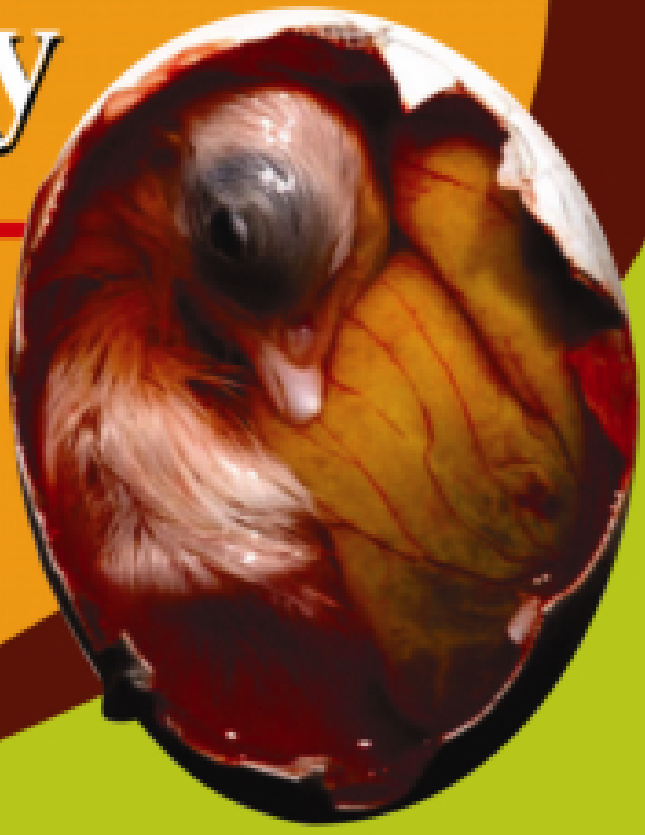
Applied and Developmental Biology



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Distributed by
Himalaya Publishing House
ISO 9001:2008 CERTIFIED

Applied and Developmental Biology

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First Edition : 2016

Published by : Mrs. Meena Pandey for **Himalaya Publishing House Pvt. Ltd.,**
"Ramdoot", Dr. Bhalerao Marg, Girgaon, Mumbai - 400 004.
Phone: 022-23860170/23863863, Fax: 022-23877178
E-mail: himpub@vsnl.com; Website: www.himpub.com

Branch Offices :

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DTP by : Priyanka and Sanhita

Printed at : Shri Krishna Offset Press, New Delhi, On behalf of HPH.

PREFACE

The first edition of this book has been designed very meticulously to introduce to readers the basic concepts of **Applied and Developmental Biology** and to meet the needs of undergraduate students of Indian universities. The present concise book has been written painstakingly for the students to let them know, that our quality of life, the strength of our economy and the very future of our society depends on the innovations and the discoveries made by scientists. Applied and developmental biology is rapidly evolving field that will continue to grow and maintain excitement over the next few decades. Applied Biology is the scientific management of a natural phenomenon covering a spectrum of areas of research to economic advantages. To provide livelihood and reduction of rural poverty is a paramount goal of the developing countries like India as the preponderating number of the poor population still resides in the countryside. The world Bank, for example, estimates that more than 70% of the world's poor live in rural area. A number of initiatives and strategies have been taken to address this concern besides creation of avenues for employment both under Agriculture and non-Agriculture sector. Applied Biology depicts the economic biology, which is one of the best suited occupation for ideal growth and development. With the launching of massive developmental schemes, it is expected to gain an accelerated tempo of sericultural activities in the country, paving way for doubling the employment opportunities in phased manner, and therby, it may set to bring a soothing touch to the burning problem of acute unemployment in rural India and thus can check the rural migration to urban areas to a certain extent. Apiculture today is the scientific management of a natural phenomenon covering a spectrum of areas of research and technology development to economic advantages. Beekeeping is a technology that is simple, easily accessible and affordable, especially in rural areas. It utilizes only the naturally available resources which otherwise go waste. The potential of beekeeping is yet to be tapped for increasing opportunities for gainful employment and income generation in the rural areas, in spite of which apiculture continue to remain a minor cottage industry, possibly because of the poor scientific support and organizational infrastructure provided to this industry. Developmental biology is not confined to the study of the development of embryo but portrays the genetic, biochemical and morphological aspects of the entire developmental period of the individual organism. According to (Lewis Wolpert, 1986), it is not birth, marriage, or death, but gastrulation, which is truly the most important time in your life. Gastrulation is a key process in embryonic development-it's how a simple ball of cells begins to grow into a fully functioning being. The present book also attempts to illustrate some human disorders in seperate chapters, depicting whys and hows of human disorders viz. Diabetes, Malaria, Liver toxicity and Tuberculosis. Development of novel drugs is sine qua non and it is possible only after knowing the whys and hows of the disorders. The practical icthyology chapter has been designed to meet the needs of students studying Zoology at both Undergraduate and Postgraduate levels in Indian Universities.

