Chapter 1

INTRODUCTION
1.1 Mosquito as a Vector:

Mosquito-borne diseases such as Malaria, Filaria, Yellow Fever, Dengue, Chikunguniya, Japanese encephalitis, etc. cause extensive morbidity and mortality, globally. In India there are estimated 29 million cases of filariasis and about 1.5 million reported cases of malaria annually (http://www.searo.who.int/EN/Section313). Mosquitoes are classified in the family Culicidae within the order Diptera. Some 3,490 species are currently formally recognized (Harbach & Howard, 2007) under 44 genera. Female mosquitoes of fewer than 150 species, largely confined to the genera of Anopheles, Aedes, Armigeres, Coquillettidia, Culex, Culiseta, Haemagogus and Mansonia are vectors of causative organisms of these diseases.

Anopheles stephensi a sub-tropical species, distributed throughout the Middle East and South Asia region, is a primary vector of malaria in urban areas and breeds mainly in man-made breeding sites containing freshwater (Kumar et al., 2007). Culex quinquefasciatus on the other hand breeds in organically rich polluted waters and is the principal vector of Wuchereria bancrofti, the causative organism of Bancroftian filariasis (Curtis & Feachem, 1981). Dengue is a viral disease transmitted by the domestic container breeding and anthropophilic mosquito Aedes aegypti which is its principal vector. Chikunguniya and yellow fever is also vectored inter alia by Aedes aegypti.

1.2 Mosquito Biology:

Mosquito adults are slender, long-legged, two winged insects easily recognized by their long proboscis and the presence of scales on most body parts. The hindwings have developed into a pair of halteres, small knobbed structures that function as vibrating structure helping in balancing.
Both male and female mosquitoes feed on nectar and only female sucks blood to obtain supplemental nutrition (proteins and iron) to develop eggs.

Larvae are distinguished from other aquatic insects by the absence of legs, the presence of a distinct head bearing mouth brushes and antennae, a bulbous thorax that is wider than the head and abdomen, posterior anal papillae and either a pair of respiratory openings (subfamily Anophelinae) or an elongate siphon (subfamily Culicinæ) borne near the end of the abdomen. The immature stages of mosquitoes occupy a spectrum of aquatic environments like temporary or permanent bodies of ground water, leaf axils, tree-holes, rock-holes, crab-holes, bamboo internodes, bromeliads and aroids, fruit shells and husks, fallen leaves, spathes, flower bracts, snail shells, pitcher plants, and artificial containers.

1.3 Control of Mosquito Vector:

Control of mosquito-borne diseases is possible by elimination of the causative organism or control of their mosquito vectors. As affordable solution based on inexpensive vaccines is currently unavailable for protection from most of these diseases, treatment of infected individuals and effective control of vectors is the mainstay of mosquito-borne disease control programmes. Many vector control strategies are available for both adults and larvae. Rapid development of resistance to insecticides have made search for bio-control imperative.

1.4 Bio-control of vectors:

Biological control by harnessing natural enemies and entomopathogens is of interest because of their target precision, handler safety, ecological safety and host specificity. Biological agents such as fishes, bugs, mesocyclops, bacteria (Kumar et al., 1994,
1996; Becker & Ascher, 1998), protozoa (Chapman, 1974; Legner, 1995), nematodes (Kaya & Gaugler, 1993) and fungi (Scholte et al., 2004) have been used as arsenal for mosquito control. Vector control in rice fields was achieved by using the oomycete Lagenidium giganteum (Hallmon, et al., 2000). The commercial success of some entomopathogenic fungi in pest control/integrated vector control makes them an attractive option for vector control (Butt et al., 2001).

