Mosquitoes are small well-adapted insects with three pairs of long legs, a pair of membranous wings for efficient flight and another pair of wings that is modified as small knob like structures known as halteres for balancing. The systematic position of mosquitoes in the animal kingdom is as follows

- **Phylum:** Arthropoda
- **Class:** Insecta
- **Subclass:** Pterygota
- **Division:** Endopterygota
- **Order:** Diptera (comprising two winged insects or true flies)
- **Subdivision:** Nematocera
- **Superfamily:** Culicoidea
- **Family:** Culicidae

There are more than 3200 species of mosquitoes distributed worldwide belonging to 37 well-recognised genera. Mosquitoes exploit a variety of habitats as developmental sites for their immature forms and are ubiquitous in distribution except in extreme conditions like Arctic and Antarctic regions. They are well adapted to rural, forested, suburban and urban conditions although different species show predilection for different ecological niches. The females of all species except those of genus *Toxorhynchites* seek animal or human blood meal for the completion of their gonotrophic cycle i.e. for the production of eggs and are accordingly labeled as zoophilic or anthropophilic respectively. Some species are host-specific while others may secure blood meal from a variety of hosts depending upon their availability. The females of some of these species particularly those belonging to genera *Anopheles*, *Culex*, *Aedes* and *Mansonia* not only possess great
nuisance potential but also transmit parasitic and pathogenic diseases of great socioeconomic importance in humans (Table 1.1).

These diseases have posed a grave threat to human health over the centuries. Historically, malaria transmitted by Anopheles mosquitoes has been the greatest single cause of mortality in the human beings, maiming and destroying number of civilizations and repulsing mightiest of the armies, a great demoralizer and a strong contributor to human misery and poverty. Currently, it threatens 2400 million (about 40%) people in the world. According to the recent World Health Organization estimates, there are 300-500 million clinical cases and 1.5 to 2.7 million deaths annually attributed to malaria, especially among young children and expectant mothers mostly in Africa, Asia and Latin America (WHO web site). Malaria is still prevalent in over 100 countries and *Plasmodium falciparum*, that causes malignant malaria and most of the malarial deaths is transmitted in 92 countries. Epidemiological indices of malaria in India too show an increasing trend (Sharma, 1999a). There are 15 million cases & 19,500 deaths estimated due to malaria in India by WHO SEA Regional Office, although the National Anti Malaria Programme (NAMP) reports only 1.5-3.0 million cases with few thousand deaths annually (Sharma, 1999b).

On the other hand, major human viral diseases that are transmitted by various mosquito species include yellow fever, dengue/dengue haemorrhagic fever and several forms of encephalitis including zoonotic encephalitis. Yellow fever viruses are transmitted by *Aedes aegypti* and *Aedes albopictus*. According to WHO estimates there are 200,000 cases of yellow fever and 30,000 reported deaths annually attributed to this disease alone.
Table 1.1: Some important mosquito vector genera, the causative organisms and the diseases caused by them in humans.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Vector Genera</th>
<th>Causative Organism</th>
<th>Disease</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Anopheles</em> spp. (about 50)</td>
<td>Protozoa <em>(Plasmodium vivax, P. falciparum, P. ovale &amp; P. malariae)</em></td>
<td>Malaria</td>
</tr>
<tr>
<td>2.</td>
<td><em>Aedes</em> spp.</td>
<td>Viruses DEN group serotypes 1,2,3,4 (family Flaviridae; Group B)</td>
<td>Dengue, Yellow fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yellow fever virus (family Flaviridae Gp. B)</td>
<td>Yellow fever</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Alphavirus Gp. A (family Togoviridae)</td>
<td>Eastern Equine Encephalitis</td>
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<tr>
<td></td>
<td></td>
<td>JE virus Gp. A</td>
<td>Japanese Encephalitis</td>
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<tr>
<td></td>
<td></td>
<td>St. Louis virus Gp. B (Flavivirus)</td>
<td>St. Louis Encephalitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>West Nile virus Gp.B (Flavivirus)</td>
<td>West Nile Encephalitis</td>
</tr>
</tbody>
</table>
Fortunately, indigenous transmission of this disease has not been reported so far from India although there is lurking fear of its introduction and transmission due to the wide distribution of its natural vector *Ae. aegypti*. Dengue (DEN)/ Dengue haemorrhagic fever (DHF) also primarily vectored by *Ae. aegypti*, constitute an important burden on mankind in terms of morbidity and mortality. About 1.5 billion people in the tropics mainly in Asia including India, the western Pacific, the Caribbean, as well as central and South Africa live under the risk of dengue infection (Becker & Margalit, 1993). In the 1990’s cases with haemorrhagic manifestations have increased to an alarming rate in India. During August to November, 1996 Delhi witnessed an outbreak of DHF with over 90,000 admissions and about 400 deaths with average case fatality rate of 4.3 percent (Sharma, 1997).

