groove	$1/mm^2$	
Type-I	11 Tarsal Segment of Meso-thoracic Leg	52.5	2.5	1.25	5	Longitudinal Groove	$2/mm^2$	
Type-I	12 Coxa/Trochontar of Pro-thoracic Leg	60	2.86	0.71	4.29	Longitudinal Groove	Innumerable	

Sensilla	Location	Length (μ)	Breadth Base (μ)	Breadth Tip (μ)	Diameter of the Socket (μ)	Surface Feature	Total Number	Any other special Characteristic
Type-I	13 Tip of the Fifth Tarsal Segment of the Pro-thoracic Leg	34	2	1	3	Longitudinal Groove	4/mm^2	
Type-III	1 Femur of Pro-thoracic Leg	66.67 to 68.33	2.5 to 3.33	0.83	5	Longitudinal Groove	1/mm^2	
Type-III	2 Tip of the Fifth Tarsal Segment of Pro-thoracic Leg	56.67	2.22	0.56	4.44	Longitudinal Groove	3/mm^2	
Type-IX	1 First Tarsal Segment of Pro-thoracic Leg	25.56 to 27.78	3.33	0.56	4.44 to 5.55	Longitudinal Groove	2/mm^2	
Type-XII	1 Surface of the Probosis	5.5 to 12.5	0.5 to 1	0.25 to 0.5	3	Longitudinal Groove	1/mm^2	
Type-XIII	1 Tibia/Tarsal Joint of Meta-thoracic Leg	74.29	2.86	0.71	4.29	Longitudinal Groove	Innumerable	Rough Edges

Fig.70(a) SEM picture of the surface of antenna of *A. vagus* showing the sensilla trichodea type-I x 540 µn.

Fig.70(b) SEM picture of the base of sensilla trichodea type-I located on the surface of antenna of *A. vagus* x 3200 µn.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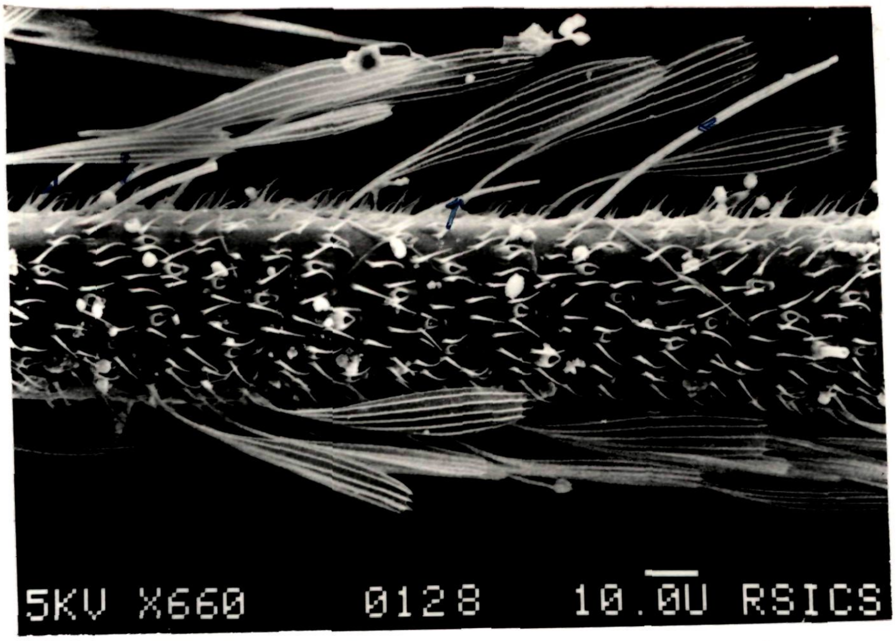
15KV X3200 0122 10.0U RSIC

Fig.71 SEM picture of the surface of palpi of *A. vagus* showing the sensilla trichodea type-I x 660 µn.

Fig.72 SEM picture of the coxa of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 660 µn.

Fig.73 SEM picture of the trochontar of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 660 µn.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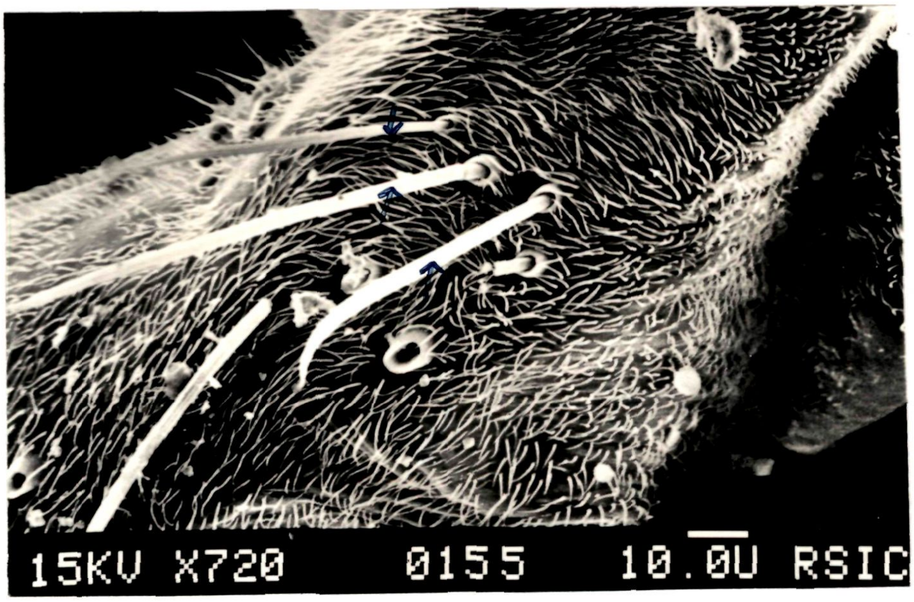
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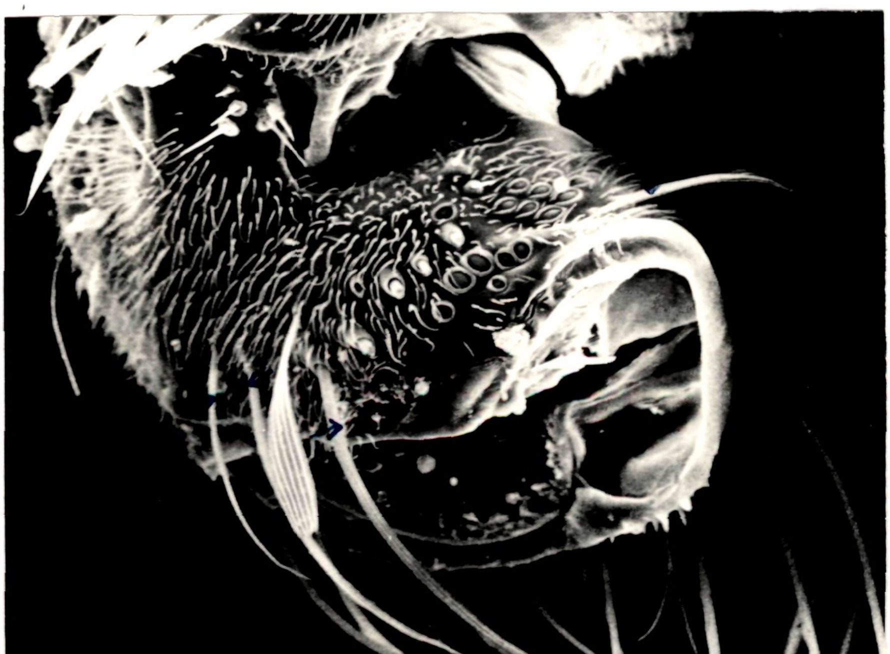
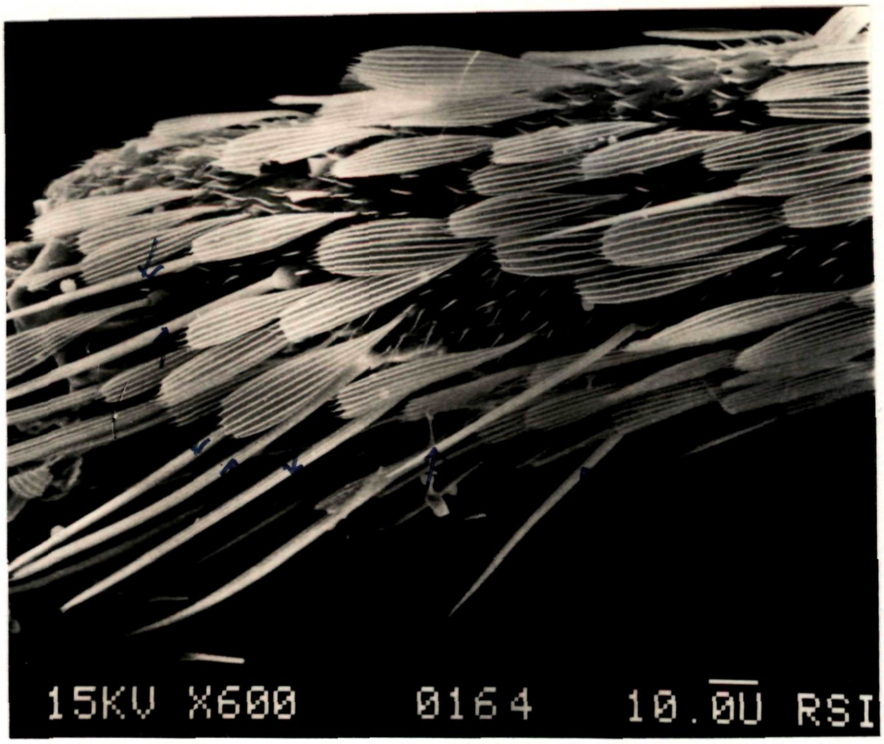

KV X660 0156 10.0U RSICS

Fig.74 SEM picture of the femur/tibia joint of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 600 µm.

Fig.75 SEM picture of the tibia of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 720 µm.

Fig.76 SEM picture of the tarsus of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 540 µm.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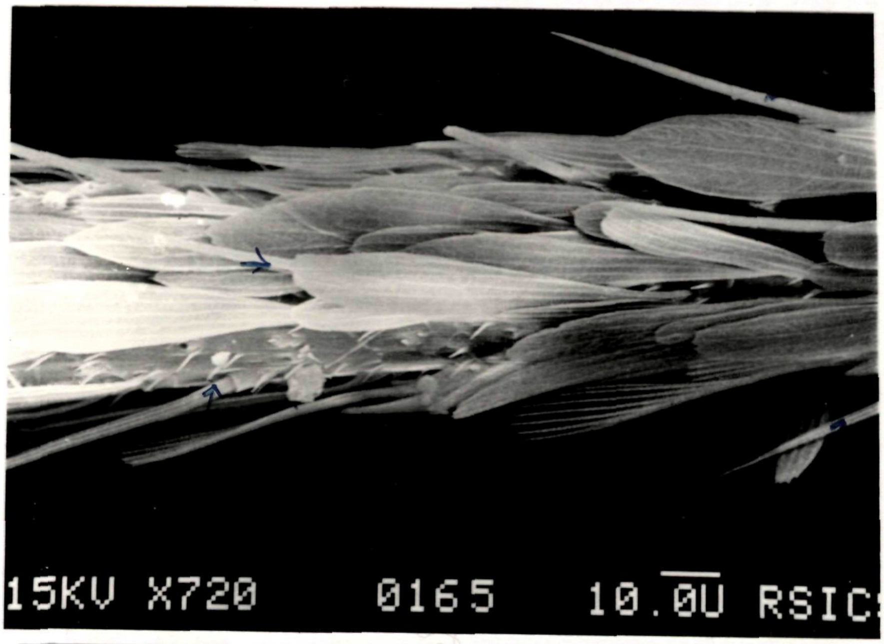
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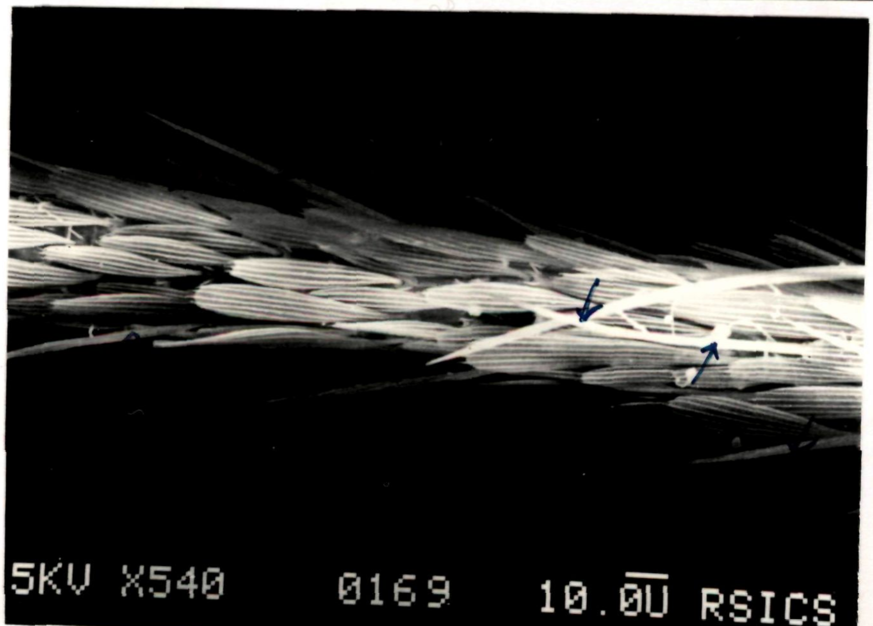
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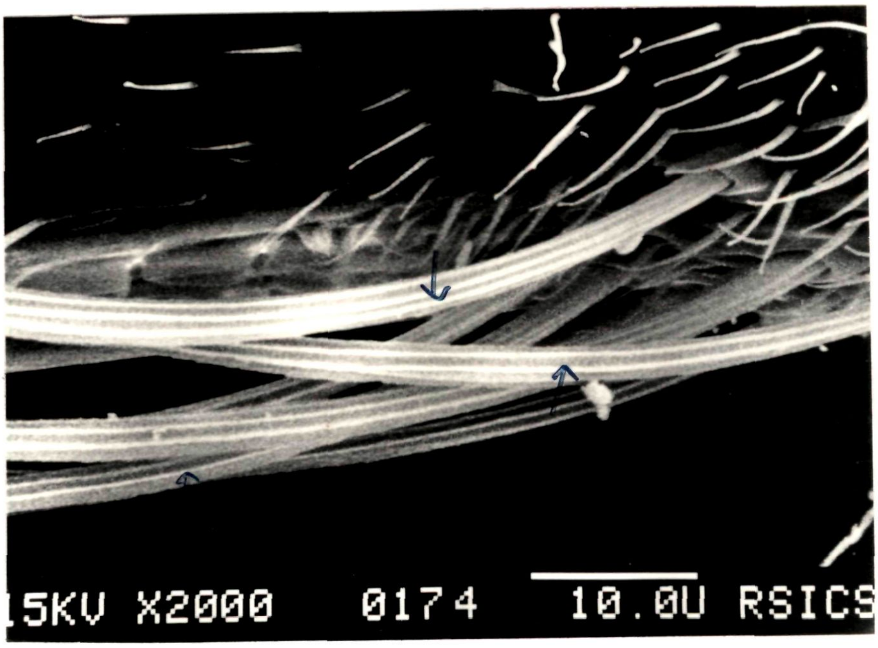
Fig.77 SEM picture of the coxa/trochontar of meso-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 940 µn.

Fig.78 SEM picture of tibia of meso-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 1000 µn.

