CHAPTER I
INTRODUCTION

Biological control is a method of suppressing or controlling the population of undesirable insects, other animals or plants by the introduction, encouragement, or artificial increase of their natural enemies to economically non–important levels. It has been used widespread as an important component of integrated pest management (IPM) programs (Weeden et al., 2007). Biocontrol agents may exhibit itself as a predators or parasites or pathogens that either kill the harmful organisms or interfere with its biological processes (Freedman, 1976; Zhang et al., 2007; Van Lenteren, 2000; Luff, 1983). Biological control is one of the safest methods of pest control as the biocontrol agents are non toxic and do not leave back any residue. The history of biological control was initiated in 1762 with the introduction of a bird called Mynah bird (Acridotheres tristis) from India into Mauritius for the management of the sugar cane red locust Nomadacris septemfasciata. (Shahid et al., 2012).

Biological control can be divided into two approaches: classical and inundative. Classical biological control involves the introduction of a natural enemy of the target pest from its native range into the exotic range, ideally resulting in a self sustaining, balanced system in which the pest population is maintained at a non damaging or sub economic level, as its fitness or competitive edge is eroded (Evans and Ellison, 1990). Inundative control, on the other hand, involves the mass production, formulation and application of a product
(mycoinsecticide) that can be marketed and employed like a conventional chemical pesticide.

Microbial control (one way biological control) has been advocated as one of the alternatives of chemical control of different insect pests. Microbial control refers to the exploration of the disease causing organisms to reduce the population of insect pests below the damaging level. Different types of pathogens present in the environment like viruses, bacteria, fungi, protozoa, rickettsia, mycoplasma, nematodes etc. have been causing epizootics in natural pest population and hence these act as the natural enemies of pests and are used as biopesticide.

Microbial agent is a stable formulation of one or several microbes designed for large-scale application. For the successful application of any microbial agent in pest control, infrastructure and technical efficiency are essential along with it should satisfy at least two other criteria from the following: practical efficacy (easy and cheap uptake), commercial viability (profitability), sustainability (long -term control) and /or public benefit (safety) (Gelernter and Lomer, 2000; Shahid et al., 2012).

Many biological control agents have been evaluated to determine their efficacy for control of mosquito vectors at larval stages including *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphericus* (*Bs*) (Fillinger et. al. 2003). Biological control agents are non–polluting, not harmful to human health and other non-target organisms. Thus, it is environmentally safe and acceptable. Usually they are species specific to target pest and weeds. The biological control practices reduce the use of environmentally and ecologically unsuitable chemicals and thus it leads to the establishment of natural balance. The biological pest control measure also minimizes the problems of increased resistance in the pest
population for chemical insecticides and other such types of agents. Environmental methods and biological control are alternatives to chemical control and are key components of the integrated pest control strategy. The use of vertebrate and invertebrate predators, microorganisms, viruses, botanicals and entomopathogens as biological control agents and their role in integrated pest control programs have been reviewed with emphasis on different species of pest population including mosquitoes.

Entomopathogenic fungi are important natural regulators of insect populations and are common and widespread in almost all classes of insects. The word “entomopathogen” or “entomogenous micro-organism” has been derived from two Greek words “entomon” meaning insects and “genes” meaning arising in. Therefore, the meaning of the word “entomopathogen” or “entomogenous microorganism” is that microorganisms which arise in insects. The insect born entomopathogenic fungi represent a sustainable solution towards integrated pest management programs having no side effect on human life and other non-target organisms. At present approximately 700 species of entomopathogenic fungi are known from 90 genera exhibiting in a wide range of adaptations and infecting capacities (Khachatourians and Sohail, 2008).

Some of the fungi can instantly kill the insect and some other show absolute parasitic mode of action and kill the host slowly. Absolute parasites are the fungi which live in association with a host and benefit at the host’s expenses (Smith et al., 1981). Entomopathogenic fungi regulate a very wide range of insect populations including lepidopterous larvae, aphids and thrips which are of great concern in agriculture worldwide (Roberts and Humber, 1981). Some of the
genus like *Lagenidium*, *Coelomomyces* and *Culicinomyces* can also infect insects like mosquitoes etc.