Another viral disease, Japanese Encephalitis (JE) is transmitted by *Culex vishnui* group of mosquitoes viz., *Cx. vishnui, Cx. pseudovishnui, Cx. tritaeniorhynchus* and *Cx. gelidus*. According to current WHO estimates, there are 30,000-50,000 cases of Japanese Encephalitis annually (WHO, website). According to Sharma (1997), since 1973 a large-scale outbreak of JE had occurred in the Northern and North Eastern parts of the India. By 1995 JE spread to Western Coast of India and North- Western plains of Punjab & Haryana. The annual incidence in the country ranged from 1700-1750 cases with case fatality rates from 35-40 %. Another form of encephalitis West Nile Encephalitis is caused by West Nile Virus and is transmitted by mosquitoes of *Culex* spp. the main vector being *Culex univittatus*. The recent outbreak of this disease occurred in the US in 1999. Birds constitute the vertebrate host reservoir for the West Nile Viruses in nature that also infects
humans and pigs. There are other zoonotic viral diseases transmitted by mosquitoes in humans, the true incidence of which is not known (Table.1.1).

Lymphatic filarial disease caused by nematodes, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* affects 90 million people in Asia, Africa and South America alone. Millions of people are directly exposed to infection through various mosquito genera of both Culicine and Anopheline, the most important being *Culex quinquefasciatus* and *Mansonina annulifera* and *M. uniformis*. There are estimated 120 million cases of filariasis in the world annually (WHO, website). Filariasis is now widespread in India. There are already 26.42 million infected and 20.40 million chronic cases in the country (Sharma, 1997).

Despite considerable national and international efforts aimed at suppressing these vector-borne diseases, they still continue to pose serious threat to human health. Control of these diseases is possible either through the elimination of causative organism or their mosquito vectors. Affordable prophylactic solution based on inexpensive vaccines remains unavailable for protection against most of these diseases. In addition to chemotherapy, vector control is an important and affective protective measure that attacks the problem at its root.

A multitude of mosquito vector control strategies have been developed and are currently being used in different parts of the world. Besides various techniques for exclusion and personal protection, mosquitoes can be controlled either in immature forms or adults in breeding habitats, inside dwellings or in harborage places. Traditionally adult control has been the popular method employed in malaria control programmes in the rural areas of developing countries, whereas larval control measures have been instituted for the
control of malaria, filariasis, dengue and encephalitis, especially in urban and peri-urban communities (Mulla, 1991).

Larval control depends to a large extent on the application of chemical larvicides to water where mosquitoes breed. During the earlier period of the development of chemical insecticides for insect control, the chlorinated hydrocarbons such as DDT, BHC, Methoxychlor and others were popular. However the very property that made these chemicals useful i.e. long residual action and toxicity for wide range of organisms, brought about serious environmental problems. A switch over to organophosphorous insecticides was made using malathion, fenitrothion, temephos and others. Another range of compound used in mosquito larval control are the synthetic pyrethroids such as cypermethrin and bifenthrin but were also found to be toxic for some fish and crustaceans. Insect growth regulators (IGR’s) are also known to be highly effective against mosquito larvae and are considered safer to humans and other non target fauna (Lacey & Mulla, 1991).