Fig.79 SEM picture of the tarsus of meso-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 400 µn.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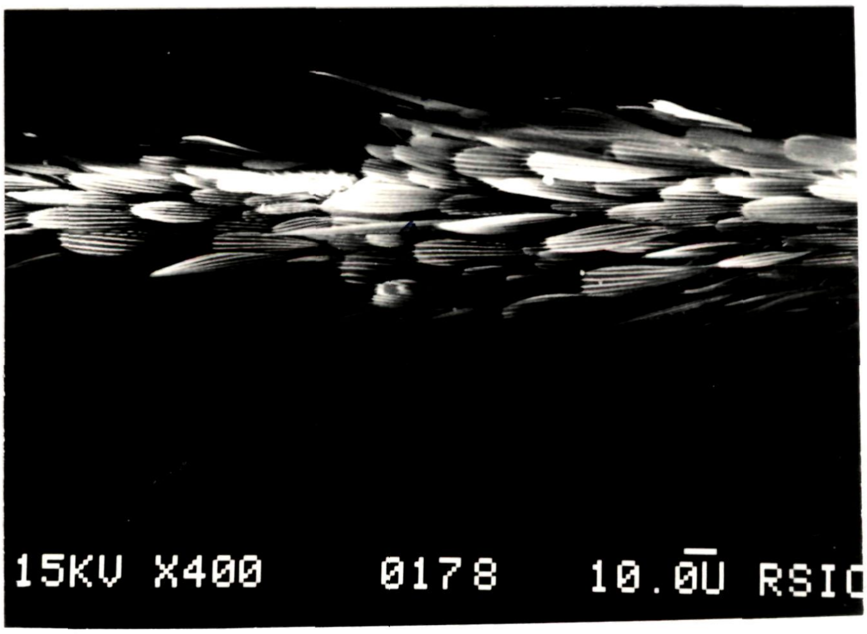
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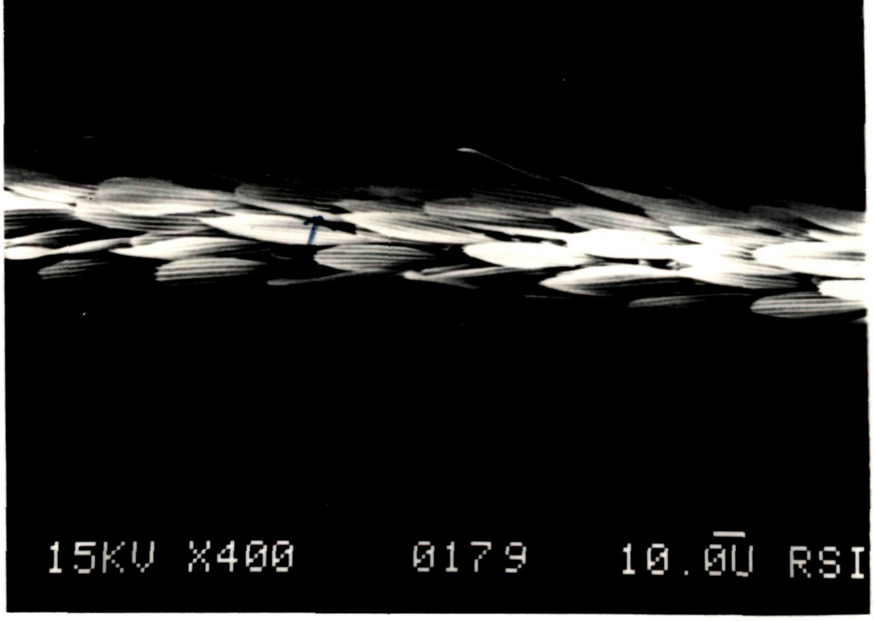
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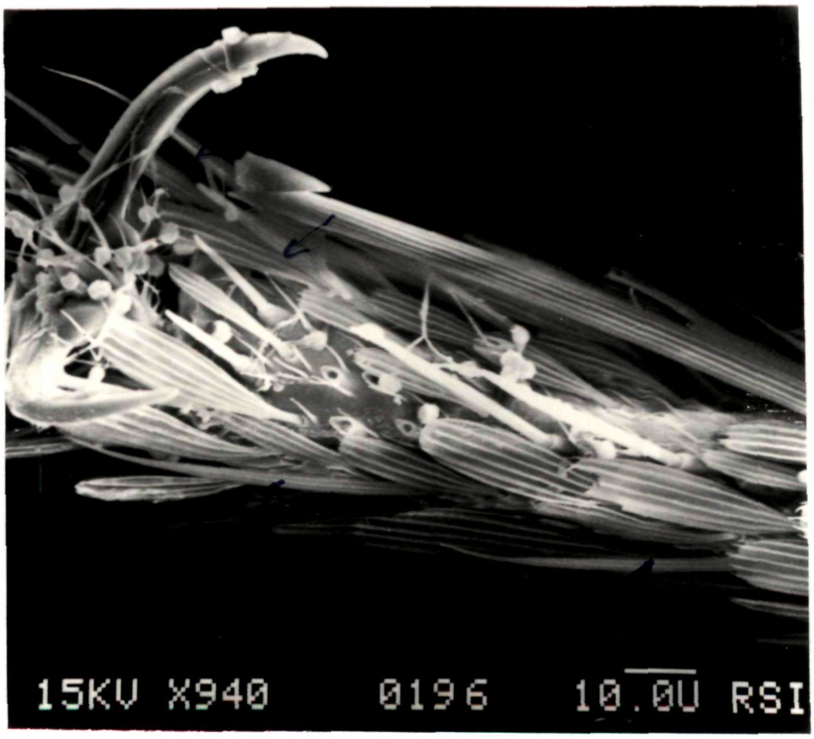
Fig.80 SEM picture of the coxa/trochontar of pro-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 660 µn.

Fig.81 SEM picture of tip of the last tarsal segment of pro-thoracic leg of *A. vagus* showing the sensilla trichodea type-I x 940 µn.


5KV X660 0180 10.0U RSICS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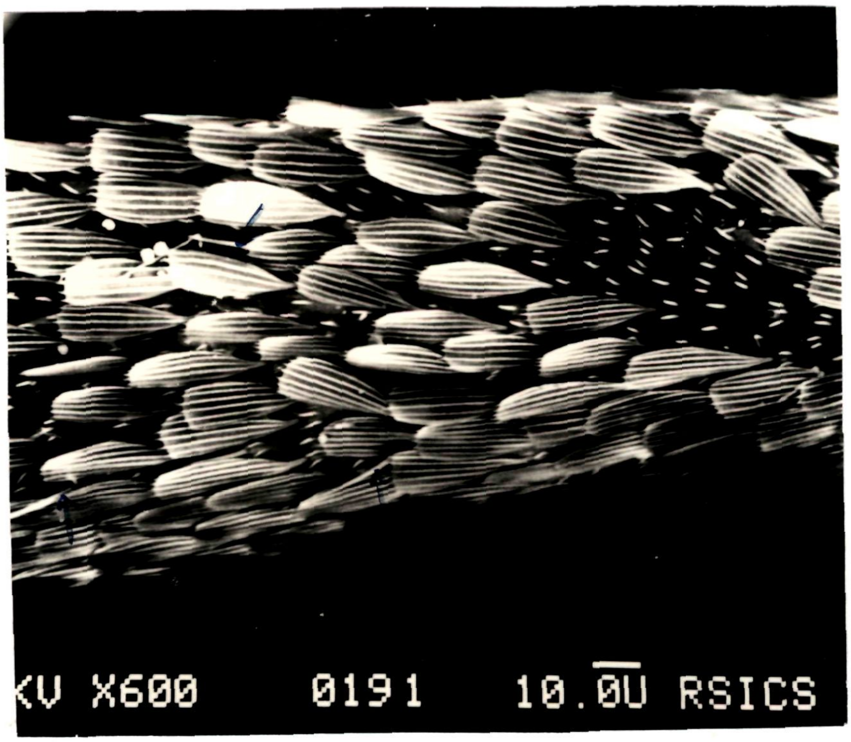
Sensilla trichodea type-III : The shaft is tapered, socketted and has a prominent longitudinal groove. The sensilla is located on the femur of pro-thoracic leg and fifth tarsal segment of pro-thoracic leg (Figs.82a,b). The length of the sensilla shows a variation of 56.67 m to 68.33 m, the width at the base also range from 2.22 m to 3.33 m. The diameter of the socket also vary between 4.44 m to 5 m (Table-9a).

Sensilla trichodea type-IX : The shaft is divided into three parts at the anterior region, and having a faint, longitudinal groove and with a swollen socket (Fig.83). The sensilla is located on the tarsal segment of pro-thoracic leg. The length range from 25.26 m to 27.78 m, the width at the base is approximately 3.33 m and that at the tip is 0.56 m. The diameter of socket vary between 4.44 m to 5.55 m (Table-9a).

Sensilla trichodea type-XII : The shaft is slightly tapered, thick-walled, broad socketted and with longitudinal groove. These sensilla are located on the surface of the probosis (Figs.84a,b). The length range from 5.5 m to 12.5 m, the width at the base range from 0.5 m to 1 m and that at the tip between 0.25 m to 0.5 m. The diameter of the socket is approximately 3 m (Table-9a).

Fig.82(a) SEM picture of femur of pro-thoracic leg of *A. vagus* showing the sensilla trichodea type-III x 600 µn.

Fig.82(b) SEM picture of the fifth tarsal segment of pro-thoracic leg of *A. vagus* showing the sensilla trichodea type-III x 940 µn.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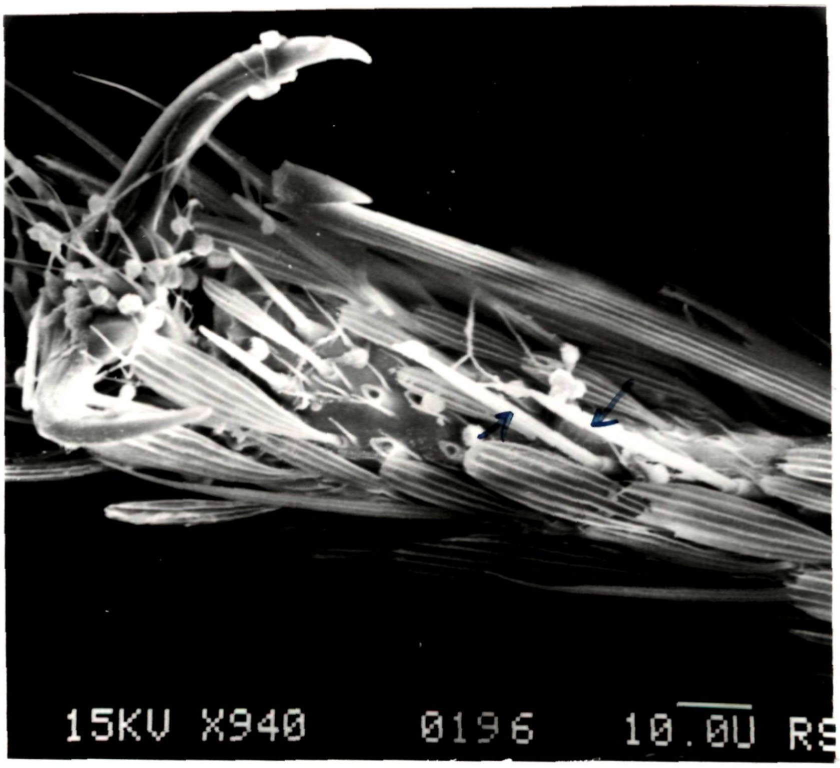
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Fig.83 SEM picture of the tarsal segment of pro-thoracic leg of *A. vagus* showing the sensilla trichodea type-IX x 860 µn.

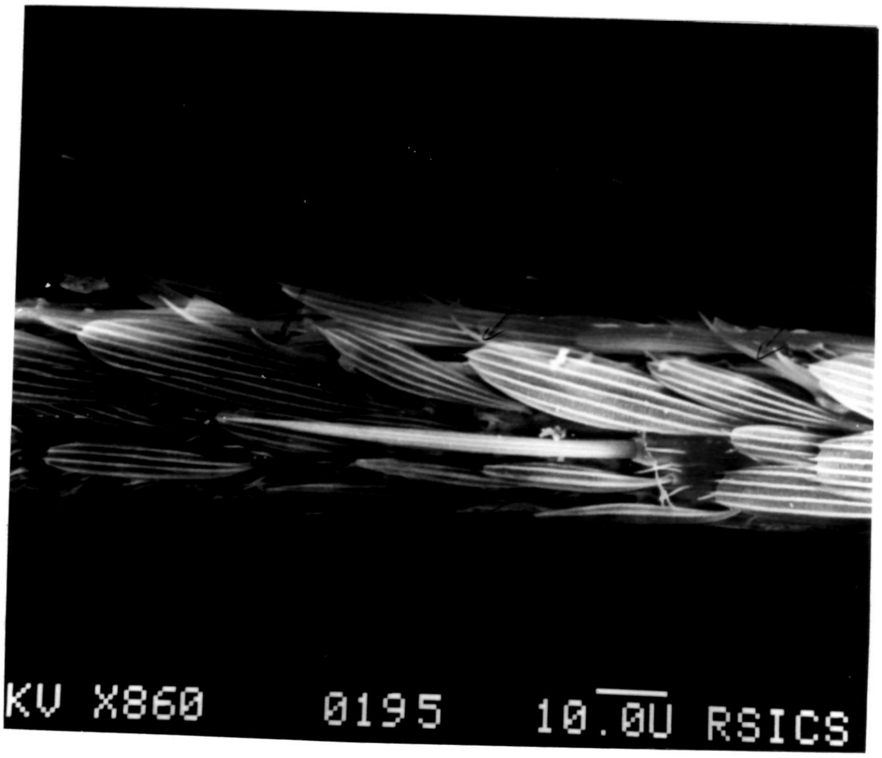
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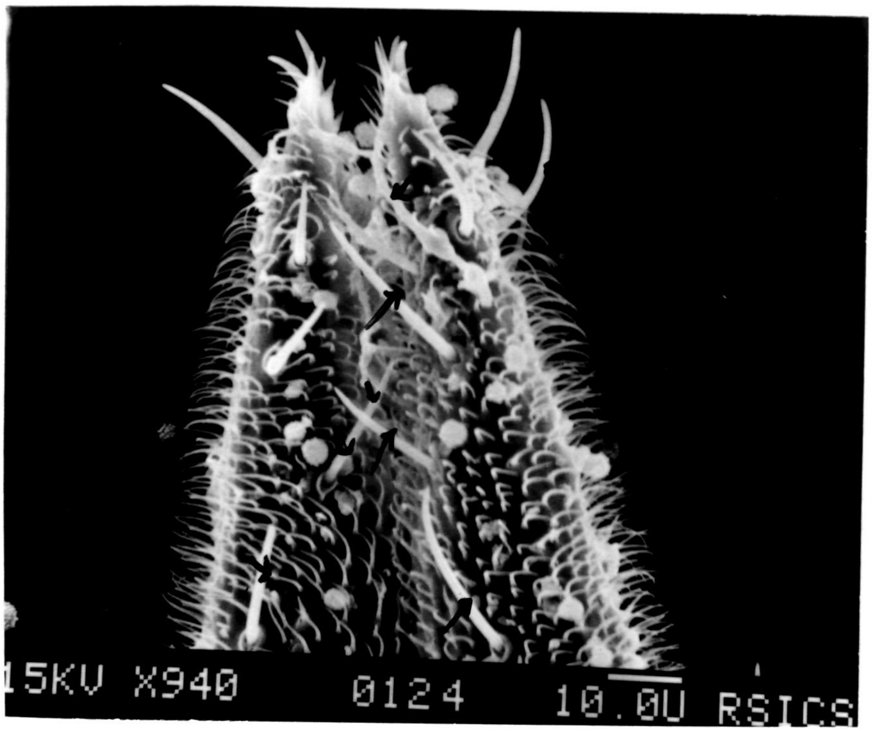
Fig.84(a) SEM picture of the surface of the probosis of *A. vagus* showing the sensilla trichodea type-XII x 940 µn.