Dr. Arshed Iqbal Dar

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5 Chapter

MALARIA

Malaria is a disease caused by Apicomplex protozoans, represented by 150 species of *Plasmodium*, transmitted by the bites of mosquito vectors to man, simians, rodents, birds, and reptiles. Malaria is present all over the tropics where four species infect humans, *P. falciparum, P. vivax, P. malariae* and *P. ovale*. Over 90% of cases occur in sub-Saharan Africa, causing over two million deaths each year with high mortality among children (WHO, 1997). Malaria is also endemic in South East Asia, in Central and South America and Oceania. After the African countries, India and Brazil are presently the regions of highest endemicity in the World, (WHO, 1997). Three *Plasmodium* species occur in humans in Brazil: (i) *P. falciparum*, the most deadly if not treated; (ii) *P. vivax*, the most prevalent causing about 80% of the current cases, according to the Ministry of Health reports, and (iii) *P. malariae,* of lowest prevalence, presently under-diagnosed in Brazil due to the method used, the Giemsa stain thick blood smear. This method of blood concentration is ideal for malaria diagnosis being highly sensitive and specific, as reviewed by (Avila and Ferreira, 2000). Misdiagnosis of *P. malariae* as *P. vivax* does occur unless the typical small mature *P. malariae* schizonts are found. Although treatment is successful with chloroquine for both species of parasite, *P. vivax* requires also a 14-day treatment with primaquine to prevent late relapses caused by liver hypnozoites. In addition, resistance of *P. vivax* to chloroquine and mefloquine has been demonstrated in Brazil (Alecrim *et al.,* 1999). A more precise specific diagnosis requires the thin blood smear, and it should be ideal to implement it as a routine. Although adding a thin blood smear for malaria diagnosis will be more work, and more expensive, it seems regretable missing most *P. malariae* cases in Brazil. In the thin blood smear, the typical *P. malariae* equatorial asexual parasites allow a correct result, or using the polymerase chain reaction (PCR), non-practical in routine tests. A semi-nested PCR in blood of 96 malaria patients from Rondonia, Western Brazilian Amazon, showed 10% positive for *P. malariae* (Cavasini *et al.,* 2000), none of which were identified by local microscopists, in spite of being highly trained in malaria diagnosis; therefore, there is a technical limitation of the thick blood smear test. The total number of acute malaria cases in Brazil is on the increase in endemic areas, 99% being transmitted in the Amazon region. This reflects the socio-economic decline of the

rural south and east, with consequent waves of migration of workers to mining and agricultural projects in the north, where a larger population is exposed to endemic transmission. Transmission has never been interrupted in the Amazon where the waves of immigrants in the last decades, mostly non-immune individuals from areas where there is no malaria, have aggravated the situation. In addition, drug resistance of *P. falciparum*, reviewed in (Zalis, 2000) and *P. vivax* (Alecrim *et al.,* 1999) has made control difficult. During the malaria eradication campaign, coordinated by the WHO and the Brazilian government, transmission of the disease was eliminated in most areas of the country outside the Amazon region. During the 70s, about 70,000 cases of malaria occurred yearly a number raised gradually to 610,000.