Entomopathogenic fungi are the first micro-organisms used as a biological control agent against insects. Fungal strains or isolates may be specific to their host or may be widely distributed in nature (Khan *et al.*, 2012). They possess a negligible amount of risk to the non-target organisms and hence, they have been used as a safer alternative for use in integrated pest management (IPM) than chemical insecticides (Goettel and Hajek, 2000; Pell *et al.*, 2001).

The activity of entomopathogenic fungi involves four steps: adhesion, germination, differentiation and penetration. All these steps need to be influenced by a range of integrated intrinsic and external factors. A successful infection of entomopathogenic fungi is achieved by the attachment or adhesion of spores to the host which is achieved through the secretion of some enzymes like mucilage and lectins as well as some other hydrophobic and electrostatic forces (Boucias *et al.*, 1998). After adhesion, germination of the fungi requires the assimilation of utilizable nutrients and a tolerance to toxic compounds present on the surface cuticle (Latge *et al.*, 1987). Next to the germination, appresoria develop at the end of short germ tubes which ultimately attach to the insect cuticle and forms a narrow penetration peg (Boucias and Pendland, 1982; Roberts and Humber, 1981; Wraight *et al.*, 1998; Zacharuk, 1973). Penetration is both a mechanical and an enzymatic process (Charnley, 1984; McCoy *et al.*, 1988; St. Leger *et al.*, 1988). The exact mechanism of penetration is usually peculiar to species to species. All these steps need to be regulated by a wide range of integrated intrinsic and extrinsic factors, which ultimately determine the pathogenicity (Shahid, 2012). Next to the penetration, death of the insect takes place and it depends on the type
of fungus and the type of infecting spores and more specifically on the characteristics of the host (Pendland and Boucias, 1998). After death of the insect, fungal spores arise inside the body which disperse and continue their life cycle on new hosts (Hajek and St. Leger, 1994). Entomopathogenic fungi have several strains having a potentiality to infect a whole range of insect orders and resulting in their mortality. The prevailing local isolates collected from either soil or from dead insect hosts in different geographic areas show the great potentiality to control most of the target pest.

Research has been carried out in different corners of the globe to find out commercial products from the entomopathogenic fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* etc. Both, the fungi are well characterized in respect to pathogenicity to several insect pests and also have been commercially used as agents for the biological control of agriculture pests worldwide (Inglis *et al*., 2001; Reddy *et al*., 2014). About 11 companies offer at least 16 products based on the entomopathogenic fungus *B. bassiana*. These products are used in different types of crops such as bean, cabbage, corn, potato, tomato and also used in some haematophagous insect pests and vectors of diseases like mosquitoes and flies etc. (Florez, 2002).

Fungal pathogenicity involves a combination of different factors like suitable host, ambient humidity, temperature, action of different enzymes, host immunity recognition etc. Its pathogenicity is different than bacteria and viruses in that it infects the host by breaching the host cuticle. The cuticle is composed of chitin fibrils embedded in a matrix of proteins, lipids, pigments and N-acylcatecholamines (Richard *et al*., 2010). When the fungal spores or conidia are attached on the cuticle of a suitable host, they secrete extracellular enzymes.
proteases, chitinase and lipases to degrade the major constituents of the cuticle initiating cascades of recognition and enzyme activation reactions by both the host and the fungal parasite (Samson et al., 1988). The infection initiates after the secretion of exoenzymes and according to Khachatourians (1996) it is directly proportional to a successful infection. Invasion of the insect body and circulatory system (hemolymph) occurs once the fungus has passed through the cuticle of the external insect skeleton and it is believed that both mechanical force and enzymatic action are involved in the process. There are a number of toxic compounds reported in the filtrate of entomopathogenic fungi such as small secondary metabolites, cyclic peptides and macromolecular proteins. B. bassiana is reported to produce low molecular weight cyclic peptides and Cyclosporins A and C with insecticidal properties such as beauvericin, enniatins, bassianolide (Roberts, 1981; Vey et al., 2001). The genetic study of the fungal infection process revealed that a number of genes were involved in the pathogenicity process (Cho et al., 2007) such as chitinases (Fang et al., 2005), guanine nucleotide-binding proteins and its regulator (Fang et al., 2007), adhesin which helps in attachment of spore (Wang and St. Leger, 2007a), a perilipin-like protein that regulates appressorium turgor pressure and differentiation and a cell protective coat protein helping in escaping the pathogen from the host immunity recognition (Wang and St. Leger, 2006).