Fig.84(b) SEM picture of the sensilla trichodea type-XII of *A. vagus* located on the probosis (enlarged view) x 7800 µn.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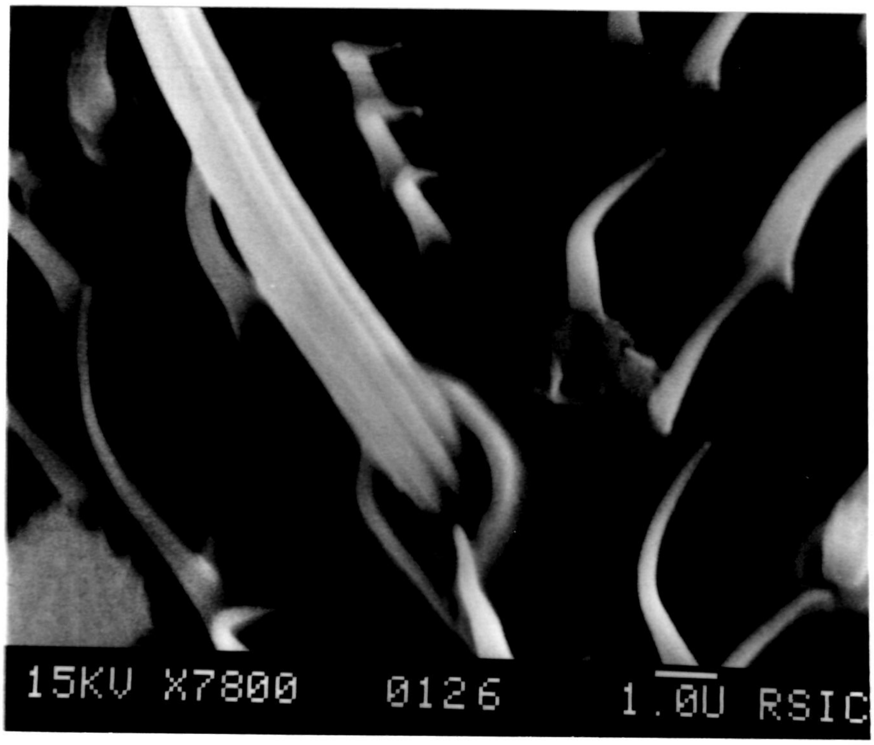
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Sensilla trichodea type-XIII : The shaft is tapered and has a faint, longitudinal groove. The sensilla is located on the tibia/tarsal joint of meta-thoracic leg (Fig.85). These sensilla are approximately 74.29 m in length and a basal diameter of 2.86 m and that at the tip around 0.71 m. The sensilla is socketted and having a diameter of 4.29 m (Table-9a).

Sensilla chaetica : There are six types (Type-I, II, III, IV, VII and VIII) of sensilla chaetica detected on the various locations of the species. The sub-types are distinguished basing on the length, width at the base and tip, diameter of the socket and the surface feature.

Sensilla chaetica type-I : The shaft is slightly tapered, smooth and almost curved at the anterior region. The sensilla is located on the dorsal surface of the abdomen (Fig.86a), general surface of the wing (Fig.86b), coxa of meta-thoracic leg (Fig.86c), trochontar of meta-thoracic leg (Fig.86d) and ventral view of thorax (Fig.86e). These sensilla range from 1.67 m to 7.65 m in length, the width at the base range from 0.21 m to 0.71 m and that at the tip between 0.10 m to 0.36 m. These sensilla are socketted having a diameter between 0.38 m to 1.17 m (Table-9b).

Fig.85 SEM picture of the tibia/tarsal joint of meta-thoracic leg of *A. vagus* showing the sensilla trichodea type-XIII x 1600 µm.

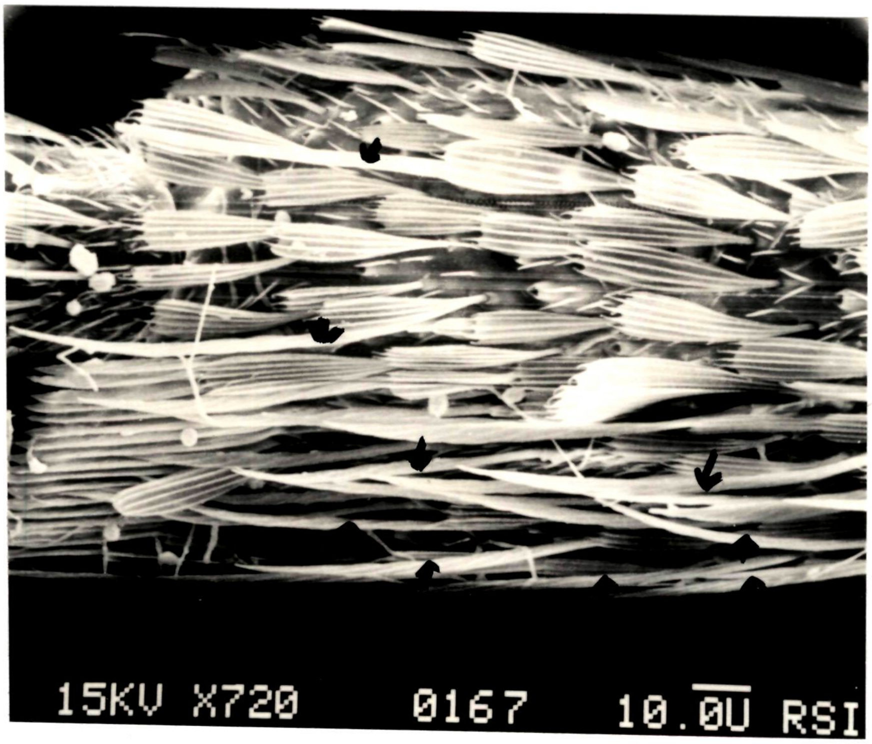
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Fig.86(a) SEM picture of the surface of abdomen (Dorsal) of *A. vagus* showing the sensilla chaetica type-I x 4400 µn.

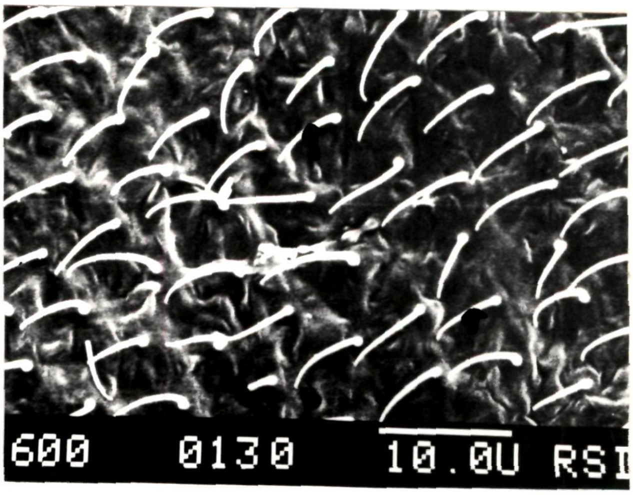
Fig.86(b) SEM picture of the surface of wing of *A. vagus* showing the sensilla chaetica type-I x 1600 µn.

Fig.86(c) SEM picture of the coxa of meta-thoracic leg of *A. vagus* showing the sensilla chaetica type-I x 720 µn.

Fig.86(d) SEM picture of the trochontar of meta-thoracic leg of *A. vagus* showing the sensilla chaetica type-I x 660 µn.

Fig.86(e) SEM picture of the ventral surface of thorax of *A. vagus* showing the sensilla chaetica type-I x 1200 µn.


0113 1.0U RSICS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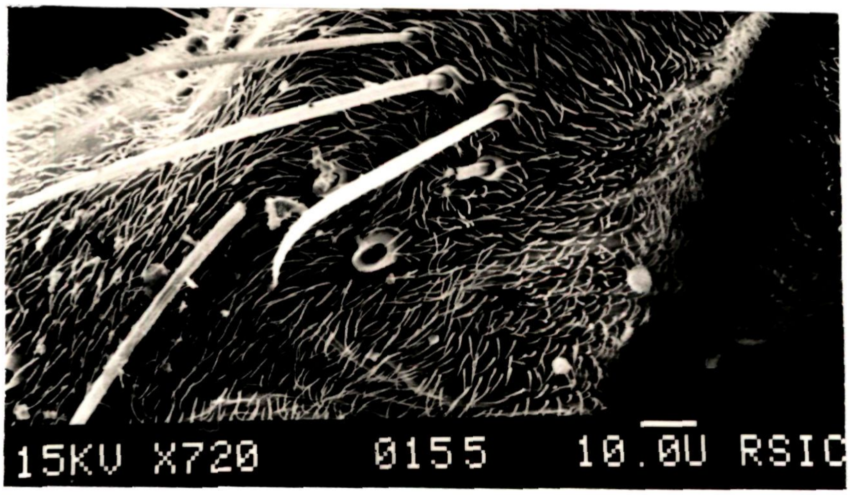
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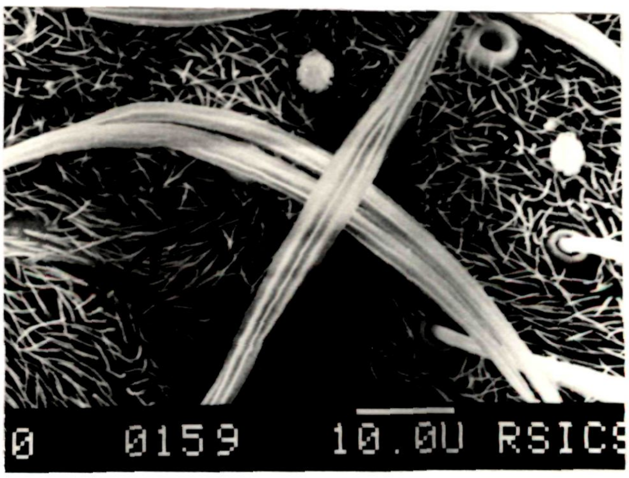

0 0159 10.0U RSICS

Sensilla chaetica type-II : The shaft is tapered, smooth and non-socketted. These sensilla are located on the coxa/trochontar of meta-thoracic leg (Fig.87a), coxa/trochontar of meso-thoracic leg (Fig.87b) and coxa/trochontar of pro-thoracic leg (Fig.87c). On the coxa/trochontar of meta-thoracic, the length range from 2.86 m to 8.10 m, the width at the base range from 0.47 m to 0.48 m and that at the tip between 0.19 m to 0.24 m (Table-9b). Those located on the coxa/trochontar of meso-thoracic leg, the length vary from 1.71 m to 4.28 m, the width at the base range from 0.29 m to 0.43 m and that at the tip at 0.14 m to 0.28 m (Table-9b). Whereas, those in the coxa/trochontar of pro-thoracic leg, the length range from 3.71 m to 5.71 m and that at the tip is approximately 0.18 m (Table-9b).

Sensilla chaetica type-III : The shaft is smooth, non-socketted and with a blunt tip. These sensilla are located on the surface of palpi (Fig.88a), ventral view of abdomen (Fig.88b) and tarsal segment of meta-thoracic leg (Fig.88c). The length vary from 4.44 m to 30 m, the width at the base range from 0.37 m to 2.14 m and that at the tip between 0.19 m to 1.43 m (Table-9b).

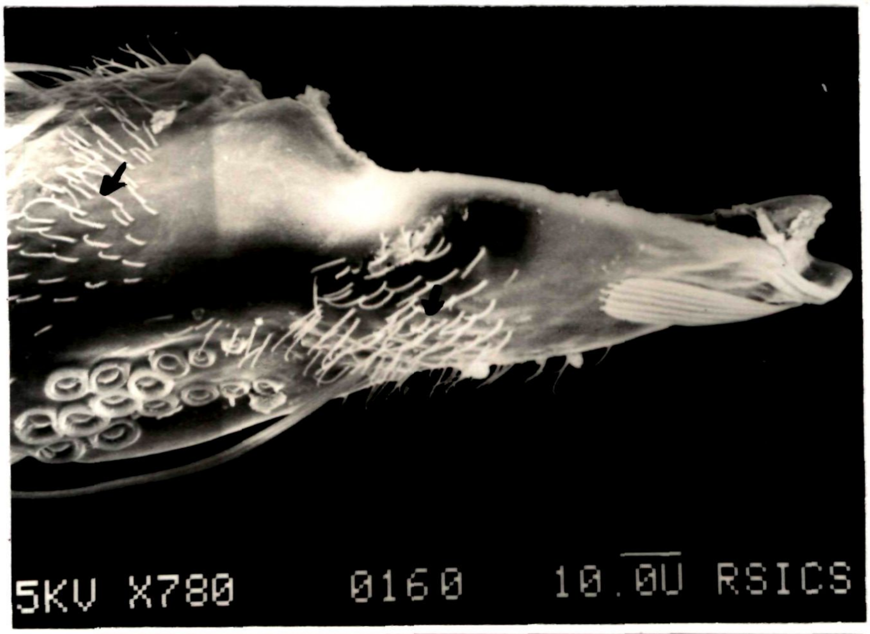
Sensilla chaetica type-IV : The shaft is smooth, non-socketted and with a pointed tip. These sensilla is located on the last dorsal abdominal segment (Fig.89a) and dorsal

Fig.87(a) SEM picture of the coxa/trochontar of meta-thoracic leg of *A. vagus* showing the sensilla chaetica type-II x 660 µn.

Fig.87(b) SEM picture of the coxa/trochontar of meso-thoracic leg of *A. vagus* showing the sensilla chaetica type-II x 780 µn.

Fig.87(c) SEM picture of the coxa/trochontar of the pro-thoracic leg of *A. vagus* showing the sensilla chaetica type-II x 660 µn.


10.0U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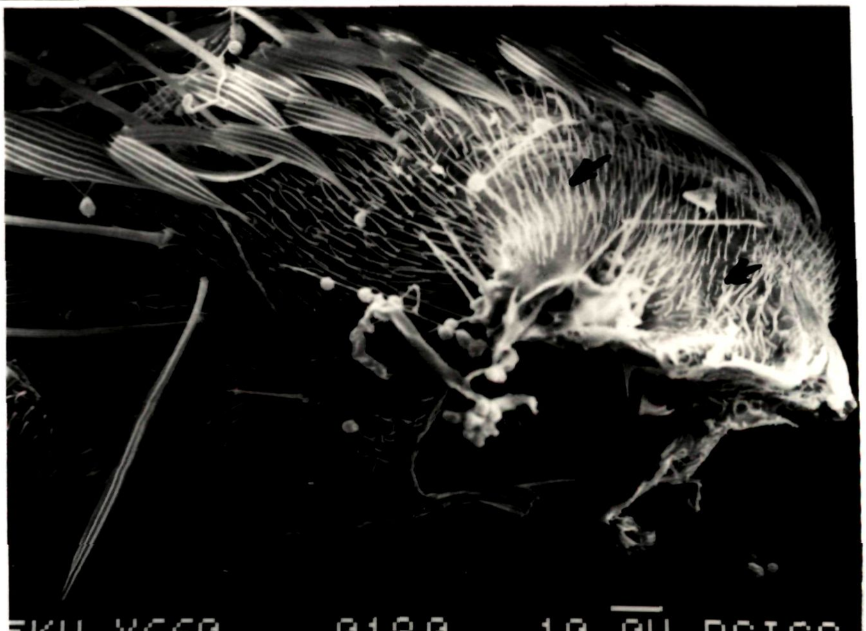
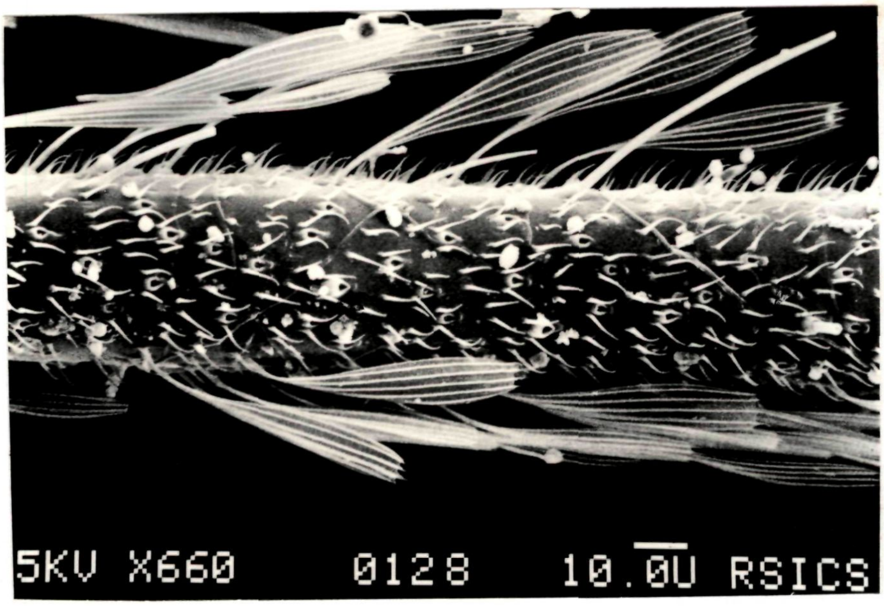

5KV X660 0180 10.0U RSICS

Fig.88(a) SEM picture of the surface of palpi of *A. vagus* showing the sensilla chaetica type-III x 660 µn.

