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2. Review of Literature

2.1 Fungi

Fungi are considered as one of the major clades with eukaryotic life. They have a wider geographical distribution compared to plants and other organisms (Hernández-Restrepo et al., 2017). This hyper diverse group of microorganisms spread in various terrestrial and aquatic ecosystems by employing different strategies such as pathogenic, saprobic or symbiotic and are able to adapt themselves or escape from changing environmental conditions. The number of species for this remarkable and diverse group has been estimated to be between 1.5 and 5.1 million since many habitats have remained unexplored (Hawksworth, 1991; Blackwell, 2011). Fungal mode of growth varies and can range from unicellular to multicellular forms with the ability of producing fruiting bodies. Based on phylogenetic analysis, fungi are divided into four main groups including: Ascomycota, Basidiomycota, Chytridiomycota and Zygomycota and the majority of plant pathogenic fungal species belong to these groups (Hawksworth, 1991; Agrios, 2005). Among soil microbial community, fungi take place after bacteria as the second most abundant group of soil microbiota. However, fungi sometimes exist as the most dominant microbial biomass in a specific soil. Some of them are plant pathogens and can cause severe economic losses. Serious economic losses on vegetables and crops can happen due to soil borne diseases. Some of the important pathogenic fungal genera are *Fusarium*, *Pythium*, *Rhizoctonia* and *Sclerotinia*. Handling and controlling soil borne disease is normally difficult because pathogens can survive and build up their populations through their persistent structures for several years (Lumsden and Ayers, 1975; Hancock, 1977; Peethambaran and Singh, 1977).

Some fungi are responsible for decomposing plant residues (Zhang et al., 2014b; Hernández-Restrepo et al., 2017). The filamentous hyphae of fungi enable them to explore and exploit more environments for food and translocate and store deficient nutrients to distant parts of the soil where nutrients may be lacking, allowing reproduction and growth to continue. Moreover, fungal hyphal filaments decompose plant residues and organic materials by releasing enzymes which breakdown all these material to the absorbable form for fungi (Zhang et al., 2014b). Some fungi play a role as parasites of other fungi, plants, nematodes and insects. Improving soil structure and aeration is another role of the soil fungi. Some of them may form mutualistic relationship with plant and help the plant to uptake more nutrients (Win et al., 2012).

2.2 Role of fungi in soil

Soils are the foundation of all terrestrial ecosystems and are colonized by a variety of microorganisms such as bacteria and fungi, with an estimated 10^7–10^9 distinct bacterial species and 1.5-5 million fungal taxa worldwide (Blackwell, 2011). Soil microorganisms certainly play a central role in soil processes including recycling, transformation and making nutrients (Jackson et al., 2013; Tardy et al., 2015; Huang et al., 2016). Soil fertility and available nutrients for plant growth play an important role and provide the optimum conditions for increasing crop yields. Soil with higher fertility has more diverse mass of microbes, which lead to less availability of niches for pathogens to compete and less availability of nutrients for pathogens to utilize and proliferate (Martin and Hancock, 1986; Manjunath et al., 2017). Similarly, plant nutrition impacts environmental sustainability, cropping systems and human health (El-Ramady et al., 2014). Soil fertility depends on the proper balance of available nutrients during a particular time of a season for crop growth. However, sufficient quantities of these nutrients cannot guarantee their availability for plant as the other factors such as presence of salt or toxic elements or soil pH may restrict plant growth (El-Ramady et al., 2014). Availability of nutrients and acquisition by plants can be influenced by soil, plant and microbes. These factors vary from one ecological region to the other or even from one field to the other field in the same area. Plant diversity can be mediated through negative or positive feedback mechanisms of soil microbial communities.