Various well-recognised problems associated with the use of chemical insecticides are-

a) Resistance

b) Environmental pollution and food web contamination

c) Broad spectrum effect e.g. Killing of useful fauna

d) Cross-resistance to same class of insecticides and faster development of resistance to other classes of insecticides

e) Rising cost of material and application

f) High refusal rates among communities for indoor spraying
g) Excito-repellent effect and changing vector resting behavior

Due to the above constraints, the emphasis from chemical control of vectors has shifted to other safer biological control methods particularly the use of natural enemies and pathogens. In the recent decades, research has been intensified to discover, isolate and develop various bio-control agents and employ them in the vector control programmes. Among these agents of mosquitoes that hold great promise for large scale use are the bacteria (*Bacillus thuringiensis* & *B. sphaericus*); fungi (*Coelomomyces* spp., *Culicinomyces clavisporus*, *Lagenidium giganteum*, etc.); nematodes (*Ramanomermis* spp. & *Octomyomermis* spp.); viruses and many species of larvivorous fishes (e.g. *Gambusia affinis*, *Lebistis reticulatus*, *Rasbora daniconius*, *Aplocheilus blockii*, *Oryzias melastigma*, *Puntius* spp., etc.).

Spore forming bacteria of genus *Bacillus* admirably fulfill the criteria of a microbial larval control agent. Ever since the discovery of *B. sphaericus* by Kellen & Meyers (1964) and evidence of its insecticidal property provided, a large number of toxic strains of this species have been isolated. The other species of *Bacillus* that is widely used in the bio-control programmes is *Bacillus thuringiensis israelensis* (serotype H-14) which was first isolated in Israel from dead *Culex pipiens* larvae and its larvicidal activity was tested (Goldberg & Margalit, 1977). *Bti* is almost an ideal microbial control agent because of its high larvicidal activity towards mosquitoes and safety to non-target insects and mammals. It is easily fermented to a stable sporulating stage and can be readily formulated (Davidson, 1990). *Bacillus thuringiensis israelensis* and *B. sphaericus* act as stomach poisons than as infectious pathogens (WHO, 1983).
At present a large number of Bs & Bt strains have been isolated, extensively studied, commercialized and used in mosquito control programmes (Hougard, 1990; Mulla, 1991; Rodriguez et al., 1998 and Brown et al., 1998 & 1999). There are extensive reports on the successful small and large scale field trials undertaken for the control of mosquitoes from India using Bt and Bs formulations of Russian origin (Dua et al., 1993; Bhalwar et al., 1993; Kumar et al., 1994, 1995, 1996, 1997 & 1998; Shukla et al., 1997).

Both Bti and Bs have been reported to be safe to man and other non-target fauna (Shadduck et al., 1980; Siegel & Shadduck, 1990; Lacey & Mulla, 1990; Drobiewsky, 1994). Recently there are some reports of the development of resistance in mosquitoes to these biocides. This problem can be managed by their judicious application in the vector control programmes (McGaughey & Whalon, 1992; Ferre et al., 1995 and Poopathi et al., 1999).

Although a wide variety of mosquito-pathogenic bacteria have been isolated from several geographic zones, yet there is a need to isolate and deploy indigenous potent strains in vector control programmes due to the restrictions imposed on the use of imported strains and their prohibitive costs. Thus the present study was initiated with an aim to find some local entomopathogenic Bacillus strains from Goa. Additionally, there is a possibility of finding new pathogenic bacilli or new serotypes of both Bti and Bs with characteristics that make them more suitable for development and field application.

For the fulfillment of this objective, survey and collection of the soil samples from the breeding habitats of the mosquitoes such as ponds, lakes, paddy fields, mangrove areas and other stagnant waters were carried out in Goa. The soil samples from various sites were screened for the presence of mosquito-pathogenic bacilli. The spore-forming bacilli
were isolated from these samples, identified and characterized. Purification, storage and culture of the promising bacilli were done. Small-scale laboratory trials were conducted to determine the efficacy of the new Bacillus isolates pathogenic to the larvae of three most important vector mosquito species viz., *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. The material and techniques used as well as results of these studies have been discussed in detail in the following chapters.