1.5 Fungi as bio-control agents:

Amongst entomopathogenic fungi, Hyphomycetes have been found to be effective as bio-control agents (Ferron, 1978; Tanada & Kaya, 1993; Hajek & St. Leger, 1994; Wraight & Carruthers, 1999; Keshava Prasad & Bhat, 2007; Mohanty & Prakash, 2010). A recent report focuses on the use of entomopathogenic fungi for mosquito control (Scholte, et al., 2004). Out of the 90 fungal genera reported to be pathogenic to insects and mites, more than 20 genera are reported to be mosquito-pathogenic, prominent amongst them are Leptolegnia, Pythium, Lagenidium, Crypticola, Coelomomyces, Conidiobolus, Entomophthora, Erynia, Smittium, Culcinomyces, Beauveria, Metarhizium, Paecilomyces, Penicillium, Aspergillus, Trichophyton, Verticillium, Fusarium, Chrysosporium and Tolypocladium.

Many entomopathogenic fungi produce insecticidal toxins in liquid culture (Charnley, 2003). Tolypin from Tolypocladium niveum was reported to be larvicidal to the larvae of mosquitoes and blackflies (Matha et al., 1988). In the Culex pipiens autogenicus larvae the depsipeptide beauvericin from Beauveria bassiana caused ultrastructural changes in the midgut epithelium and mortality (Zizka & Weiser, 1993). Extracellular fungal metabolites active against mosquito larvae have been reported from India (Vijayan & Balraman, 1991; Mohanty & Prakash, 2004; Govindarajan et al., 2005; Mohanty et al., 2008b; Soni & Prakash 2010). It
is important to know the action of fungal secondary metabolites on insects if they are to be used effectively in vector control programmes. A successful fungal vector control agent apart from killing selectively mosquitoes and no other organisms, is effective over a large range of salinities, temperatures, relative humidities and breeding sites with variable water quality, is easily and cost-effectively mass-produced and formulated, retains prolonged activity during storage (long shelf life), shows residual activity and persistence in the mosquito population after introduction and is not harmful to humans and other non-target organisms. None of the mosquito-pathogenic fungi presently known exhibit all these characteristics, but they all exhibit at least some.

1.6 Present Work:

It was felt that there is a need to isolate, identify and use the promising indigenous mosquito-pathogenic fungal strains and also to gain an insight into pathogenicity, virulence, invasive process, active principles and bio-safety of promising fungi to non-target organisms. Hence, the present work was taken up with the following objectives:

1. Isolation of entomopathogenic fungi; sourcing and maintaining mosquito-pathogenic fungal isolates from the Fungal Culture Collection of National Institute of Malaria Research, Campal, Panaji, Goa and Botany Dept. of Goa University, Taleigao Plateau, Goa.

2. Preliminary bioassay of a few known fungal isolates against laboratory reared mosquito larvae of the three vector species.

3. Studies on mode of action of the fungus to study the invasive and pathological process involved at the histological level of the host.
4. Bioassay of promising isolates against laboratory reared 3\textsuperscript{rd} instar larvae of *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* (Linnaeus).

5. Bioassay of crude metabolites of highly effective isolates against vector species with an attempt to separate active fraction/s.

6. Identification of enzymes released by the effective fungal isolates and qualitative analysis of the enzymes (1 or 2 most efficacious isolates).


The thesis has been organized into 6 Chapters. Besides a brief introduction in this chapter, a review of work done so far on mosquito vectors and biocontrol, isolation and mode of action of entomopathogenic fungi, enzymes, toxins produced by them, biology of mosquito-pathogenic fungi, epizootology of fungal diseases in insects, factors affecting infectivity, host response to fungal invasion, application of the mycoinsecticides and their persistence and recycling in the environment, bio-safety of non target organisms and integrated use of mycoinsecticides in vector management is presented in Chapter 2. Materials used and methods followed in the work have been elaborated in Chapter 3. Results are presented in the Chapter 4 of the thesis in several parts. This is followed by discussion in the 5\textsuperscript{th} Chapter. 6\textsuperscript{th} Chapter encompasses a brief summary of the work carried out. A comprehensive list of bibliography is given at the end of the thesis. Research articles published during the tenure of this work are appended to the thesis at the end.