Fungi along with bacteria are one of the most important groups of organisms that play critically important and different ecological roles in maintaining sustainability, vitality and health of ecosystem. They perform important services and different activities related to water dynamics and holding capacity, soil nutrient transformation and provision, soil structure stabilization and quality, organic matter decomposition and formation, recycling of wastes and detoxification, converting plant-derived compounds into usable forms, contributing to disease suppression and plant protection against phytopathogens, promoting plant health and growth, bioremediation, filtering of contaminants, carbon storage and regulation of greenhouse gas emissions and other ecosystem biochemical processes (De Gannes et al., 2013; Peay et al., 2013; Acosta-Martínez et al., 2014; Huang et al., 2015). However in some cases, they are responsible for plant diseases and devastation of crops. Soil fungi are normally endemic to a specific region and their community dispersal varies in nature and are influenced by several factors such as climate (Al-Azizi et al., 2013; Bernard et al., 2014; Esmaeili Taheri et al., 2017).

2.3 Type of fungi

2.3.1 Beneficial fungi

The ecological relationships and interactions with the surrounding environment can lead to beneficial to detrimental outcomes under particular environment and conditions (Pérez-Brocal et al., 2013). Symbiosis is defined as association among partners namely a host and symbiont. The host is the producer of resource while the symbiont is considered as the consumer of resource and may provide services (Pérez-Brocal et al., 2013). Also, switching between symbiosis and parasitism is possible. A variety of beneficial fungi may live on plant parts as contributor to provide plant nutrition or protect plants against pathogens (Chandna et al., 2014; Yurnaliza et al., 2014; Vasumathi et al., 2017).

Fungi play indispensable and critical roles in terrestrial ecosystems as decomposers, biogeochemical nutrient cyclers, soil aggregators, pathogens, and mycorrhizal symbionts (Barea et al., 2011; Bagyaraj, 2014; Guo et al., 2015; Heilmann-Clausen et al., 2015; Kohler et al., 2015). Three functional groups of fungi exist in soil ecosystems are: the saprotrophs, the mycorrhizas, and the lichens (Otten et al., 2004; Abed et al., 2013; Heilmann-Clausen et al., 2015). Saprophytic fungi can be named as the largest group of fungi with the ability to grow on dead organic matter (Al-Mazroui and Al-Sadi, 2016; Hernández-Restrepo et al., 2017). Saprotrophic fungi often dominate the surface layers of the soil profile, where they decompose shed plant litter, fallen trees and debris of animals or insects.

The term mycorrhiza (meaning fungus roots) was introduced by Albert Bernard Frank in 1885 (Siddiqui and Pichtel, 2008). Mycorrhizae are classified based on the arrangement of their hyphae in or around plant roots (Smith and Read, 2008). Currently, ectomycorrhizae and arbuscular mycorrhizae are described as the most abundant types of mycorrhizae, which occupy a majority of plant species except Brassicaceae and Chenopodaceae families (Smith and Read, 2008; Bagyaraj, 2014). Arbuscular mycorrhizal fungi (AMF) account for a frequency up to 50% of soil microbial biomass. Additionally, they can be found among the other plant species such as gymnosperms, bryophytes, and ferns (Lipson and Kelley, 2014). They enhance and modulate plant growth and tissue nutrient content which lead to an increase in their survival rates under various stressful conditions (Barea et al., 2011; Bagyaraj, 2014; Lipson and Kelley, 2014; Van Geel et al., 2015). In contrast, just 3% of vascular plants are associated with ectomycorrhizal fungi but they are dominant in forest ecosystems (Lipson and Kelley, 2014). Arbuscular mycorrhizae are classified as obligate biotrophs while ectomycorrhizae are considered as saprotrophs and they can access more to soil nutrients compared to AM fungi. Mycorrhizal symbioses have been accepted universally as fundamental potent for soil health and quality restoration which result in better nutrient capture for plant (Smith and Read, 2008; Barea et al., 2011). Mycorrhizal hyphae significantly enhance the surface area for plant roots by changing their physiology and architecture. Mycorrhizal fungi produce several times more hyphae which efficiently acquit mineral nutrients under even nutrient limiting conditions, activate dissolvable nutrients and transport them back to the plant (Siddiqui and Pichtel, 2008; Smith and Read, 2008; Bagyaraj, 2014; Cavagnaro, 2014; Kohler et al., 2015; Van Geel et al., 2015). Numerous findings provided insight onto the propensity of mycorrhizal fungi in bioprotection against biotic and abiotic stresses such as pathogens, pests, drought, salinity (Smith and Read, 2008; Barea et al., 2011; Heilmann-Clausen et al., 2015).