Malaria is a major parasitic disease in the world, especially in Africa. It is responsible for 500 million new cases and 2 to 3 million deaths every year, mostly among children under five years and pregnant women (WMR, 2008). *Plasmodium falciparum,* the most widespread etiological agent for human malaria, has become increasingly resistant to standard antimalarials, e.g., chloroquine and antifolates. Consequently, new drugs or drug combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use. In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and upto 80% of the African population use traditional medicines for primary health care (WHO, 2002). Since little scientific data exist to validate antimalarial properties of these medicinal plants, it is important that their claimed antimalarial properties are investigated, in order to establish their efficacy and determine their potential as sources of new antimalarial drugs (such as artemisinin isolated from *Artemisia annua*). Malaria is the world's most devastating disease (Rodriguez-Acosta *et al.,* 1998; Klayman, 1989). An estimated 2 billion people are exposed to be global endemic among which 500 million are affected by this disease yearly (Gentilini, 1995). The presence of *Plasmodium falciparum* in some areas of the world is closely linked to the presence of vectors and to favourable conditions for their developmental cycle.

HISTORICAL BACKGROUND

Most of the drugs currently available to treat malaria are quinoline derivatives modeled on the quinine molecule, found in the bark of *Cinchona spp.* trees found in high altitudes of South America. This genus is said to have been named after the Spanish Countess of Chinchon who was successfully treated with the powdered bark (Garnham, 1966). In the XVI century, the plant bark and seeds were taken to Europe by the Jesuits and used for centuries to treat human malarial with efficacy. Cinchona plantations were established in Java and, until the Second World War, were the source

of quinine. The quinine molecule inspired the synthesis of chloroquine and this drug became the chief replacement for quinine, during the Second World War. Chloroquine was close to the ideal antimalarial drugs and was used for decades due to its high efficacy against all species of malaria parasites, its low toxicity, low cost and high tolerance; it is still widely used to treat malaria in areas where notable drug resistance has not yet appeared. Among the modern antimalarial compounds isolated from plants artemisinine is the most important one at present, being discovered and characterized by Chinese scientists. This substance comes from *Artemisia annua L.,* a plant used for thousand years to treat malaria according to the Chinese *Materia Medica*.

According to the WHO report, about 80% of the products in the US market were of plant origin and their sails exceed 65 billion US dollars annually. Krettli *et al.* (2001) have reviewed the overall situation of malaria in India and Brazil. Artemisinine, an antimalarial drug was isolated by the Chinese scientist who was quite effective against *P. falciparum* till few years back, but recently Artemisia resistant *P. falciparum* and *P. vivax* have been reported by Zalis, (2000).

More than 40% of the world's population, much of it socio-economically and politically challenged, live in areas where malaria, alone or together with HIV/AIDS and tuberculosis, is a significant health risk (Bates *et al.,* 2004 and Stratton *et al.,* 2008). According to the World Health Organization (WHO), approximately 250 million clinical cases of malaria occur every year. Malaria is estimated to kill nearly one million people annually, with most of the deaths occurring in children under 5 years of age in sub-Saharan Africa (Roca-Feltrer *et al.,* 2008). If children survive multiple infections, such exposure leads to a natural immunity that limits the severity of the disease later in life. However, this immunity wanes in the absence of continued exposure to malaria infections. Additionally, pregnant women and newborns have reduced immunity, and therefore are vulnerable to severe complications of malaria infection and disease (Rogerson, 2010 and Schantz-Dunn *et al.,* 2009). Malaria is primarily caused by four species of the protozoan parasite *Plasmodium: P. falciparum, P. vivax, P. malariae,* and *P. ovale,* which are transmitted by over 70 species of *Anopheles* mosquitoes (Service *et al.,* 2002). These parasite species occur sympatrically both in human populations and within infected individuals, with *P. falciparum* and *P. vivax* being the predominant species (Gurarie *et al.,* 2006 and Zimmerman *et al.,* 2004). Approximately, 80% of all malaria cases and 90% of malaria-attributed deaths occur in Africa, and are caused by *P. falciparum*. Outside of Africa, *P. vivax* is the most widespread species, occurring largely in Asia, including the Middle East and the Western Pacific, and in Central and South America (Baird *et al.,* 2007). This parasite species causes a relatively less lethal form of the disease compared with *P. falciparum* (Baird *et al.,* 2007 and Price *et al.,* 2007).