Fig.88(b) SEM picture of the surface of abdomen (ventral) of *A. vagus* showing the sensilla chaetica type-III x 3600 µn.

Fig.88(c) SEM picture of the tarsal segment of meta-thoracic leg of *A. vagus* showing the sensilla chaetica type-III x 2600 µn.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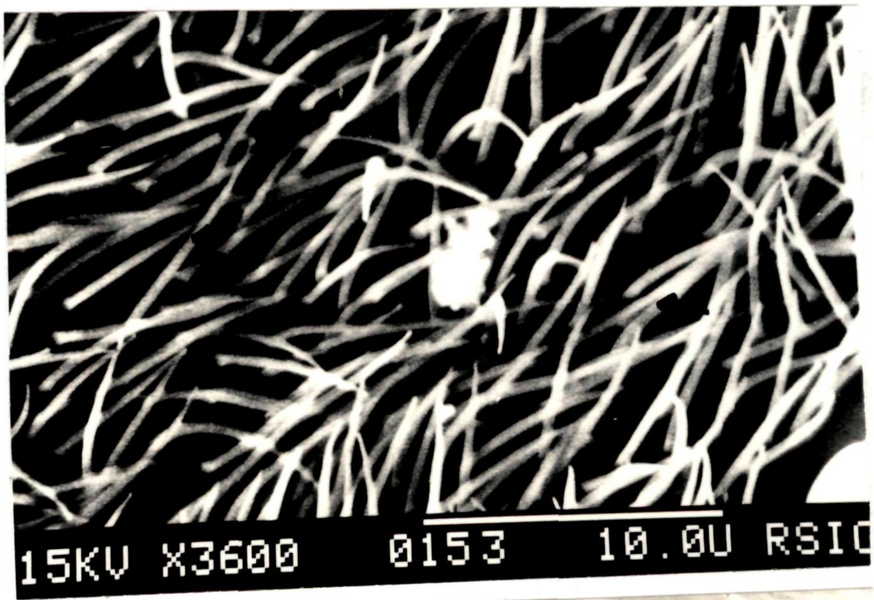
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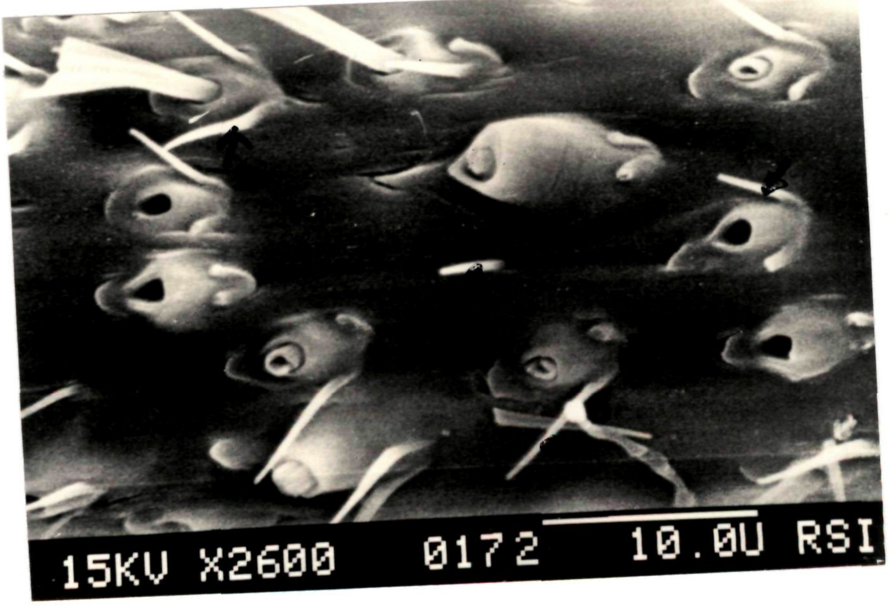
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Table-9(b)
Distribution Pattern and Dimension of Different Types of Sensilla Chaetica of *Anopheles vagus*

Sensilla	Location	Length (µ)	Breadth: Base (µ)	Breadth: Tip (µ)	Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
Type-I	1 Dorsal surface of of Abdomen	2.8 to 4	.4	.2 to .3	.5	Smooth	Innumerable	Socket-Round
Type-I	2 General surface of the wing	4.71 to 7.65	0.59	0.29	0.17	Smooth	Innumerable	Socket-Round
Type-I	3 Coxa of the Meta-thoracic Leg	5	0.31	0.16	0.63	Smooth	Innumerable	Socket-Round
Type-I	4 Trochontar of Meta-thoracic Leg	5.71	0.71	0.36	0.71	Smooth	Innumerable	Socket-Round
Type-I	5 Ventral view of thorax	1.67	0.21	0.10	0.38	Smooth	Innumerable	Socket-Round
Type-II	1 Coxa/Trochontar of Meso-thoracic Leg	0.71 to 4.28	0.29 to 0.43	0.14 to 0.28		Smooth	Innumerable	Pointed Tip and Non-socketted
Type-II	2 Coxa/Trochontar of Pro-thoracic Leg	3.71 to 5.71	0.26 to 0.36	0.18		Smooth	Innumerable	Pointed-Tip and Non-socketted
Type-III	1 Surface view of Palpi	5.71 to 30	0.71 to 2.14	0.35 to 1.43		Smooth	Innumerable	Blunt-Tip and Non-socketted
Type-III	2 Ventral Segment of Abdomen	5 to 6	0.38 to 0.75	0.25 to 1.75		Smooth	Innumerable	Blunt-Tip and Non-socketted
Type-III	3 Tarsal Segment of Meta-thoracic Leg	0.44 to 6.66	0.37 to 1.11	0.19		Smooth	Innumerable	Blunt-Tip and Non-socketted
Type-IV	1 7th Dorsal Segment of Abdomen	5	0.42	0.21	0.53	Smooth	Innumerable	Tip-Pointed and socket-round
Type-IV	2 Dorsal view of Thorax	3	0.21	0.11	0.23	Smooth	Innumerable	Tip-Pointed and socket-round

Sensilla	Location	Breadth			Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
		Length (µ)	Base (µ)	Tip (µ)				
Type-VII	1 Tibia/Tarsal Joint of Meta-thoracic Leg	37.5	1.88	0.63	3.62	Smooth	Innumerable	Rough-edges
Type-VII	2 Tibia/Tarsal of Pro-thoracic Leg	40.63	1.88	0.63	3.64	Smooth	Innumerable	Rough-edges
Type-VIII	Surface view of Probosis	1.89 to 5.44	0.33 to 0.44	0.11 to 0.5	0.55 to 1.22	Smooth	Innumerable	Tip-Portion Pointed

surface of the thorax (Fig.89b). These sensilla range from 3 m to 5 m in length, the width at the base is between 0.21 m to 0.42 m and those at the tip between 0.23 m to 0.53 m (Table-9b).

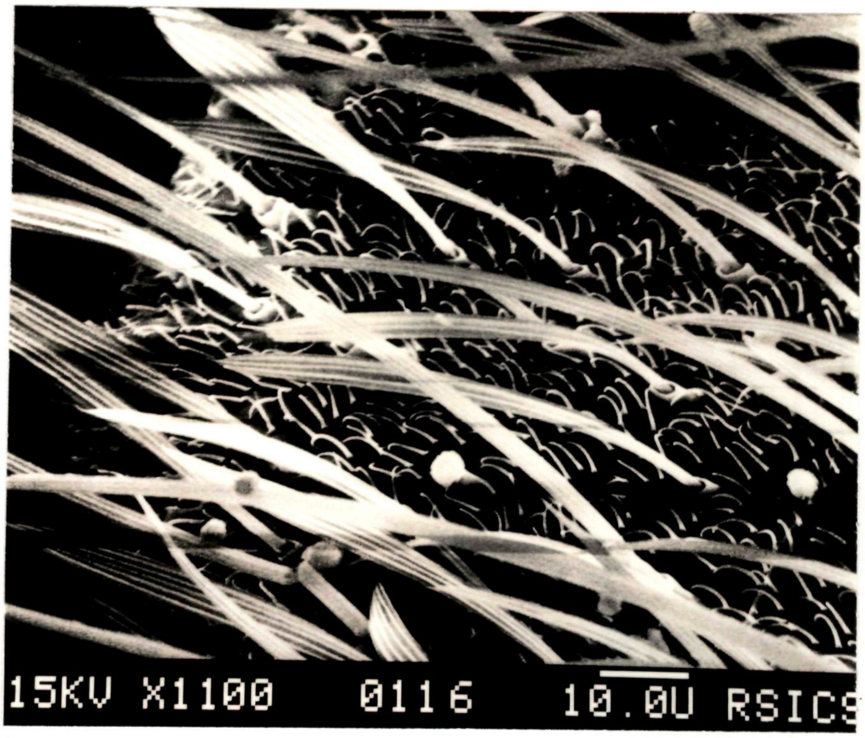
<u>Sensilla chaetica type-VII</u> : The shaft is smooth, socketted, pointed, and with rough edges. These sensilla are located on the tibia/tarsal joints of meta- and pro-thoracic legs (Figs.90a,b). These sensilla are long and large, and ranging in length between 37.5 m to 40.63 m, the width at the base is approximately 1.88 m and that at the tip at 0.63 m. The diameter of the socket range from 3.62 m to 3.64 m (Table-9b).

<u>Sensilla chaetica type-VIII</u> : The shaft is pointed socketted and the surface feature is smooth. These sensilla are located only on the surface of the probosis (Figs.91a,b). The length of the sensilla range from 1.89 m to 5.44 m, the width at the base vary between 0.33 m to 0.44 m and that at the tip between 0.5 m to 0.11 m. The diameter of the socket range from 0.55 m to 1.22 m (Table-9b).

Spinules : One types of spinules have been detected (Type-II) on the different locations of the body. The sub-types are recognised on the basis of their length, width at the base and tip, diameter of the socket and surface feature.

Fig.89(a) SEM picture of the last abdominal segment (Dorsal) of *A. vagus* showing the sensilla chaetica type-IV x 1100 µn.

Fig.89(b) SEM picture of the dorsal surface of thorax of *A. vagus* showing the sensilla chaetica type-IX x 1200 µn.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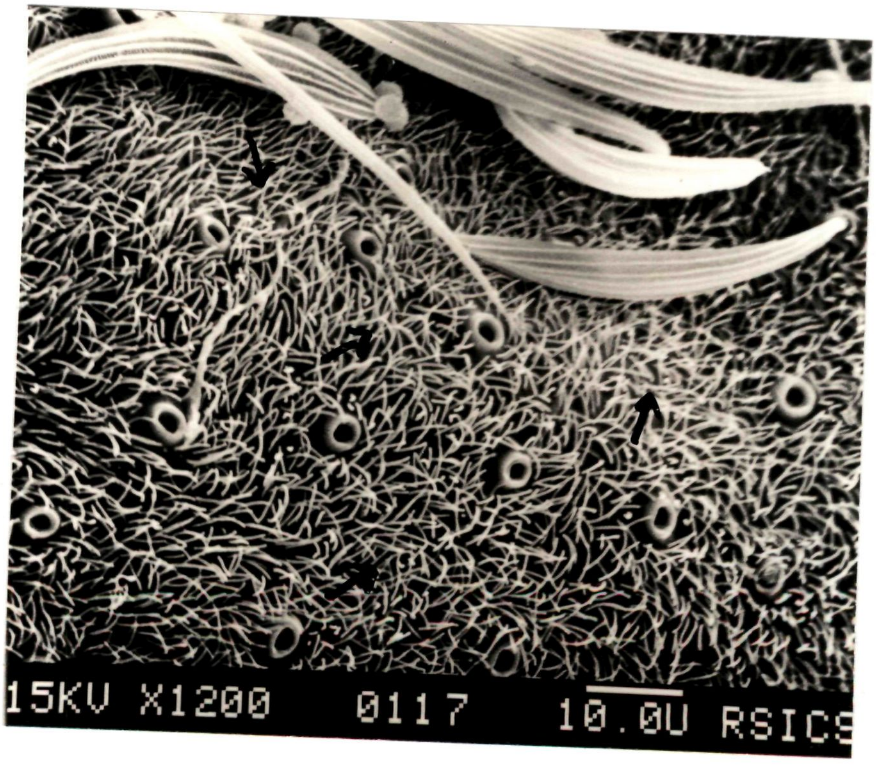
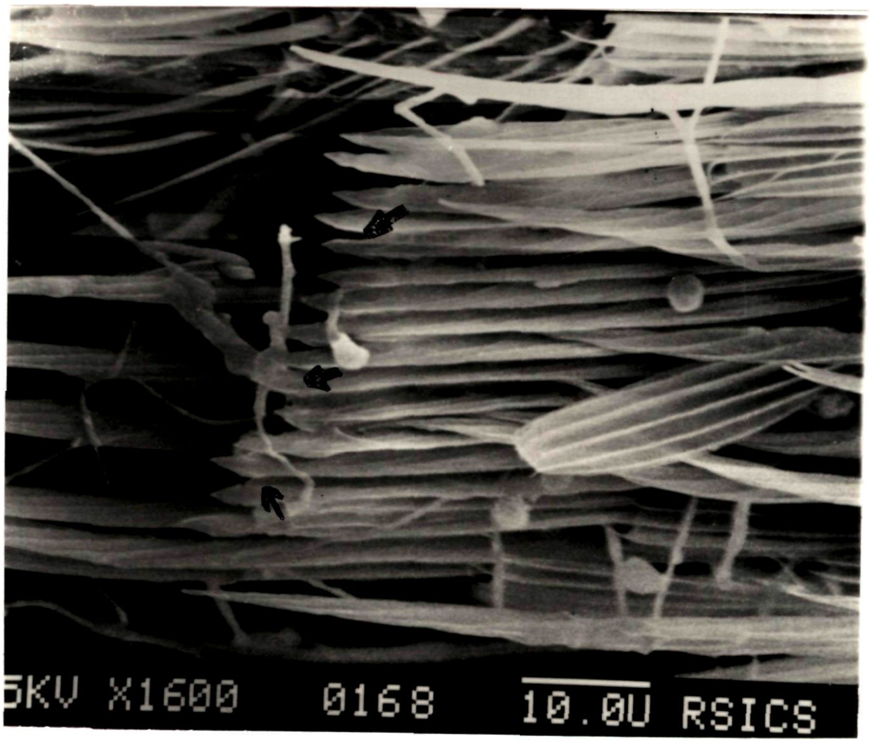

15KV X1200 0117 10.0U RSICS

Fig.90(a) SEM picture of the tibia/tarsal joint of metathoracic leg of *A. vagus* showing the sensilla chaetica type-VII x 720 μn.

