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

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-eminence 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