Overview of Antimalarial Drugs

Today when many mosquito vectors are resistant to insecticides (Greenwood *et al.,* 2008 and Hemingway *et al.,* 2000) and an effective vaccine is not yet available (Greenwood *et al.,* 2008 and Girard *et al.,* 2007), chemoprophylaxis/chemotherapy remains the principal means of combating malaria. Over the past 60 to 70 years, since the introduction of synthetic antimalarials, only a small number compounds, belonging to three broad classes, have been found suitable for clinical usage (Schlitzer *et al.,* 2008 and White *et al.,* 2008). These classes are described below.

Quinine and Related Drugs

Quinine, originally extracted from cinchona bark in the early 1800s, along with its dextroisomer quinidine, is still one of the most important drugs for the treatment of uncomplicated malaria, and often the drug of last resort for the treatment of severe malaria. Chloroquine (CQ), a 4-aminoquinoline derivative of quinine, has been the most successful, inexpensive, and therefore the most widely used antimalarial drug since the 1940s. However, its usefulness has rapidly declined in those parts of the world where CQ-resistant strains of *P. falciparum* and *P. vivax* have emerged and are now widespread. Amodiaquine, an analogue of CQ, is a pro-drug that relies on its active metabolite monodesethylamodiaquine, and is still effective in areas of Africa, but not in regions of South America. Other quinine-related, commonly used drugs include mefloquine, a 4-quinoline-methanol derivative of quinine, and the 8-aminoquinoline derivative, primaquine; the latter is specifically used for eliminating relapse causing, latent hepatic forms (hypnozoites) of *P. vivax*. Halofantrine and lumefantrine, both structurally related to quinine, were found to be effective against multidrug resistant falciparum malaria (Schlitzer *et al.,* 2008, Wernsdorfer *et al.,* 1991, and Wiesner *et al.,* 2003). While lumefantrine, in combination with an artemisinin derivative, artemether (Coartem), is recommended by the WHO for treating uncomplicated falciparum malaria, halofantrine generally is not recommended because of serious side-effects and extensive cross-resistance with mefloquine (Croft *et al.,* 2007).

Antifolate Combination Drugs

Antifolate drugs include various combinations of dihydrofolate reductase enzyme (DHFR) inhibitors, such as pyrimethamine, proguanil, and chlorproguanil, and dihydropteroate synthase enzyme (DHPS) inhibitors, such as sulfadoxine and dapsone. With rapidly growing sulfadoxinepyrimethamine (SP) resistance, a new combination drug, Lapdap (chlorproguanil-dapsone), was tested in Africa in the early 2000s, but was withdrawn in 2008 because of hemolytic anemia in patients with Glucose-6-phosphate dehydrogenase enzyme (G6PD) deficiency (Luzzatto *et al.,* 2010).

Artemisinin and its Derivatives

Artemisinin drugs, which originated from the Chinese herb qing hao (*Artemisia annua*), belong to a unique class of compounds, the sesquiterpene lactone endoperoxides. The parent compound of this class is artemisinin (quinghaosu), whereas dihydroartemisinin (DHA), artesunate, artemether, and β-arteether are the most common derivatives of artemisinin; DHA is the main bioactive metabolite of all artemisinin derivatives (artesunate, artemether, β-arteether, etc.), and is also available as a drug itself.