Fig.90(b) SEM picture of the tibia/tarsal joint of prothoracic leg of *A. vagus* showing the sensilla chaetica type-VII x 1500 μn.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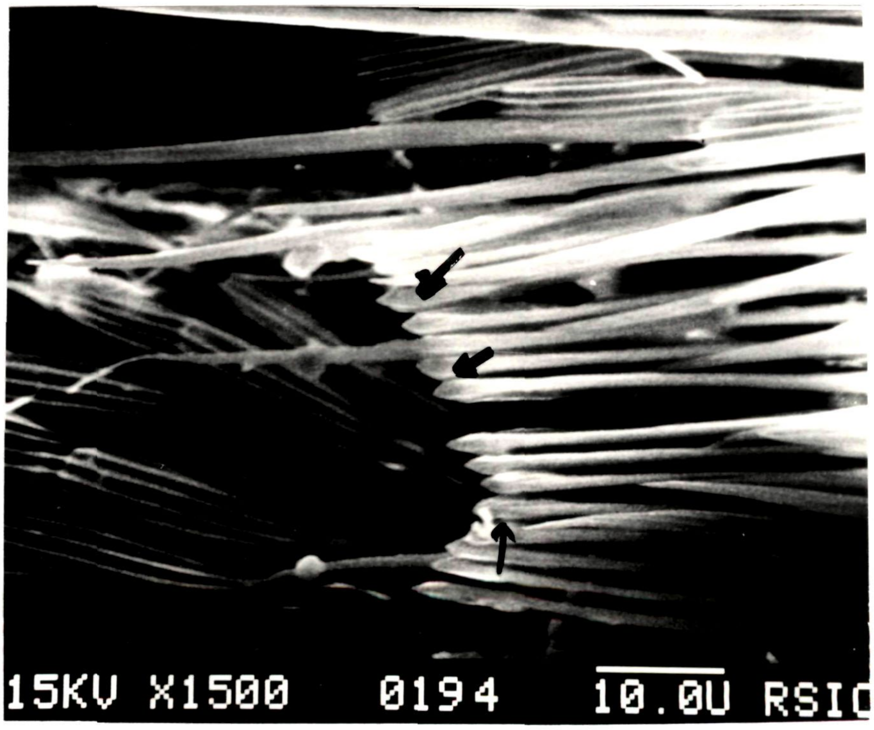
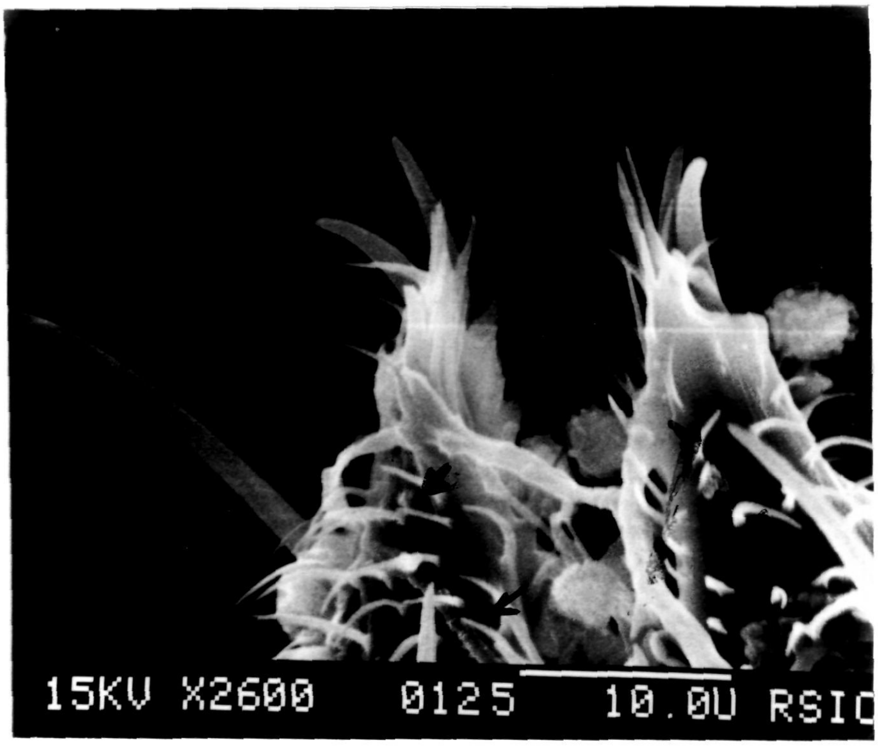

15KV X1500 0194 10.0U RSIC

Fig.91(a) SEM picture of the probosis of *A. vagus* showing the sensilla chaetica type-IX x 940 µn.

Fig.91(b) SEM picture of the probosis of *A. vagus* showing the sensilla chaetica type-IX x 940 µn (Enlarged).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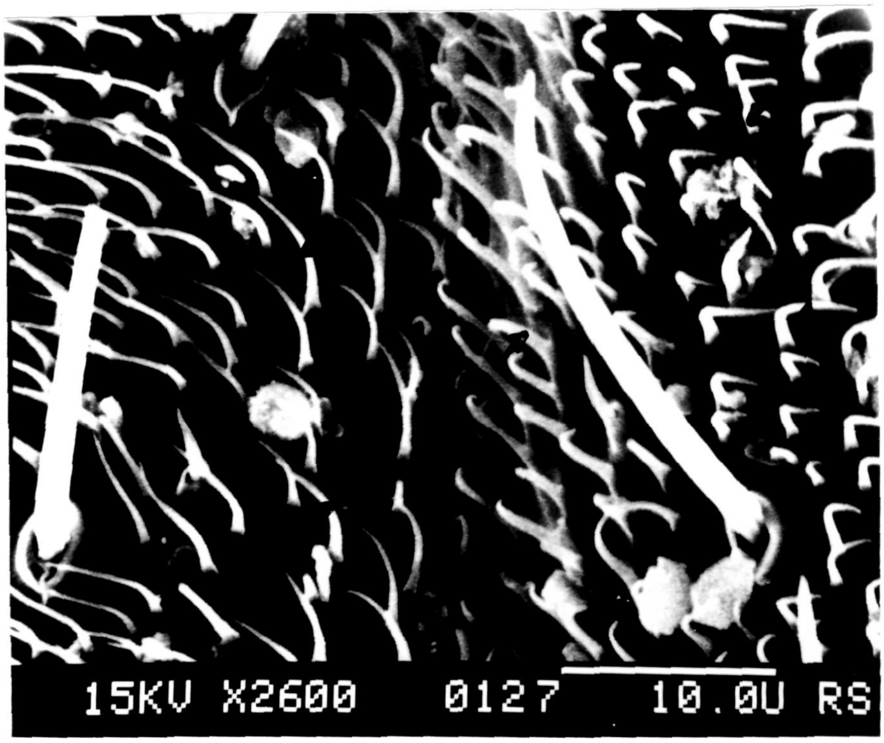

15KV X2600 0127 10.0U RS

Spinules type-II : The shaft is slightly tapered, non-socketted and surface feature is smooth. These sensilla are located on the tibia of meta-thoracic leg (Fig.92a), femur of meta-thoracic leg (Fig.92b), and femur of pro-thoracic leg (Fig.92c). These sensilla range from 2.86 m to 5.77 m in length, the width at the base vary between 0.36 m to 0.83 m and those at the tip between 0.18 m to 0.42 m (Table-9c).

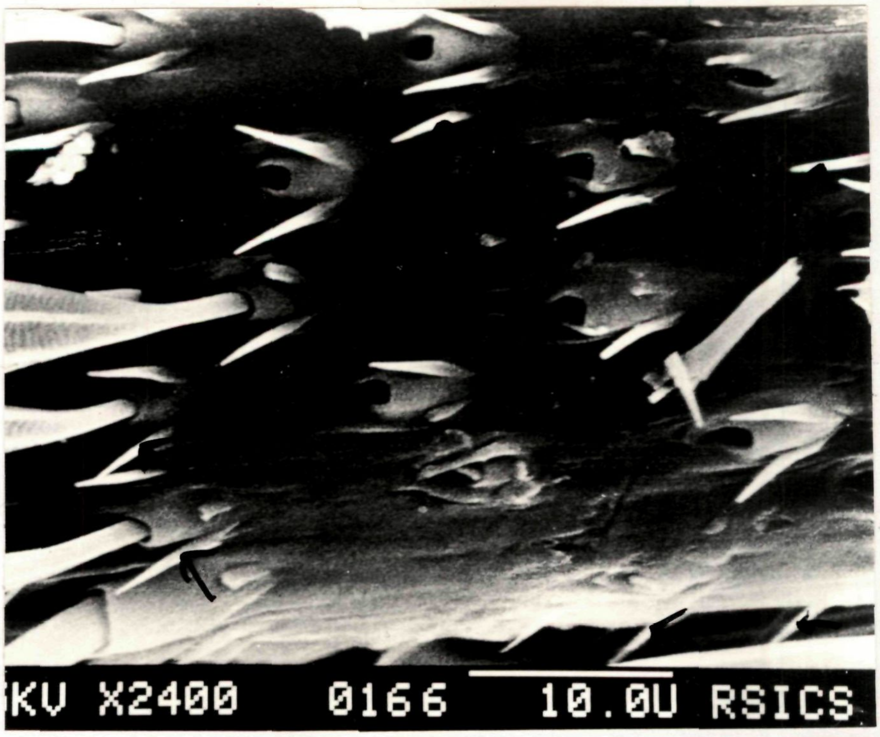
Hair-Plate : These sensilla have been detected on the coxa/trochontar of meta-thoracic leg, trochontar of the meso-thoracic leg, coxa/trochontar of pro-thoracic leg and the adjoining region of the wing to thorax. There are three types (Type-I, II, III) which are distinguished basing on the length, breadth at the base and tip, surface feature and the shape and diameter of the socket.

Hair-plate type-I : These sensilla are smooth, tapered, the hair-shaft point distally and is distinctly curved towards the anterior region of its length. The tip portion of the shaft is also round and the socket are absent. These sensilla is located only on the trochontar of meso-thoracic leg (Fig.93). The sensilla range in length from 5 m to 6.07 m, the width at the base at approximately 0.54 m and that at the tip at 0.36 m (Table-9c).

Fig.92(a) SEM picture of the tibia of meta-thoracic leg of *A. vagus* showing spinules type-II x 2400 µn.

Fig.92(b) SEM picture of femur of meta-thoracic leg of *A. vagus* showing spinules type-II x 2600 µn.

Fig.92(c) SEM picture of femur of pro-thoracic leg of *A. vagus* showing spinules type-II x 600 µn.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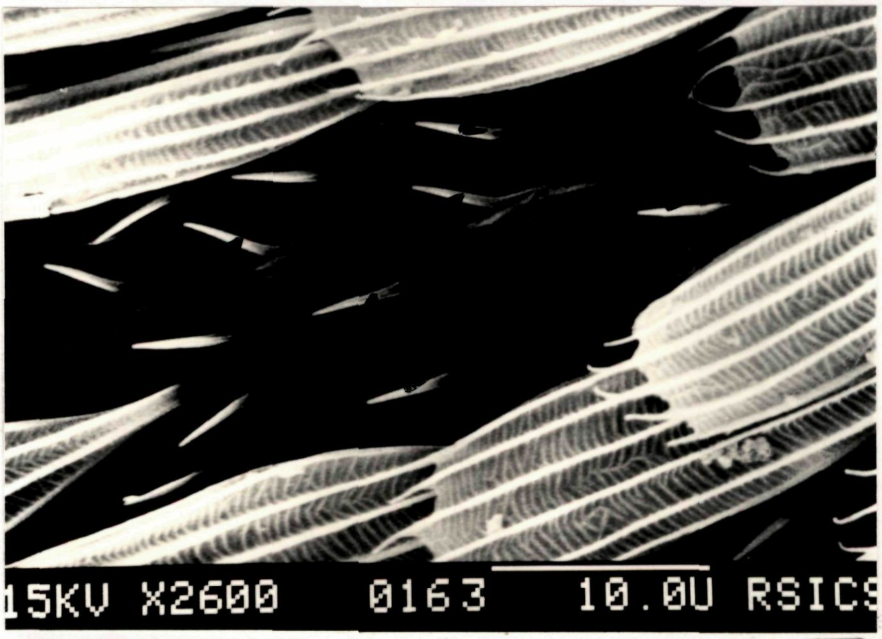

15KV X2600 0163 10.0U RSICS

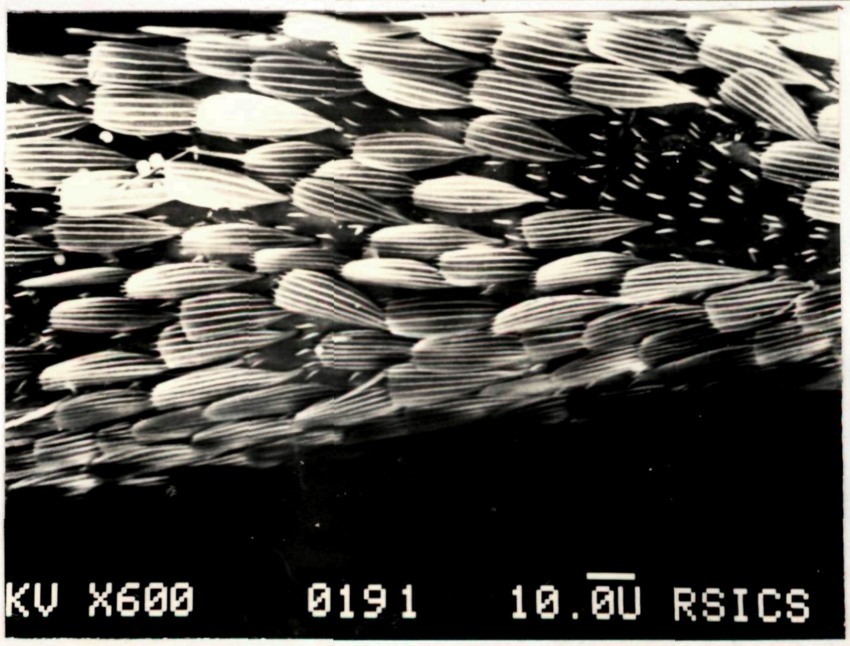

KV X600 0191 10.0U RSICS

Table-9(c)
Distribution Pattern and Dimension of Different Types of Sensilla of *Anopheles vagus*

Sensilla	Location	Breadth			Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
		Length (µ)	Base (µ)	Tip (µ)				
SPINULES								
Type-II	1 Tibia of Meta-thoracic Leg	3.85 to 5.77	0.58 to 0.77	0.38	1.15 to 1.54	Smooth	Innumerable	
Type-II	2 Femur of Meta-thoracic	3.75	0.36	0.18	1.07	Smooth	Innumerable	
Type-II	3 Femur of Meso-thoracic Leg	2.86	0.71	0.36	1.42	Smooth	Innumerable	
Type-II	4 Femur of Pro-thoracic Leg	5	0.83	0.42	1.67	Smooth	Innumerable	
HAIR PLATE								
Type-II	1 Coxa/Trochontar of Pro-thoracic Leg		2.14 to 2.86		4.29	Smooth	$4/mm^2$	
Type-II	2 Coxa of Meta-thoracic Leg	18.75	1.25	0.63	3.75	Smooth	$1/mm^2$	
Type-II	3 Joint Region of Wing to thorax	11.11	1.11	0.56	2.22	Smooth	$6/mm^2$	
BASICONICA								
Type-I	1 Antenna	10	1	0.5	3.68	Smooth	$1/mm^2$	
Type-II	1 Trochontar of Meta-thoracic Leg	3.71 to 12	0.86 to 1.43	0.29 to 0.43	0.57 to 1.43	Smooth	$8/mm^2$	
Type-II	2 Trochontar/Femur Joint of Meta-thoracic Leg	4.17 to 5	1.25	0.42	2.50 to 3.33	Smooth	$2/mm^2$	
Type-II	3 Coxa/Trochontar of Pro-thoracic Leg	12.86 to 15.71	1.43	0.36	2.86	Smooth	$2/mm^2$	

Sensilla	Location	Breadth			Diameter of the Socket (µ)	Surface Feature	Total Number	Any other special Characteristic
		Length (µ)	Base (µ)	Tip (µ)				
Type-III	1 Coxa/Trochontar of Meso-thoracic Leg	3.33	0.55	0.28	1.11	Smooth	$1/mm^2$	
Type-III	2 Tip of the Last Tarsal Segment of Pro-thoracic Leg	15	2	1	3.33	Smooth	$2/mm^2$	

Sensilla	Location	Diameter (µ)	Total Number	Any other Special Characteristic
CAMPANIFORMIA	1 Trochontar of Meta-thoracic Leg	4.29 to 7.14	16	Of the Sixteen, Six are having a Horse-Shoe Shape
"	2 Trochontar of Meso-thoracic Leg	2.86 to 7.14	15	
"	3 Coxa/Trochontar of Pro-thoracic Leg	4.29 to 5.71	15	

Hair-plate type-II : The shaft is tapered, smooth and distinctly form socket. These sensilla is localised on the coxa/trochontar of meta-thoracic leg (Fig.94a) and coxa/trochontar of pro-thoracic leg (Fig.94b). The sensilla is approximately 18.75 m, the width range from 1.25 m to 2.86 m and that at the tip is approximately 18.75 m, the width range from 1.25 m to 2.86 m and that at the tip is approximately 0.63 m. The diameter of the socket vary between 3.75 m to 4.29 m (Table-9c).