Past research has shown some promise of the use of entomopathogenic fungi as a selective pesticide. It has been applied as an effective component of bio-control strategy and also reduces the use of chemical pesticide. The Entomopathogenic fungi act as a natural enemy of the pest population by devastating them with ecofriendly manner on human health and environment.
This type of fungi have also received a lot of curiosity due to their prospect as biocontrol agent, peculiar mode of pathogenicity, broad host ranges etc. But, still they have only covered a small percentage of the global insecticide market. Entomopathogenic fungi have been found in different habitats and associated with a broad range of insect hosts (Samson et al., 1988). These are among the first organisms to be used for the biological control of pest. Currently, priority has been raised to develop entomopathogenic fungi as inundative biological control agents of insects, mites and ticks, despite the great potential for use in conservation and classical biocontrol strategies (Butt et al., 2001; Goettel et al., 2005; Vincent et al., 2007). This is normally achieved through a strategy in which pest control relies on the action of the released agent but not on successive generations of the fungus. Here, it is worth mentioning that over 170 products have been developed based on at least 12 species of entomopathogenic fungi (Faria and Wraight, 2007) out of which most of the commercially produced fungi are species of Beauveria, Metarhizium, Lecanicillium, Isaria, Nomuraea, Hirsutella etc. The genus Beauveria exhibits at least 49 species of which approximately 22 are considered pathogenic to pests (Kirk, 2003). B. bassiana is one of the most historically important and commonly used fungal species in this genus. The species name was given after the Italian scientist Agostino Bassi who first implicated it as the causative agent of a white muscardine disease in domestic silkworm (Furlong and Pell, 2005; Zimmermann, 2007). The species grows naturally in soil throughout the world and bears the entomopathogenic mode of action exhibiting pathogenic effect on various insect species causing white muscardine disease (Sandhu et al., 2004; Jain et al., 2008). B. bassiana can easily be isolated from insect cadavers or from soil in forested areas by using media as well as by baiting soil with insects.
The species has high specificity of its many isolates including the Colorado potato beetle, the codling moth, several genera of termites, American bollworm, *Helicoverpa armigera* etc. (Thakur *et al.*, 2010).

Another infective insect pathogenic fungus *M. anisopliae* is known to be pathogenic to a large number of insect species (Ferron, 1978) causing a disease known as green muscardine in insect hosts because of the green colour of its conidial cells. *M. anisopliae* was first described near Odessa in Ukraine from infected larvae of the wheat cockchafer *Anisopliae austriaca* in 1879 and later on, *Cleonus punctiventis* by Metschnikoff. It was later renamed as *M. anisopliae* by (Tulloch, 1976).