Since 2001, the WHO has recommended the use of artemisinin-based combination therapies (ACTs) for treating falciparum malaria in all countries where resistance to monotherapies or non-artemisinin combination therapies (e.g., SP) is prevalent (WHO, 2010). The rationale for the use of ACTs is based on the facts that artemisinin derivatives are highly potent and fast acting, and that the partner drug in ACT has a long half-life, which allows killing the parasites that may have escaped the artemisinin inhibition. Thus, it is thought that ACTs will delay the onset of resistance by acting as a "double-edged sword" (Bosman *et al.,* 2007 and Hastings *et al.,* 2010). ACTs, such as artemether-lumefantrine, artesunate-amodiaquine, and artesunate-mefloquine are being used in China, South East Asia, many parts of Africa, and some parts of South America. Introduction of ACTs has initiated noticeable reduction in malaria prevalence in these endemic regions of the world. Although the mechanism(s) of action is poorly understood (described below), the current high level of interest in artemisinin drugs is due to their well-recognized pharmacological advantages. These drugs act rapidly upon asexual blood stages of CQ-sensitive as well as CQ-resistant strains of both *P. falciparum* and *P. vivax*, and reduce the parasite biomass very quickly, by about 4-logs for each asexual cycle. In addition, these drugs are gametocytocidal. Thus, through rapid killing of asexual blood stages and developing gametocytes, artemisinin drugs significantly limit the transmission potential of the treated infections. These drugs have large therapeutic windows, and based on extensive human use, they appear to be safe, even in children and mid/late pregnant women. Furthermore, there is no reported "added toxicity" when these drugs are used in combination with other types of antimalarial compounds.

In addition to these three main classes of compounds, the antibiotic tetracycline and its derivatives, such as doxycycline, are consistently active against all species of malaria, and in combination with quinine, are particularly useful for the treatment of severe falciparum malaria (Schlitzer, 2008). Until recently, a combination of atovaquone, a hydroxynaphthoquinone, and proguanil (Malarone) was considered to be effective against CQ- and multidrug-resistant falciparum malaria; atovaquone resistance has recently been noticed in Africa (Musset *et al.,* 2006). Piperaquine, another member of

the 4-aminoquinoline group, in combination with DHA (Artekin), holds the promise of being successful in CQ-resistant endemic areas of Southeast Asia.

Overview of Antimalarial Drug Resistance

This limited antimalarial armament is now severely compromised because of the parasite's remarkable ability to develop resistance to these compounds (Wernsdorfer *et al.,* 1991 and Hyde, 2005). In many different malaria-endemic areas, low-to high-level resistance in the predominant malaria parasites, *P. falciparum* and *P. vivax*, has been observed for CQ, amodiaquine, mefloquine, primaquine, and *SP. Plasmodium falciparum* has developed resistance to nearly all antimalarial drugs in current use, although the geographic distribution of resistance to any one particular drug varies greatly. In particular, Southeast Asia has a highly variable distribution of falciparum drug resistance; some areas have a high prevalence of complete resistance to multiple drugs, while elsewhere there is a spectrum of sensitivity to various drugs. Until 2009, no noticeable clinical resistance to artemisinin drugs was reported. However, as described below, a number of recent studies have raised concerns about the efficacy of ACTs, particularly in Southeast Asia.