The term endophytic fungi was coined by de Bary in 1886 that refers to systemic symbiotic fungi that occupy living plant tissues (root, leaf or transmitted to their host seed) without causing any pathogenic effect (Araújo et al., 2002; Sudha et al., 2016).

Forest pathologists consider them as minor or secondary pathogens. Overall, endophytes interactions can range from mutualistic to parasitic (Lipson and Kelley, 2014). Depending on host species and fungi, endophytes play different ecological roles in different fields such as protecting their host against herbivores, pathogenic organisms and drought stress. They can also increase nitrogen uptake and stimulate root growth (Khan et al., 2016; Chhabra and Dowling, 2017; Hussin et al., 2017; Kotilínek et al., 2017; Li et al., 2017). They are famous for producing antibacterial, antifungal or insecticidal secondary metabolites for use in agriculture or biotechnology (Lipson and Kelley, 2014; Suman et al., 2016; Ting and Jioe, 2016). Noticeable insecticidal activity of endophytic fungi (*Claviceps purpurea*) against *A. gossypii* (Hemiptera: Aphididae) was reported (Zhang et al., 2010; Shi et al., 2013). Improving disease resistance, stimulating plant growth and withstanding environmental stresses are the other benefits of this group of microorganisms (Sturz et al., 2000). Endophytes are capable to enhance the plant growth and resistance by different mechanisms such as nitrogen fixation, increasing phosphorus uptake, production of plant hormones and siderophores. Endophytes play a role in phytoremediation process and enhancing tolerance of plants in soil accumulated with pollutants and contaminants. For example, cadmium tolerance in plant species inoculated with endophytes was reported by (Soleimani et al., 2010). Khan and Lee (2013) showed that the fungal endophyte, *Penicillum funiculosum* could act as a bioremediation agent in copper polluted area.

2.3.2 Plant Pathogenic Fungi

Diseases normally appear by infection and colonization of pathogens. Interestingly, certain pathogenic microorganisms are capable of infecting and colonizing species belonging to different taxonomic kingdoms. A pathogen or pathogenic microorganism is normally described as a biological agent that can cause damage on its host due to interaction between them. The damage can happen directly by the microorganism because of producing toxin or the other virulence factors or it can happen indirectly because of host immune activity (Casadevall and Pirofski, 1999). The ability of a pathogen to cause infection is defined as pathogenicity and the pathogen expresses it by means of virulence. The term ‘virulence’ defines the relative and quantitative degree of pathogenicity and it distinguishes a pathogen from a non-pathogen

(Casadevall and Pirofski, 1999). Pathogenic microorganisms that cause diseases are traditionally classified as obligate, facultative and opportunistic pathogens. Obligate pathogens are distinguished by their ability to cause infection only within their narrow host range. The groups of pathogens that can cause infection within their narrow host range but also can survive outside the host in the environment are called facultative pathogens. Opportunistic pathogens are defined as low virulence group of microorganisms that can thrive on organic substrates. They are capable of attacking injured or compromised in immune response host (Baarlen et al., 2006).

One of the well-known examples of detrimental effects of fungi is the potato late blight disease during the 19th century in Europe. This disaster was caused by a fungus called *Phytophthora infestans* on potato. This invasion led to potato famine in Ireland, nearly one million people died during 1846-1860 and more than 1.5 million people emigrated from there (Agrios, 2005). In addition, fungal pathogens can be a potential threat for natural ecosystems, trees and forest. Dutch elm disease caused by *Ceratocystis ulmi* is one of the catastrophic diseases on UK and North American landscapes (Brasier, 1990). Another detrimental example of fungi is chestnut blight fungus caused by host specific fungus (*Cryphonectria parasitica*) and as a consequence of this disease, almost all native chestnut trees (*Castanea dentata*) were destroyed in America (Hepting, 1974). Some fungal pathogens such as *Phytophthora cinnamomi* has a broad host spectrum and can attack at least 48 various families of plants (Weste and Marks, 1987). The fungus *Discula destructive* is another invasive pathogen, which caused anthracnose disease on *Cornus* spp. and nearly 80% tree mortality in the USA (Hiers and Evans, 1997). Mango wilt, caused by Ceratocystis Mangifera, killed over 200,000 trees in Oman within 5 years (Al-Adawi et al., 2006). Damping-off and vine decline diseases of cucurbits can result in 75% losses in seedlings and plants (Al-Sadi et al., 2011c; Al-Sadi et al., 2012a).