*Verticillium lecanii* is a species of insect pathogenic fungus. It is widely distributed and can cause large epizootic in tropical and subtropical regions as well as in warm and humid environment (Nunez *et al.*, 2008). The species was reported by (Kim *et al.*, 2002) as an effective biological control agent against *Trialeurodes vaporariorum* in South Korean greenhouses. The fungus attacks nymphs and adults and sticks to the leaf underside by means of a filamentous mycelium (Nunez *et al.*, 2008). From 1970 onwards *V. lecanii* was developed to control whitefly and several aphids species, including the green peach aphids (*Myzus persicae*) for use in the greenhouse chrysanthemums (Hamlen *et al.*, 1979)

*Nomuraea sp.* includes *Nomuraea rileyi* which is a dimorphic hyphomycete fungi that can cause epizootic death in various insects. The species exhibit high host specificity and infect many insect species belonging to Lepidoptera including *Spodoptera litura* and some infect species belonging to Coleoptera (Ignoff, 1981). The pathogenicity of this fungus has been reported in several insect hosts such as *Trichoplusiani, Heliothis zea, Plathypena scabra, Bombyx mori, Pseudoplusia includes, Anticarsia gemmatalis* etc.
*Paecilomyces fumosoroseus* is an entomopathogenic fungus known as one of the most important natural enemies of whiteflies worldwide. It causes Yellow Muscardine (Nunez, *et al.*, 2008) and potential against *Bemisia* and *Trialeurodes* spp. in both greenhouse and open field environments. In humid conditions, the fungus grows extensively over the leaf surface to spread rapidly through whitefly populations (Wraight *et al.*, 2000). Both the nymphs and adults of whitefly are highly affected by the mycelial threads which stick them to the underside of the leaves (Nunez *et al.*, 2008).

Effectiveness of entomopathogenic fungi against different mosquito larvae under laboratory conditions had been reported (Benserradj and Mihoubi, 2014; Madkour *et al.*, 2014; Singh and Prakash, 2012a; Carolino *et al.*, 2014) with higher variability of effectiveness at killing mosquito larvae in the field condition (Goettel, 1987).

Here, some of the advantages of entomopathogenic fungi have been summarized. Fungal biopesticides are very specific to their target host and least specific to other non-target organisms. They are environment friendly and cause no hazardous effect on animal or mammalian health. Unlike chemical pesticides, resistance to insecticide does not occur after the application of fungal biopesticide. Some of the fungal pesticides are endophytic in nature and can help in immune system activation. They can persist longtime in the environment and thus provide long-term suppression of the pest. Modern research has revealed that the pathogenicity of the fungal biopesticides is related to some genes that secrete insect toxins and thus bear great potential for future research prospects.
Recently efforts have been made to regulate the vector borne diseases in the country. Out of the several mosquito species the yellow fever mosquito *Ae. aegypti* has been receiving topmost concern due to its vectorial capacity and spreading of different diseases from dengue fever (DF) to the recently introduced zika viral disease. To get rid of these, vector control has been recommended as the most acceptable method. Till date, several mosquito control programmes have been implemented through the introduction of several pesticides, insecticides and other xenobiotics. However, frequent and indiscriminant use of chemical insecticides led to development of pesticide resistance, pest resurgence and secondary pest outbreaks along with other environmental pollution which necessitated the development and adoption of eco-friendly measures for vector mosquito control. Biological control can be utilized as an effective and environmental friendly alternative approach to minimize the mosquito population. Among several aspects of biological control, the use of fungal entomopathogens constitutes as one of the important components with added advantage over other microbial pathogens. Several workers have introduced different fungal pathogens to control mosquito populations. Considering all the above circumstances, effort has been made to evaluate the efficacy of few naturally occurring soil borne entomopathogenic fungi for the control of *Ae. aegypti* mosquito.
Aim and objectives:

Several studies have been conducted to determine the pathogenicity of entomopathogenic fungi on *Ae. aegypti* larvae. However, scarcity of information has been noticed about the natively isolated fungal toxicity on the larvae of *Ae. aegypti* in this part of the state. The main aim of the present study was to isolate, screening, identification and evaluation of efficacy of soil borne fungi against *Ae. aegypti* larvae and the following objectives were selected for the present study.

1. To isolate, screening and identification of fungal strains from soil.
2. To evaluate the efficacy of the fungal isolate against *Aedes aegypti* larvae.
3. To evaluate the effect of the potent fungal isolates on the developmental stages of *Aedes aegypti*. 