Overview of Genetic Basis for Antimalarial Drug Resistance

It is believed that the selection of parasites harboring polymorphisms, particularly point mutations, associated with reduced drug sensitivity, is the primary basis for drug resistance in malaria parasites (Hayton *et al.,* 2004 and Hyde, 2007). Drug-resistant parasites are more likely to be selected if parasite populations are exposed to sub-therapeutic drug concentrations through: (a) unregulated drug use; (b) the use of inadequate drug regimens; and/or (c) the use of long half-life drugs singly or in non-artemisinin combination therapies. In recent years, significant progress has been made to understand the genetic/molecular mechanisms underlying drug resistance in malaria parasites (Ekland *et al.,* 2007 and Kidgell *et al.,* 2006). Chloroquine resistance (CQR) in *P. falciparum* is now linked to point mutations in the chloroquine resistance transporter (PfCRT [encoded by *pfcrt*, located on chromosome 7]). *Pfcrt*-K76T mutation confers resistance *in vitro*, and is the most reliable molecular marker for CQR. Polymorphisms, including copy number variation and point mutations, in another parasite transporter, multidrug resistance (PfMDR1 or Pgh1 [encoded by *pfmdr1*, located on chromosome 5]), contribute to the parasite's susceptibility to a variety of antimalarial drugs. Point mutations in *pfmdr1* play a modulatory role in CQR, which appears to be a parasite strain-dependent phenomenon (Valderramos *et al.,* 2006). Point mutations in the *P. falciparum* DHPS enzyme (encoded by pf-dhps, located on chromosome 8) are involved in the mechanism of resistance to the sulfa class of

antimalarials, and accumulation of mutations in the *P. falciparum* DHFR domain (encoded by *pf-dhfr*, located on chromosome 4) defines the major mechanism of high-level pyrimethamine resistance. In field studies, a *pf-dhps* double mutant (437G with either 540E or 581G), combined with the *pf-dhfr* triple mutant (108N_51I_59R), was found to be frequently associated with SP treatment failure [28,29]. Orthologues of *pfcrt (pvcrt-o), pfmdr1 (pvmdr1), pf-dhps (pv-dhps),* and *pf-dhfr (pv-dhfr)* in *P. vivax* have been identified, and found to be polymorphic. However, associations of the mutant alleles of *pvcrt-o/pvmdr1* and *pv-dhps/pv-dhfr* with clinical resistance to CQ and SP, respectively, are unclear (Mehlotra *et al.,* 2009).

Finding New Antimalarial Compounds by Exploring Natural Products

Historically, drug discovery and development has greatly benefited from sourcing compounds from nature. In fact, between 1981 and 2002, 61% of new chemical entities brought to the market can be traced back to, or were inspired by, natural sources (Newman *et al.,* 2003). Malaria drug discovery is no exception. The isolation of the antimalarial drug quinine from Cinchona bark was accomplished in 1820. The bark had long been used by indigenous peoples in the Amazon region for the treatment of fevers. The Chinese herb qing hao (*Artemisia annua*) was also used as a treatment for fevers in China for more than 2,000 years, but it was not until 1972 that the active compound artemisinin was extracted, and later identified as a potent antimalarial drug. The 1990s saw a demise in research into natural products for drug discovery, due in part to the emergence of high-throughput screening and combinatorial chemistry. Today, however, the current demand for novel compounds to tackle emerging antimalarial resistance has stimulated new interest in their potential (Ginsburg *et al.,* 2011). Several screening projects utilizing different natural sources, from the rainforest to the deep sea and from exotic microorganisms to plants, have been carried out, resulting in several interesting antimalarial lead compounds with remarkable chemical diversity (Fernandez *et al.,* 2010 and Wright, 2010). Some such projects at the Medicines for Malaria Venture (MMV) are in the early-stage discovery pipeline (Olliaro *et al.,* 2009). The mode of action of compounds originating from natural products is mostly unknown, and, in order to understand the basis for their pharmacological effects, research focused on their synergistic or antagonistic interactions is needed (Efferth *et al.,* 2011). It is also clear that the much desired success of this approach faces several challenges, such as species selection criteria, screening procedures, pharmacological models and fractionation processes, as well as prediction of clinical safety and efficacy (Bourdy *et al.,* 2008 and Willcox *et al.,* 2011). Nevertheless, it is hoped that once the activity of natural medicinal products in humans is characterized, it can be used to identify new molecular scaffolds which will form the basis of the next generation of antimalarial therapies (Wells, 2011).