Furthermore, some fungi produce toxic compounds, which are harmful for plants, vertebrates and invertebrates. For instance, *Metarhizium anisopliae* produces destruxins and cytochalasins and *Trichoderma harzianum* secretes peptaibols. Toxins may play a role in pathogenesis or contribute to overcoming the host defense to the fungus (Goettel et al., 2001). The toxin of the fungal pathogen *Fusarium nygamai* has a potential to be used as a mycoherbicide against weed (*Striga hermonthica*) but this has been banned due to the threat for human, animals or vertebrates (Capasso et al., 1996; Goettel et al., 2001).

2.4 Impact of plants and microbial communities on each other

Plants depend on soil microorganisms for recycling nutrients and similarly, soil microbes rely on plants for their energy requirements. Collaboration among them results in change in soil physiochemical characters, climate and atmospheric CO_2. In comparison with bulk soils, plants release their photosynthesis products and root exudates into the soil and by this way they enhance microbial growth rates and assembly (Igiehon and Babalola, 2017). For example, arbuscular mycorrhizal fungi produce a substance called glomalin that improves soil structure, stabilizes soil aggregates and prepare more favorable structure for root growth (Lipson and Kelley, 2014). Quality and quantity of plant residues have a profound influence on microbial community. Eviner and Chapin (2003) reported the influence of plants on soil microclimate and chemistry which leads to the effect on nutrient biochemical cycling and microbial processes. However, in some cases compounds from plant residues may have negative effects on mineralization rates (Cesco et al., 2012). Generally, decomposition of plants from stressful ecosystems is harder than plants in high fertility soils. Plants surviving in stressful and unsuitable environments are more protected but produce low quality litter, which ends up to low mineralization rates. In contrast, high quality soils lead to high quality litter, more active microbial community and high mineralization rates (Lipson and Kelley, 2014). Eviner and Chapin (2003) showed that plants may regulate microbial processes due to their effect on soil water, temperature and pH. Some plants such as cucumber or tomato are capable to secrete organic acids, alter pH around the root and enhancing the growth of specific microbes. It has been demonstrated that continuous cropping of soybean in a particular land can cause transformation in the soil from bacterial community to fungal community. Continuous cropping with soybean can change soil pH from neutral to acidic which is desirable for fungi (Tanaka et al., 2012). Continuous cropping of soybean besides providing favorable conditions for beneficial fungi such as arbuscular mycorrhizal fungi, provides desirable environment for root rot causing pathogens due to the increase of some substances such as sugars and amino acids (Igiehon and Babalola, 2017). Some crops such as cereals can show inhibitory activity towards specific groups of microbes by secreting metabolites into the rhizosphere (Lipson and Kelley, 2014). Moreover, culture independent studies have shown that microbial community is different in the rhizosphere of different plant species and also transplanting a plant species in soils can change their microbial community. Similarly, previous studies showed that colonization rate among various cultivars of soybean was different (Igiehon and Babalola, 2017). However, some species such as *Rhizophagus irregularis* have broad geographical range and are not environment specific. Different plant genotypes and cultivars can be favorable for various pathogenic microorganisms (Dias et al., 2012; Lipson and Kelley, 2014).

2.5 Cultivation systems in Oman

Oman is located in the South Eastern part of the Arabian Peninsula and borders with Saudi Arabia, United Arab Emirates and Yemen in the west and south respectively (Fig. 2.1). Also it borders with Indian Ocean in the eastern side. Various crops are cultivated in the sultanate of Oman such as alfalfa, wheat, barely, maize, tobacco, etc.