Hair-plate type-III : This sensilla is located only on the adjoining region of the wing to the thorax (Fig.95). It is among the largest with length ranging from 7.79 m to 111.11 m, the width at the base is approximately 1.11 m and the tip is 0.56 m (Table-9c).

Basiconica : Three types of sensilla basiconica (Type-I, II, III) were detected in various locations on the body surface of the species. The different types were differentiated basing on their length, breadth, shape and size of the socket and also the surface feature.

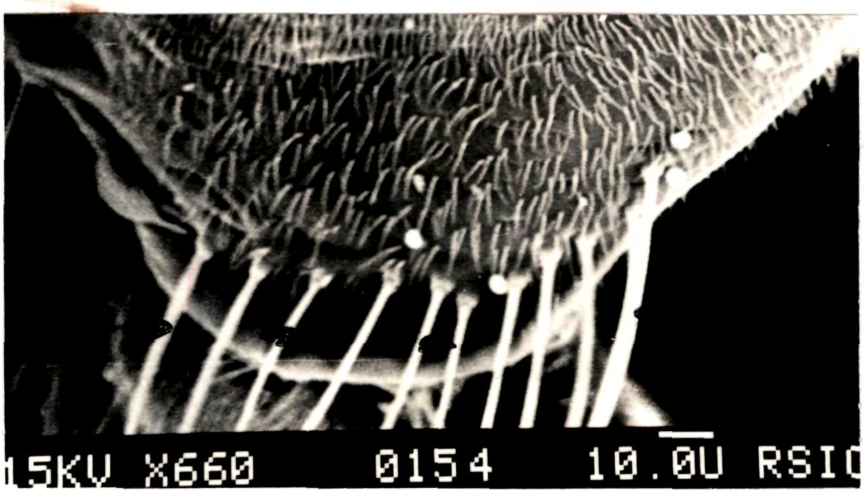
Basiconica type-I : The shaft project from a raised-based and is smooth and straight with a tapered tip. These sensilla is located on the surface of the antenna (Fig.96). These sensilla are approximately 10 m in length, the width at

Fig.93 SEM picture of trochontar of meso-thoracic leg of *A. vagus* showing the hair-plate type-I x 780 µn.

Fig.94(a) SEM picture of the coxa/trochontar of meta-thoracic leg of *A. vagus* showing the hair-plate type-II x 660 µn.

Fig.94(b) SEM picture of the coxa/trochontar of pro-thoracic leg of *A. vagus* showing the hair-plate type-II x 660 µn.

Fig.95 SEM picture of the adjoining region of the wing to the thorax of *A. vagus* showing the hair-plate type-III x 860 µn.



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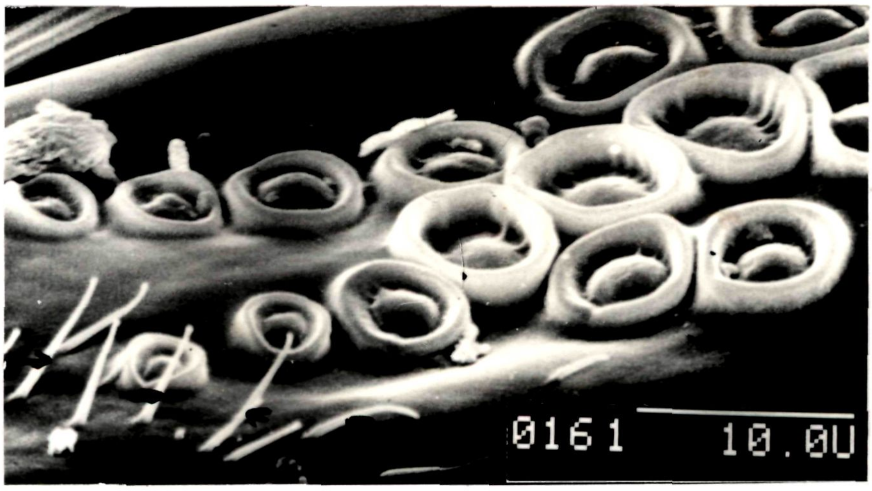
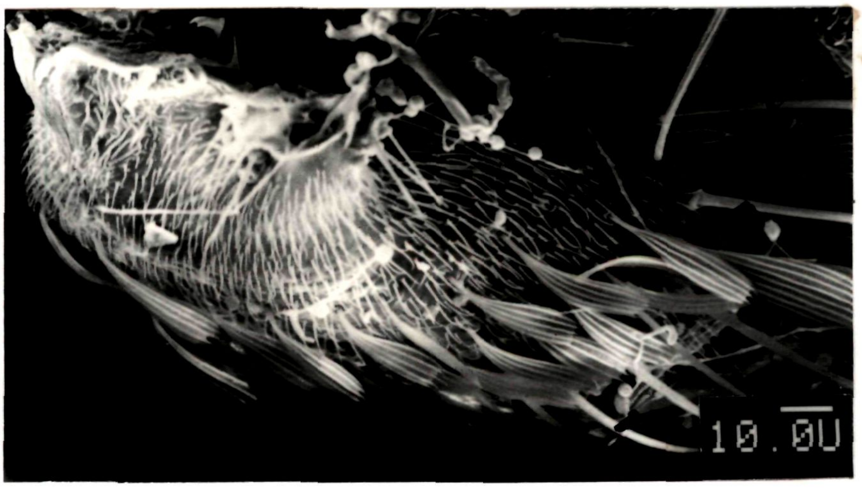


0161 10.0U



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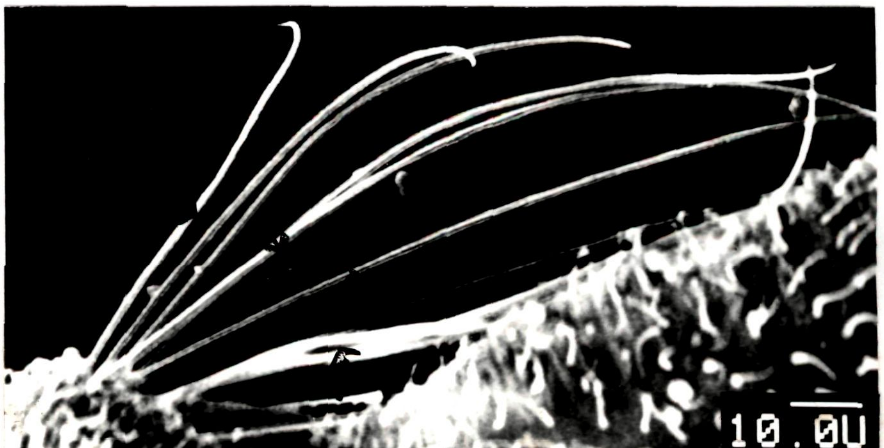


10.0U

the base is 1 m and that at the tip is 0.5 m. The basal diameter is around 3.68 m (Table-9c).

<u>Basiconica type-II</u> : The smooth, tapered shaft projects from a bulbous base. These sensilla are located on the trochontar of meta-thoracic leg (Fig.97a,b). These sensilla range in length from 3.71 m to 15.71 m, the width at the base vary between 0.86 m to 1.43 m and that at the tip ranging from 0.29 m to 0.43 m. The basal diameter vary from 0.57 m to 1.43 m (Table-9c).

<u>Basiconica type-III</u> : The shaft is smooth, socketted and with a blunt tip. The sensilla is located on the tip of the last tarsal segment of pro-thoracic leg (Fig.98). These sensilla are 15 m in length, the width at the base at 2 m and that at the tip at 1 m. The diameter of the socket is approximately 3.33 m (Table-9c).

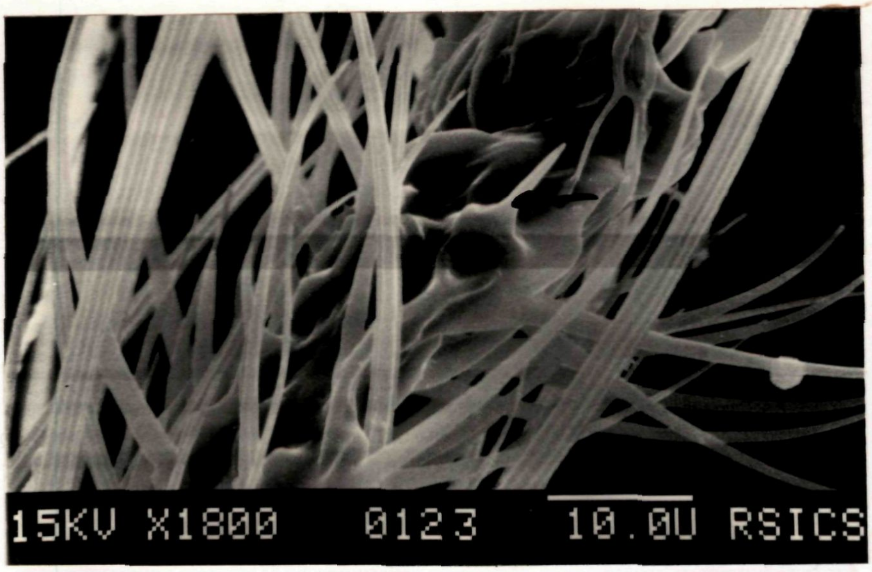
Campaniformia : The sensory structure are located on trochontar of meta-thoracic leg, trochontar of meso-thoracic leg and coxa/trochontar of pro-thoracic leg (Figs.99a,b,c). The diameter range from 4.29 m to 7.14 m.

Fig.96 SEM picture of the surface of antenna of *A. vagus* showing the sensilla basiconica type-I x 1800 µn.

Fig.97(a) SEM picture of the trochontar of meta-thoracic leg of *A. vagus* showing sensilla basiconica type-II x 660 µn.

Fig.97(b) SEM picture of sensilla basiconica type-II of *A. vagus* located on trochontar of meta-thoracic leg x 3200 µn (Enlarged).

Fig.98 SEM picture of the last tarsal segment of pro-thoracic leg of *A. vagus* showing the sensilla basiconica type-III x 940 µn.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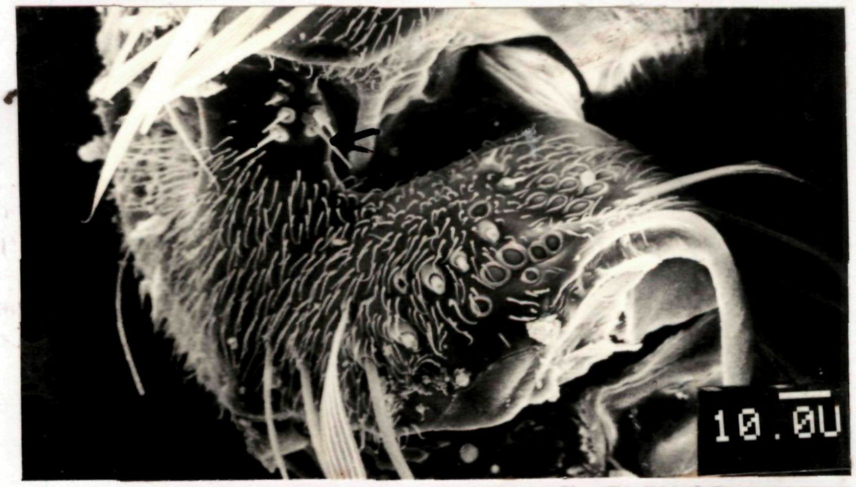
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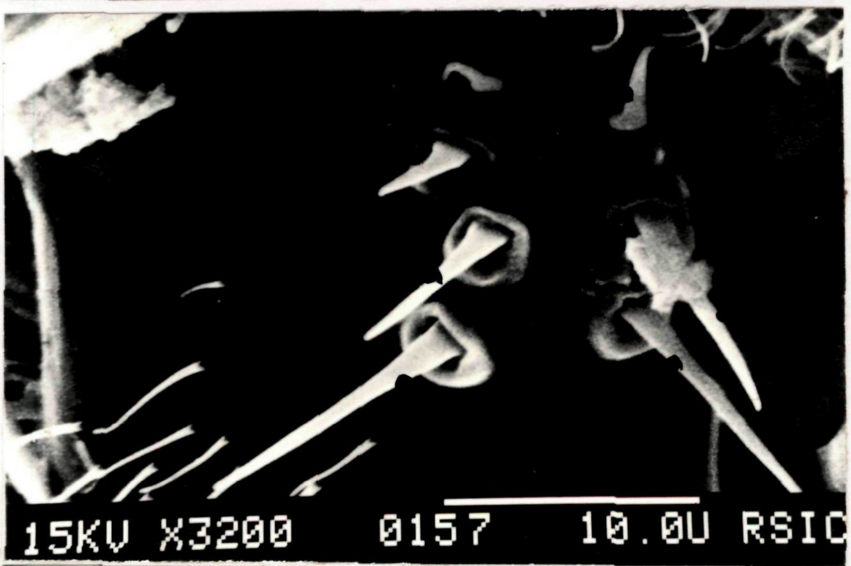
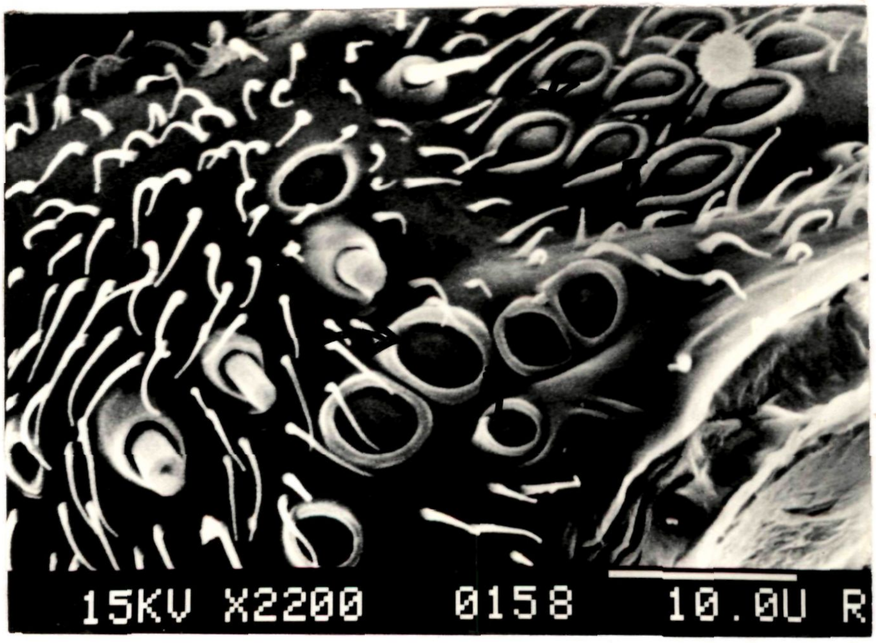

15KV X3200 0157 10.0U RSIC


10.0U

Fig.99(a) SEM picture of the trochontar of meta-thoracic leg of *A. vagus* showing the campaniformia x 2200 µn.

Fig.99(b) SEM picture of the trochontar of meso-thoracic leg of *A. vagus* showing the campaniformia x 2600 µn.