VECTOR BORNE DISEASES

Difficult to Control Despite Medical Advancements

It is estimated that every year at least 500 million people in the world suffer from one or the other tropical disease that include malaria, lymphatic filariasis, schistosomiasis, dengue, trypanosomiasis and leishmaniasis of late chikungunya, a serious mosquito borne epidemic has gained momentum in India. One of two million deaths are reported annually due to malaria worldwide. Lymphatic filariasis affects at least 120 million people in 73 countries in Africa, India, Southeast Asia and Pacific Islands. These diseases not only cause high levels of morbidity and mortality, but also inflict great economic loss and social disruption on developing countries such as India and China. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crore. Arthropod-borne diseases generally are serious. They have been and often still are difficult to control despite medical advancements. Their historical role has been important as far as man is concerned. During the past half century, the number of people at risk from tropical diseases in developing countries has increased. Although human disease transmission relationship was not realized until the last few decades, insects serve as vectors (carriers) of the major human diseases like malaria, filariasis, yellow fever, cholera and epidemic diarrhea, etc. as well as certain enteric diseases of lesser concern. As we know well that malaria, lymphatic filariasis, dengue fever, Japanese encephalitis, among other vector borne diseases, continue to be of major public health importance in India and throughout the tropical zone. Most of these major diseases have had a profound effect upon man and their civilization. Among the vectors, mosquito are of tremendous importance to man. Some species principally those of family culicidae, transmit an imposing list of human diseases including malaria, filarial, dengue and yellow fever. For this reason, mosquitoes are of utmost importance in a concentration of medical entomology. Besides the wall painful bites inflicted by the female mosquitoes, these insects are proved carriers of the above mentioned human diseases. As a direct nuisance, mosquitoes also have an economic influence. The severity of their attacks decreases property values especially in resort areas and has undoubtedly had an influence in the settlement of extreme northern areas whereas mosquitoes are usually abundant. Among these mosquito-borne diseases, lymphatic filariasis caused by waucheria beancrofti is transmitted by *Culex Spp.* especially *quinquefasciatus* and is of prime importance throughout India. The filariasis is a widely distributed infection affecting people residing both in the urban and rural areas. In the pre-historic period, the cave man used the smoke of certain plant leaves as protective methods against mosquito bite. When it was discovered that only certain species of Anopheline and Culicine mosquitoes transmit malaria and filariasis and that they are ecologically

dependent on specific condition which are required for their perpetuation and survival, a planned anti-vector disease with control method would be required. However, long before the development of organic insecticides, natural substances derived from plants, the 'first generation insecticides' like pyrethrum, retenome, etc. were successfully employed in insect control. But as a result of the introduction of new insecticides and other causes, the use of pyrethrum, retenome and other natural products was reduced and the discovery of synthetic second generation insecticides – DDT, HCH, Malathion, etc. was an outstanding advancement towards the control of mosquitoes other vector borne diseases in a more and effective and economical manner in rural and other areas. But synthetic organic insecticides although highly efficacious against target species of insects can be detrimental to be a variety of animal life including man. In addition to its adverse environmental effects from conventional insecticides, most of the major mosquito vectors and pests have become physiologically resistant to many of these compounds on prolonged application.

Criticism of conventional insecticides of the chlorinated hydrocarbons, organophosphorous compounds and carbamate groups always concentrates on the following points.

1. The products are considered to be persistent and too wide acting. The characteristics are special danger to ecosystem in which the insecticides are used.
2. The animals are too toxic for man and domestic animals.
3. Developing resistance in many insects necessitates the use of new chemicals, and this can further aggravate the toxic situation in the environment.

This criticism gave a fundamental objective of modern research for developing the safest possible insect control products and techniques; and also the need for environmentally safe, degradable and target-specific insecticides. In brief, a new life of interdisciplinary research has sprung into existence with this objective, over the years.