Figure 2.1 A map illustrating geographical locations of Oman (Source: google).

There are various biotic and abiotic constrains to agriculture in Oman. The most important abiotic problems include limited water resources for irrigation and salinization of soil, while the most challenging biotic agents include plant pests and diseases. In the summer, climate in Oman can vary from hot and humid in the coastal area to hot and dry in the interior parts and in the winter it is comparatively cool. The rainfall is low and ranges from 50-100 mm based on the area (interior or coastal area). However, some areas which are influenced by monsoon such as Dhofar mountains experience rainfall up to 300 mm. Agroclimatic regions in Oman are recognized based on cropping patterns and land and water resources potential. One of the most important crops which is cultivated in Oman is date palm (Al-Zidjali, 1995). Farms may also have citrus or various crops such as tomato and cucumber. Conventional farming system is the most common farming system in Oman and Arabian Peninsula. This farming system is defined as cultivating several crops such as date palms, acid limes, mangoes, tomatoes and cucumbers in plots and irrigation is done by flowing water or utilizing ground water resources (Al-Sadi et al., 2014; Al-Sadi et al., 2015a; Yaish and Kumar, 2015). Application of animal manures, pesticides, chemical fertilizers and composts are so common in conventional farming systems (Al-Sadi et al., 2010; Al-Sadi et al., 2011c; Al-Sadi, 2012; Al-Sadi et al., 2012b). However, few organic farms appeared in Oman during the last few years, growing several crops, mainly cucumbers and tomatoes.

2.6 Cucumbers and Tomatoes

Cucumber *(Cucumis sativus L)* is native to India or South Asia (Rubatzky and Yamaguchi, 1997), where it has been cultivated in Western Asia for 3,000 years. Cucumbers spread to other parts of the world like Europe, China, and other cucumber producing countries during different time. *Cucumis sativus* belongs to the family *Cucurbitaceae*. This family includes watermelon, cantaloupe, squash, cucumber and others. Cucumber is an annual crop that moderate temperatures and high humidity.

Cucumber is important in different parts of the world (Fig. 2.2 and 2.3). The total production of cucumbers in the world reached 80 million tons in 2016, with most production being in China (FAO, 2017). Cucumber is the most important and the most widely cultivated crop in greenhouse in Oman. Most cucumbers are produced in Al Batinah region in Oman. Cucumbers are usually produced in greenhouses three times a year (Al-Kiyumi, 2006). There are three main cultivar types of cucumbers: processing (pickling), fresh market (slicing) and greenhouse (slicing). Pickled varieties are used as salad ingredients, while slicing varieties are usually eaten as fresh. Most cucumbers in this part of the world are used in salad (Wittwer and Honma, 1979).

Figure 2.2 Cucumber production in a greenhouse (10 day-old seedlings).

Figure 2.3 Forty-day old cucumber plants in a greenhouse.

Cucumber is affected by several diseases, the most important of which are damping-off, vine decline, powdery mildew, downy mildew, root galls and viral diseases (Al-Sadi et al., 2011b; Al-Sadi et al., 2011c; Al-Mawaali et al., 2012; Al-Sadi et al., 2012a; Hatami et al., 2013; Le et al., 2015; Dombrovsky et al., 2017; Maina et al., 2017). In Oman, damping-off, vine decline, downy mildew, powdery mildew and fruit rot are the most common diseases (Al-Kiyumi, 2006; Deadman et al., 2007; Al-Sadi et al., 2011c; Al-Mawaali et al., 2012). Damping-off and vine decline diseases result in mortalities up to 75% in some greenhouses (Al-Sadi et al., 2011c). The diseases are caused by *Pythium aphanidermatum*, *P. spinosum*, *Rhizoctonia solani* and *Fusarium solani*.

Tomato originated in South America, mainly in Peru and Ecuador. Tomatoes were first domesticated and cultivated in Central America by Indian civilizations of Mexico (Rubatzky and Yamaguchi, 1997; Simone and Mullin, 1999). Then tomatoes were spread to Europe by the Spanish and then they were spread to Italy, Turkey and Asia. Tomato (*Lycopericun esculentum* Mill.) is one of the top vegetable crops worldwide (Fig. 2.4). It belongs to the nightshade family, *Solanaceae*.