Fig.99(c) SEM picture of the coxa/trochontar of pro-thoracic leg of *A. vagus* showing the campaniformia x 660 µn.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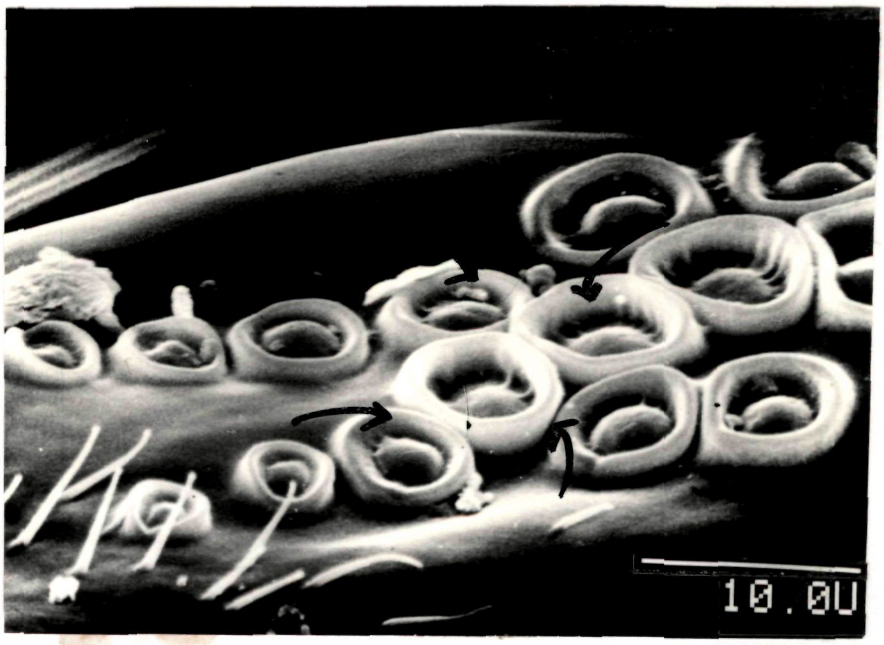
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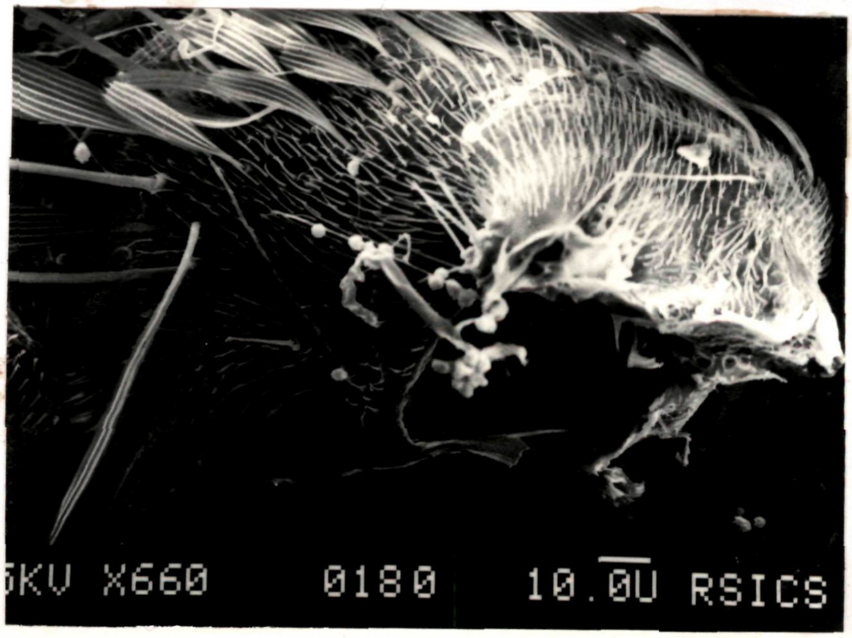
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DISCUSSION

The reaction of a complex animal to the environment, that is, its adjustments to external changes by movement or other forms of response activated from within, is dependent on two accessory conditions - (1) the outer surface must be in part at lease "sensitive" to environmental changes, and (2) the sensitive areas must be in connection with the motor mechanism. Insects depend to a large extent on their sensory system to locate their food sources, oviposition sites and mating partners. The behaviour physiologist seek to explain animal behaviour patterns in terms of physiological processes and activities. The analysis of environmental cues which elicit behaviour responses in animal has revealed that often a stimulus situation is characterised by one or a few simple factors. The function of different types of sensilla located on the body surfaces of *Anopheles annularis* and *Anopheles vagus* may be inferred by using the morphological criteria and also according to the results obtained by previous authors in other insects. The morphology and location of sensilla in general in other insects shows that they are highly specialised to meet the requirement for their adaptation to the micro-environment. The interesting observation about the sensilla of certain insects is that they possess some very long sensilla although their number in general are not much. Reduction in the number might have been compensated by specialization in position, directionality and structural features of individual sensilla in different parts of the body surface. The longest sensilla of *Anopheles annularis* range from 13.75 µ to 145 µ and that of *Anopheles vagus* from 5.5 µ to 194.3 µ. In contrast to this, the sensilla of other insects such as *Apis* is reported to be about 10 µ to 170 µ (Lindauer and Nedel, 1959; Market, 1962); that of *Periplaneta* about 30 µ to 50 µ (French and Wong, 1976); that of *Drosophila* about 80 µ long (Singh and Nayak, 1985); that of *Heliathis* from 80 µ to 165 µ (Baker and Ramaswamy, 1990), and that of *Graphium sarpedon* from 150 µ to 416 µ (Dey *et al.*, 1995). The arrangement and size of the different types of sensilla located on the different regions of the body surface of both species (*Anopheles annularis* and *Anopheles vagus*) remain more or less constant with very little individual variations. However, since the present study was confined to the sensilla of female member, it is not known how the sensory system is modified in male for specific purpose. But it is expected that there may be significant differences in the occurrence and distribution of sensilla between female and male of both the species (*Anopheles annularis* and *Anopheles vagus*), particularly in the tarsi, mouth parts (Ichinose and Honda, 1978; Chun and Schoonhoven, 1973; Kusumi and Shibuya, 1989; Altner *et al.*, 1985; Sutcliff, 1994), which will be taken up in detail in our future studies.

Sensilla trichodea : There are thirteen types of sensilla trichodea detected on the body surfaces, of which, type-I to XI (eleven types) are located on the *Anopheles annularis*, and five types (Type-I, III, IX, XII and XIII) were detected on the *Anopheles vagus*. The different types were differentiated depending upon the length, the sculpturing pattern of the surface, shape and size of the socket, and the diameter of the base and tip. As pointed out by the different authors that the flexible base and the structural features of the sensilla indicate that they function as mechano-receptors (Thum, 1964, 1965; Gaffel and Handers, 1972; Gnatzy and Tautz, 1980; French and Sanders, 1981; Altner *et al.*, 1985; Dey *et al.*, 1995). In certain insects the sensilla trichodea are known to occur over the entire body and are devoted to the reception of simple touch. However, different types of sensilla trichodea may perform different functions. As for example in honeybee antenna, they are used to sense surface thickness; in many insects the sensilla trichodea constituting the hair-plate in the joint region of leg, antenna and neck act as proprioceptors or position detectors. In *Annopheles annularis*, the sensilla trichodea types-I, II, III, IV, VI, VII, VIII and in *Anopheles vagus*, the sensilla trichodea types-I, III, XIII, probably play the role of mechano-receptor. This can be inferred from the structural feature of the sensilla and also the distribution pattern. Since these sensilla are not group as in the case of hair-plate, it is expected that they are phasic in their response to mechanical stimuli (Schmidt and Smith, 1987). Apart from its mechano-sensory role, sensilla trichodea representing type-II located on the tibia/tarsal joint of pro-thoracic leg (Fig.45) and types III and XIII located on the tibia/tarsal joint of meta-thoracic leg (Figs.36,85), probably also function as a proprioceptor that inform the insect of the relative positions.

The sensilla trichodea representing type-V, located on the third tarsal segment of pro-thoracic leg (Figs.48a,b,c) and the type-IX located on the first tarsal segment of pro-thoracic leg (Figs.52,83) possibly are the tactile receptor. Each sense organ consist of a hinged-hair, 13.75 μ long, 28.21 μ long and 25.26 μ to 27.78 μ long, and probably with a nerve fibre ending on the hinge, flanked by five foliate (Figs.48,a,b,c) and two foliate (Figs.52,83) outgrowth of the cuticle. Although transmission electron microscopy of the same has not been done, from the morphological similarity of the structure with that of similar sensilla (Menon, 1951; Halcrow, 1953), suggest the existence of nerve-connection. These hairs are said not to come into contact with the surface except when the insect on water.

The sensilla trichodea types-X, XI, XII localised on the probosis, palpi and antenna (Figs.53;54a,b,c,d,e,f;84a,b) appears to play a chemo-sensory function. The shape of the sensilla, the structural feature of the socket, the length of the hair and location (Tables 8a,9a) suggest that they may also function as the chemo-sensory organ (Zacharuk, 1985; Stadler, 1984; Faucheux, 1991).

It is generally true that identifiable differences in morphology correspond to differences in sensory function (Sutcliffe, 1994). Thus, the variety of sensilla trichodea in mosquitoes may probably serve many specific sensory functions. Number of physiological studies that have been done on certain species of mosquitoes confirmed that some of the sensilla trichodea types and sub-types respond to various stimuli. Briefly, apart from its mechano-sensory function, sensilla trichodea of certain types, have been found to respond to oviposition site-related compounds (Davis, 1976; Bently *et al.*, 1982), essential oils (often associated with nectar sources - Lacher, 1967; Davis, 1977), fatty acids and oils associated with skin, and to certain repellents (Lacher, 1971; Davis and Robert, 1972). Interestingly, none of the sensilla trichodea have been found to be sensitive to lactic acid (Sutcliffe, 1994).

Sensilla chaetica - The sense organs included here are merely trichoid sensilla in which the external process is bristle-like or spine-like rather than typically setiform.

The presence of sensilla chaetica type-I in the wing surface, coxa/trochontar of meso-thoracic leg and trochontar/femur joint of pro-thoracic leg, and the sensilla chaetica type-IV located on the dorsal surface of the head, thorax and abdomen on both the species - *Anopheles annularis* and *Annopheles vagus* may probably play a mechano-sensory role. The morphological feature are consistent with the "flexible socket" - mechano-sensilla of insect as reviewed by Altner and Loftus (1985).

The sensilla chaetica type-II located on the trochontar of meta-thoracic leg in *Anopheles annularis*, and on the coxa/trochontar of meso- and pro-thoracic leg in *Anopheles vagus*, are unsocketted hairs, the precise function of which is not known. However, they may play some mechanical role since they have been shown to be non-innervated (Schmidt and Smith, 1985c). Another role of these unsocketted hairs in the legs may be to prevent the leg surface from adhering to wet or sticky substances and also to prevent the legs from adhering to each other.

In both the species (*Anopheles annularis* and *Anopheles vagus*), the sensilla chaetica type-III found on the tarsal segment, femur, palpi, ventral surface of abdomen, coxa and trochonta, appears to be mechano-sensory as well as contact chemo-sensory in nature. In this context it is to be noted that the sensilla chaetica of this type in a wide variety of insects representing different orders have been shown to be contact chemo-sensilla (Kaestner, 1972; Wigglesworth, 1972; Horn, 1982). Further, it has been shown that tactile hairs especially those located on the tarsal segments can mediate tibial reflexes (Runion and Usher Wood, 1968). Whereas, sensilla chaetica of this type which are mechano-sensory in nature probably play the same role in getting information about contact with substrate and also the surface texture. The contact chemo-receptors may help the insect in the perception of the host and may respond specifically to chemical odour from host to non-host (Roessingh *et al.*, 1991; Colvert, 1974). In many cases these receptor also response to the presence of dissolved carbohydrates (Anderson, 1932; Marshall, 1935; Dethier, 1955; Handen and Heuman, 1971) and are also involved in feeding response of the insects. In both the species (*Anopheles annularis* and *Anopheles vagus*) sensilla chaetica type-III are well developed and their position suggest that they are in contact with the substrate when the insect walks or rests. Thus, they may be essential to the insect apart from the obvious mechano-sensory role since they may also function as contact chemo-receptor, such as gustatory sensilla (Smith *et al.*, 1976; Hatfield, 1976). In this context it is worth mentioning that the contact chemo-reception of this type of sensilla has been demonstrated in some lepidoptera species located on the tarsi (Mitchell and Seabrook, 1974; Anderson and Hallberg, 1990).

Sensilla chaetica type-V, VII which was observed in tibia-tarsal joint of pro- and meta-thoracic leg in both the species - *Anopheles annularis* and *Anopheles vagus* is quite interesting as far as its structural characteristic are concerned. The apex of the sensilla is pointed and spear-shaped. This structural peculiarity of the sensilla and its occurrence only in the pro- and meta-thoracic legs suggest that it may play some specific role. In this context it may be noted that the legs of most insects play an important role in grooming behaviour (Tembrook, 1982). In case of some hymenopterans the legs are modified for grooming and they are involved in cleaning the antenna, abdominal tip region and the wing surface. The presence of sensilla chaetica type-V in pro- and meta-thoracic legs and its absence from meso-thoracic leg further supports its involvement in grooming behaviour.

The external morphology, general distribution and relatively large number of the sensilla chaetica type-VI and VIII on both the species - (*Anopheles annularis* and *Annopheles vagus*) and its location on the probosis, palpi and antenna, are indicative of a role as tactile mechano-sensilla. The tactile mechanism, however, may serve in a capacity that is not functionally one of touch. Static reactions in arthropods, for example, are brought about through organs that are essentially tactile in structure. Organs of this kind have been described in other coleopteran species, for example, the sensilla chaetica of *Oryzaephilus surinamensis* (L.) as reported by Arbogas *et al.* (1972).

Sensory peg - Sensory pegs are innervated hairs reduced in size, and there is no sharply dividing line between sensilla basiconica and sensory peg, either in the character of the external morphology or in the anatomy. In a typical sensory peg, the structure are the thin-walled hair and their function are possibly olfactory chemo-receptors, or may be sensitive to water-vapour (Christopher, 1960).

Spinules - These are the various hair sense organs which are generally classified anatomically according to the structure of the external process, on a more fundamental and apparently physiological basis, they may be separated into two groups, according to whether the sensillum contains a single sense cell or a group of sense cells. The spinules type-I and II (Figs.62,63,92), located on the femur of pro-, meso-, and meta-thoracic legs, are the organs of single sense cell and evidently their function could have on a mechanical function in stimulating the nerve. Whereas, the type-III

(Fig.64) that occurs in group or clusters, may be pervious to odor or taste (Schneider, 1923).

Hair-plate - The different types (Type-I, II and III) of hair-plate on both the species (*Anopheles annularis* and *Anopheles vagus*) located on the tip of the last tarsal segment, coxa/trochontar of meta-thoracic leg, pro-thoracic leg, ventro/lateral region of thorax and the adjoining regions of the wing to the thorax probably function as a proprioceptors that inform the insect of the relative position (Schmidt and Smith, 1987).

Basiconica - The external feature of these sensilla is somewhat peglike or conical structure. Judged from morphological features of the sensilla basiconica type-I located on the antenna of both the species (*Anopheles annularis* and *Anopheles vagus*) they are usually considered to be chemo-sensilla, or olfactory receptors as reported by Bland (1983) and Smith *et al.* (1976), respectively. The sensilla basiconica type-II and III located on the trochontar of meta-thoracic leg and tip of the last tarsal segment of pro-thoracic leg in *Anopheles vagus*, appears to be involved as in olfactory sensilla for host recognition Dyera and Seabrook (1975) and Smith *et al.* (1976). From the behavioural studies on *Oryzaephilis surinamensis* (L.), Arbgast *et al.* (1972) also reported that sensilla basiconica of these types, may have a hygro-receptive function. The electrophysiological studies of such sensilla of *Thanasimus formicarius* (L.) also suggest it to be olfactory chemosensilla (Hansen, 1983). It is to be noted here that the sensilla basiconica type-II and III, both of which are probable olfactory chemo-sensilla are similar in external appearance and distribution to the long sensilla basiconica of flea-beetles (Smith *et al.*, 1976; Alm and Hall, 1986).