Figure 2.4 Tomato production in an open field.

Tomatoes are rich in vitamins, minerals, sugars, essential amino acids and dietary fibers (Rubatzky and Yamaguchi, 1997). Tomato fruits are consumed fresh in salads or cooked. They can be processed into juices and ketchup. Total worldwide production of tomatoes in 2016 reached 177 million tones, with China, India, USA, Turkey and Egypt being the top producers (FAO, 2017). In Oman, tomatoes are the top vegetable crop in terms of production, with a total production of 111000 tones in 2016 (FAO, 2017). It is mostly grown in open fields.

Tomatoes are affected by several diseases. Leaf curl viral diseases are common in different countries and can result in serious losses (Ammara et al., 2015; Al-Shihi et al., 2016). Early blight and late blight cause economic losses to growers (Gannibal et al., 2014). In Oman, viral diseases as well as early and late blight diseases are common and cause economic losses (Moghal et al., 1993; Al-Shihi et al., 2014; Khan et al., 2014). Soil borne diseases are also present but their effects are less compared to foliar diseases (Moghal et al., 1993).

2.7 Damping-off disease

Damping-off is a serious disease of several vegetable crops, forest trees and other plants in tropical and temperate climates all over the world (Al-Hinai et al., 2010; Deadman et al., 2010; Al-Azizi et al., 2013; Hatami et al., 2013; Peña et al., 2013; Saleh et al., 2013).

In cucumber, damping-off is a serious problem in the USA, Canada and Oman (Hendrix and Campbell, 1973; Plaats-Niterink, 1981; Sumner et al., 1983; Al-Sa'di et al., 2007).

Pythium species are the most common species associated with damping-off disease of cucumbers (Hendrix and Campbell, 1973; Stanghellini and Phillips, 1975; Abol-Wafa et al., 1976; Plaats-Niterink, 1981; Sumner et al., 1983; Howard et al., 1994; Paul et al., 1995; Gubler and Davis, 1996; Paul et al., 1996). Nevertheless, the disease can sometimes be caused by *Rhizoctonia solani* and some *Fusarium* species (Lida et al., 1983; Howard et al., 1994).

At planting, cucumber seeds can immediately become infected by *Pythium* species which gives rise to softening and brown discoloration of the seeds, shrivelling followed by disintegration. Upon seed germination, the emerging radicle and plumule can be attacked, resulting in a soft rot and dark brown-to-black, water-soaked lesions that rapidly spread over the entire seedling killing it before it emerges out of the soil (pre-emergence damping-off). In many cases, infection by *Pythium* species occurs after germination, leading to appearance of post-emergence damping-off disease (Fig. 2.5). Infection usually occurs at the roots, at or just above the soil level, resulting in water-soaked areas on the emerging stem where the basal part becomes thinner as a result of degradation of the tissue by enzymes which leads to collapse of the seedling. When cells of stems and main roots develop secondary thickenings with age, infection may become restricted to feeder roots weakening the plant but not killing it in most cases (Hendrix and Campbell, 1973).

Figure 2.5 Damping-off symptoms of cucumber.

Development of damping-off disease in usually starts with random infections of cucumber seedlings followed by systematic expansion of infected plants (Deadman et al., 2002; Deadman et al., 2005). This is usually attributed to the distribution of different levels of inoculum in the soil and the suggested involvement of secondary inoculum in the expansion of the focal points (Deadman et al., 2002). The level of *Pythium* inoculum in soil often shows both spatial and temporal fluctuations. Spatial distribution is not just a characteristic of the variations in the level of inoculum between fields or greenhouses, but most importantly variations within the same greenhouse and also the depth at which the inoculum is present (Martin and Loper, 1999). Vertical distribution of the inoculum levels in soil can vary based on the distribution of roots and crop debris that are available for saprophytic colonization by *Pythium* species (Pankhurst et al., 1995b; a; Martin and Loper, 1999). Temporal variations can be expressed by differences in the amount of inoculum throughout the year (Hancock, 1977), which are affected mainly by the availability of host, organic substances and conditions favouring growth and reproduction. In the absence of a host, *Pythium* species can survive saprophytically on organic matter in the soil (Hendrix and Campbell, 1973; Martin and Loper, 1999) or as resting structures for a few days to several years (Hoppe, 1966; Stanghellini and Burr, 1973) and may become a source for infection once a host is available.