Campaniformia - The sense organs included in this class have been called vesicles, organs of Hicks, papillae, cupola organs, dome organs, umbrella organs, bell organs and sense pores. For this reason the organs are appropriately termed in general as sensilla campaniformia (Berlese, 1909). The external parts of the campaniform organs are, in some cases, small dome-like papilla but slightly convex swelling (Fig.68b) usually about 7.89 μ in diameter. Others are minute disc, slightly sunken into the body wall, resembling the surface view of vacant hair fellicks (Fig.99c) usually about 4.29 μ diameter, though they are usually distinguished from the cuticular hair sockets by a more elliptical or oval form. Newton (1931) express the opinion that these sense cells are both chitinogenous and receptive in function. Various investigators, however, have found traces of other cells in the campaniformia sensilla, and Sihler (1924) describes in *Periplanata* a large cell ending in the canal of the cuticula, which is traversed by the distal process of the sense cell. McIver (1985) and Pringle (1938a), pointed out that the sensilla campaniformia which are located on the various parts of the insects body, such as wings, ovipositors, antenna, mouthparts, legs, etc. results from cuticular stress due to compression and stretching of the surrounding. The function of the sensilla campaniformia is a controversial point and opinions differ in this. McIndoo (1914, 1915) has given much experimental reasons for believing that the organs are receptors of odor stimuli. However, in the later years, different authors stress that the functions are size dependent effect on the sensitivity of the receptor (Pringle, 1938; Moran and Rowley, 1975). In both the species (*Anopheles annularis* and *Anopheles vagus*) this sensilla appears either singly or in groups of 5 to 11 and of different shape and size, which can be correlated to the size dependent effect on the sensitivity of the receptor. The campaniformia sensilla is believed also to possess the directional sensitivity which has been described by Pringle (1938); Chapman (1965); Thurm *et al.* (1975); Heinzel and Gewecke (1979); Zill and Moran (1981). The more or less circular shape of the sensilla in the present case indicates that it responds to stress in all directions. The position and orientation of the sensilla on the coxa/trochontar of *pro-, meso-* and *meta-thoracic* legs, as observed in the present study, suggest that they monitor strains that develop during dorsal bending of the tibia and femur. In addition, campaniformia sensilla could also provide information about muscular force required to maintain a fixed relation between body parts despite changes in the insects' position relative to gravity (Horn, 1985).

Pit Sensilla - Cuticular pits or pits organs are opening on the cuticular surface. In the present study, the cuticular opening distributed over the surface of the antenna are similar to the cuticular openings as reported by Alm and Hall (1986) on *C. nenuphar*, the epidermal duct openings by Bland (1983) on *H. pestica* and the hypodermal gland pores by Moeck (1967) on *Trypodendron lineatum* (Oliver). It is unlikely that these cuticular openings are pore or wax canals, since Scheie *et al.* (1968) have confirmed with the help of scanning electron microscopy, that pore canals or wax canals themselves do not open out onto the cuticle surface. Pore canal and dermal gland duct opening are, however, seen on the inner surface of the cuticle but not on the outer surface. The cuticular pits may be either sensilla ampullacea or sensilla coelonica. This differentiation can be achieved through structural studies on sensory peg within the pit (Dietz and Humphreys, 1971) which are different in sensilla ampullacea and sensilla coelonica. In our present study scanning electron microcopy of the pit organ could not reveal enough of the peg to differentiate between sensilla ampullacea and sensilla coeloconica. However, it is known that pit organs function as carbon-dioxide, temperature and humidity receptors in some hymenoptera (Lacher, 1964). Similar roles can be assigned to the cuticular pits located on the antenna of both the species (*Anopheles annularis* and *Anopheles vagus*).

SUMMARY

Mosquitoes are known to be the best group of insect because of their importance to man as pest and vector of the most distressing disease, and the literature on them is extremely large and varied. But there is a paucity of information on the biology and the ultrastructural studies of indigenous mosquitoes in the highlands of the North-Eastern Region of our country. Such a knowledge, is of utmost importance not only from the academic view point, but also in its utility in the ever-increasing technological efficiency for evolving judicious control measures. The state of Meghalaya which lies in between Brahmaputra Valley of Assam in the north and the Surma Valley of Bangladesh in the south, comprises mostly of hilly terrains, offers an excellent scope for studies of available mosquito species. The traditional practice of spraying D.D.T. for its control is part of the stresses to which fauna of this region are usually subjected to.

The present thesis highlights certain aspects of the biology and also the ultrastructural studies of two individual species, *Anopheles annularis* and *Anopheles vagus*.

It is known that environment plays a great role on the life of insects. Therefore, such a study at Byrnihat area with its geographical location at 26°58"N 91°53'02"E and the altitude of 182 meters mean sea level, helps us to understand and acquire precised information on the manner in which they survive in diverse eco-system.

The data for the present study were collected from May 1992 to June 1994. In Byrnihat area the various climatic factors, viz. rainfall, air temperature - maximum, minimum, humidity, average sunshine hours, total sunshine hours and wind velocity, fluctuated in the range of 0.0-620 mm, 13°-29°C, 11°-28°C, 60.0-86.0%, 1.20-8 hours, 100-250 hours and 2-6.20 km./hr. respectively.

Physiographically, the landmasses are mostly lateritic type derived largely from granite, schist rocks and vary from sandy red and clay-loamed to sandy lime. Lithologically, the Byrnihat area is mainly sandy type and includes green stones, granites and upper cretaceous sandstones. The soil in the basin are more fertile than the upland soils since much of the bases and organic matter from the top soil of the latter gets washed away due to high rainfall.

The natural forest in and around the Byrnihat area is now confined to small patches. Whereas, large one of this region is under secondary growth of forest on the abandoned *jhum* land. The degree of disturbance and species composition of two forest stands showed great variations. The overall practice of agricultural activities are dependent on rainfed agriculture.

In Byrnihat area, the various physico-chemical variables, viz. water temperature, pH, carbon-dioxide, specific conductivity, total alkalinity, phosphate-phosphorous, Ammonia nitrogen concentration, oxidisable organic matter fluctuated in the range of 11°-31°C, 5.8-7.8, 1.5-5.0 mg/l, 21.2-62.3 µmho/cm, 15.1-50.0 mg/l, 0.23-1.30 mg/l, 0.052-0.097 mg/l and 2.62-8.6 mg/l.

Information on the occurrence and characteristic of the miscellaneous habitat apart from the rice-field and pond form an interesting observation. These miscellaneous habitats are found to serve the purpose of breeding. These accessory structure are the depressions provided in nature by the process of denudations by the active weathering and various human activities, the presence of these habitat thus becomes an added advantage to the mosquitoes population.

Monthwise averages of the larvae obtained during the period of investigation, reveals that there is close variations on both the habitats, viz. rice-field and miscellaneous structure. The average number of larvae collected from the rice-field vary between 1.5-32.6 and that from the miscellaneous habitats the range occurs between 1-41. The analysis of variance carried out specifically for the miscellaneous habitat reveal no significant interaction between villages and months, but a significant effect of months. And consideration at the individual levels shows that in all the stations a significant interaction of both months and field.

The trend of the relative abundance of *Anopheles* adult at Byrnihat area revealed that preferred abundance of the diversity vary from season to season, exhibiting a significantly positive correlations with rainfall, temperature, and humidity. The total population of *Anopheles* species collected exhibit a seasonal fluctuation with the maximum at 280 (10.82%) in the month of May and minimum of 21 (0.81%) and 22 (0.85%) in the months of December and February. The total number of individual *Anopheles* species also revealed the occurrence of a maximum of 20 species in the month of June and a minimum of 5 species in the month of December.

The monthly total Man Hour Density (MHD) of the species occurring exhibit a maximum in May (61.50) and a minimum in December (4.13) respectively. And the total Man Hour Density of the individual species which shows the maximum are that of *Anopheles phillipinensis* (3.2), *A. vagus* (2.32) and *A. hyrcanus* (2.15), whereas the minimum are that of *Anopheles varuna* (0.06), *A. subpictus* (0.09) and *A. stephensi* (0.11), respectively.

Considering the collections of adults at the individual level, each of the four stations has also been analysed separately to determine the pattern of changes from month to month. Apart from the differences in the total number collected, the total number of species occurring also shows a variation among the individual stations. At Harilibhogan, the total number of species occurring varied from 15 types in the month of May and 4 types in the months of December and February. At Norbong the fluctuation range shows a maximum of 16 species in the months of May and August and a minimum of 3 species in December. Whereas, at Garo-basti and Amjok the occurrence revealed a similar pattern, with 16 species in the month of August and a minimum of 3 species in the month of December.

The monthwise total Man Hour Density (MHD) also shows a similar pattern with wet season maxima and dry cool seasons minima in all the study area, exhibiting two peaks of abundance, viz. May and August in all the stations corresponding to the main rice plantation season and also a heavy rainfall season. Considering the individual stations separately, the range of variation are Harilibhogan (54-3), Norbong (73-5), Garo-basti (59-3) and Amjok (60-4), respectively.

The Man Hour Density (MHD) of the individual species at every station also explained a variation in numbers among the species and also from one station to another. The range of variation at Harilibhogan are 3-0.03, Norbong 4.5-0.03, Garo-basti 3.4-0.06 and Amjok 3.6-0.09, respectively. Thus, comparatively, Norbong and Amjok support quite a large number of *Anopheles* collectively and also the individual *Anopheles* species throughout the annual cycle though there exists seasonal fluctuation also.

The external morphology of various types of sensilla located on the body surface of the female mosquitoes of two dominant species - *Anopheles annularis* and *A. vagus*, reveal their overall behavioural and adaptability qualities. The different types of sensilla differ with the functions they are responsible for. Sensilla trichodea, chaetica, sensory pea, spinules, hair-plate, basiconica, and campaniformia, etc. with different shapes and sizes and numbers plus the surface feature are present in the mouth parts, wing, head, thorax, adbdomen and on the pro-, meso- and meta-thoracic legs of both the species. This arrangement also varies between the two species - *Anopheles annularis* and *A. vagus*. In other words, these sensilla are also known as chemoreceptors, photoreceptor, mechanoreceptors and preprio-receptor, depending upon their respective function. Mosquitoes (*Anopheles annularis* and *A. vagus*) behaviour and adaptability mainly depends on the alertness of these receptors. Thus, it can be said, that the body of the mosquitoes are covered with innumerable hairs and that it is difficult to disturb the insect without the reaction of these hairs, which will instead lead to the response and alertness of these animals. That is why they are considered to be very intelligent due to their sensitiveness and their fast response to any stimuli applied to or against them.

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APPENDIX

LETHAL EFFECT OF SYNTHETIC JUVENILE HORMONE-II ON LARVAE OF *ANOPHELES* MOSQUITOES

Introduction

The basic concept of the mode of action of hormones in development among most species of insects are well illustrated by several authors - Fakuda (1942), Akai (1965), Kuruta (1978), Daillie (1979), Garel (1983), Sehnal (1985), Kadono-Okuda *et al.* (1986), Sakurai and Imokawa (1988) and Sehnal (1990). Twenty years ago, the promise of juvenile hormone as an insecticide was evident in test performed on the first hormonally active extracts prepared from male *cecropia* moths. When applied topically to silkworm pupae, the hormone penetrated the unbroken integument and caused lethal effect or derangements of metamorphosis. Subsequently, these findings have been confirmed in tests of the authentic juvenile hormone of *cecropia* silkworm, as well as of naturally occurring analogues and synthetic materials showing juvenile hormone activity.

The most active synthetic material available appears to be a product formed from treatment of ethanolic solutions of farnesoic acid with hydrogen chloride. This crude synthetic material shows a high degree of juvenile hormone activity when tested on immature insects ranging from the most primitive wingless *Thysanura* to the most highly evolved *Hymenoptera* (Spielman and Williams, 1966). Mosquito larvae contain the normal insect endocrine organs but practically not much information is known on the hormonal control of growth. In this chapter, we would like to describe the action of synthetic juvenile hormone-II (JH-II) (methyl cis-10,11-epoxy-3,7,11-trimethyl-trans, trans-2,6-tridecadie-noate) on the *Anopheles* mosquitoes larvae.

Materials and Methods

The synthetic juvenile hormone-II (JH-II) (methyl cis-10,11-epoxy-3,7,11-trimethyl-trans, trans-2,6-tridecadie-noate) was procured from Sigma Chemical Company, U.S.A.. The hormone was dissolved in acetone and stored in the refrigerator. Various dilutions were made (from 1.0 µgm/ml of acetone to 5.0 µgm/ml of acetone). In each experiment, a measured volume (0.05 ml to 0.3 ml) was stirred into the contents of glass jars containing 200 ml of distilled water a d homogeneous group of 10 to 20 mosquito larvae were introduced at specific stages in development. In control experiments the water was treated with solvent alone. The temperature was maintained at 21° ± 2°C, and food (pulverised pelletlets of rabbit chow and yeast) was added after the first day of experiment.

Results and Discussion

Larvae at the outset of the final (fourth) larval stage were placed in 200 ml of water containing 5 µgm of synthetic hormone; all underwent pupation, but no adult mosquitoes emerged. Metamorphosis was blocked ranging from pupae to full form pharate adults incapable of escaping from the old pupal cuticle. In parallel experiments in which the water was renewed daily and treated with fresh hormone, about one-fourth failed to pupate and of the remainder which pupate, only a few were able to begin adult development before their development was blocked. The same results was obtained in experiments performed on mature fourth-stage larvae.

In control experiments in which larvae were exposed to the solvent alone, virtually all individuals underwent normal metamorphosis.

The effects of synthetic hormone were studied in further detail. Homogeneous groups of larvae were exposed for 1 day of synthetic JH-II in 200 ml of water and then transferred to distilled water. Results show that the mature fourth stage larvae just prior to metamorphosis are most sensitive to hormone treatment [Table-10(a)]. Sensitivity at this stage was calibrated by exposure to graded doses for 1 day. Fourty per cent were killed with 10.0 µgm/ml of water [Table-10(b)].

When the material (JH-II) was dispersed in water, the hormone was fully effective for at least 1 day. However, by the end of 1 week, the dispersion became relatively ineffective, presumably because of breakdown of hormone by bacteria and other agents.

Males which survived and emerged as adults in the presence of low doses of hormone were affected in a surprising way. After prior exposure to hormone, many are unable to accomplish the 180° rotation of the genitalia which is necessary for successful reproduction. In this context it is also worthwhile to mention the ability of hormone to block the embryonic development of mosquito eggs.

Summary

Emergence of adult mosquitoes is blocked after addition of 10.0 µgm/ml of synthetic juvenile hormone-II (JH-II) in water to the IV stage larvae of the mosquito, *Anopheles*. Development is arrested at stages ranging from pupa to fully formed pharate adults incapable of escaping from the pupal exuvium. IV stage larvae just prior to metamorphosis are most sensitive. 40 per cent were killed after being exposed for 1 day with 10.0 µgm/ml. of juvenile hormone in water. The active material also inhibits the hatching of mosquito eggs.

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Table-10(a)

Effects of Juvenile Hormone-II (JH-II) on the Metamorphosis of *Anopheles* Larvae (for the control solvent alone was applied to the Mature-IV Stage Larvae)

Dose (µgm/l)	Stage	Treated Animals	
		Number	Emerging Adults (%)
6.0 µgm/ml	Early 4th Stage Larvae*	12	75
7.0 µgm/ml	Middle 4th Stage Larvae**	15	93
8.0 µgm/ml	Late 4th Stage Larvae***	17	31
9.0 µgm/ml	Mature 4th Stage Larvae****	30	100
10.0 µgm/ml	Pupae	5	100
Control		50	100

*Adult Eyes Invisible; ** Eyes Linear; *** Eyes Crescentric **** Eyes with Truncate Apex.

Table 10(b)

Effects of Exposure to Juvenile Hormone-II (JH-II) on the Metamorphosis of Mature Stage of *Anopheles* Larvae

Dose (µfm/ml)	Larvae (No)	Emerging adults (%)
4.0 µgm/ml	14	90
5.0 µgm/ml	12	91
6.0 µgm/ml	18	58
8.0 µgm/ml	40	88
10.0 µgm/ml	70	0