2.8 Cultivation systems versus fungal diversity

The aim of organic farming system is to optimize output, food safety and maintain soil fertility, soil health and productivity by optimizing soil biological activity. Organic farming system is practiced by crop selection and rotation, tillage, water management and augmentation of beneficial microorganisms to act as biopesticide without causing environmental pollution (Sreenivasa, 2012). Some major concerns regarding conventional farming system are the cost of chemical fertilizers, their effect on human and animal health, non- target microorganisms, biodiversity, environmental pollution and global warming. The sustainability in farming system can be obtained when nutrients are supplied by organic fertilizers or in combination with chemical fertilizers. Crop combination is a key factor in organic farming system, which leads to improvement of soil microflora, enzymes and proper use of land and water resources. Sreenivasa (2009) demonstrated a positive correlation among application of organic matter and soil fertility and biodynamics. Studies conducted to compare the effect of organic and inorganic fertilizers on land revealed that the amount of available C, N, P, enzyme activity and soil microflora was higher in organic manure applied farm (Sreenivasa, 2009). Organic farming system consists of numerous principles. For example, in this system healthy soils are obtained by incorporation of crop residues, biofertilizers, and green manures, adoption of crop rotation and better use of natural resources. Healthy plants are obtained by avoiding diseases and pests by using safe treatments such as traps, biopesticides and applying multiple cropping or crop rotation to destroy disease habitat. All agricultural operations and managements have to be done in harmony with agroecosystem and encouraging biodiversity. Crop production should be done based on National Standards for Organic Production (Sreenivasa, 2012). Organic system is considered as more profitable system and several studies have shown and compared the effect of organic farming system on evenness, richness, and dispersion of soil microorganisms (Fließbach et al., 2007). Similarly, it has been shown that adaptation among natural residues on plants and crops and compatibility between the biofertilizers and soil condition occurs more rapidly while the adaptation in commercial agriculture takes place slowly (Pagano et al., 2017).

Proper soil management and strategies to stimulate soil microbes can have an impact on soil decomposition. For example, applying perennial cultivated pastures and legumes results in change in soil organic matter content, higher C sequestration, improved sustainability, and reduction of greenhouse gases (Pagano et al., 2017). Some tropical pastures such as *Urochloa decumbens* have large root systems which contribute to greater amount of root decomposition, better gas exchange and water infiltration (Alvarenga et al., 2005). Climate change influences soil moisture and temperature and this will have an effect on the way plants respond to climate change. Plants may tolerate and persist but trees cannot cope with climate change easily (Booth and Tranter, 2015). Different microbial species such as non-pathogenic *Fusarium oxysporum* or rhizobacteria can be used to protect plants from stress or promote their growth (Lugtenberg, 2015).

On the other hand, conventional or modern agriculture depends on chemical fertilizers and pesticides to maximize crop production and cover food demands since human population is growing (Santos et al., 2012; Saramanda and Kaparapu, 2017). Soil as a sink of pesticides residues may eventually affect microorganisms (Jain et al., 2014). Abdel-Mallek et al. (1994) showed that the degree of pesticide effect on microorganisms depends on environmental factors, concentration, toxicity, persistence and bioavailability of pesticides. Exploitation of industrially manipulated chemical fertilizers leads to water and air pollution including atmospheric CO_2, soil degradation, emission of greenhouse gases and reduced biodiversity (DeLonge et al., 2016). It has been demonstrated that some pesticides are able to disturb molecular interactions between rhizobacteria and plants and inhibit nitrogen fixation process (Saramanda and Kaparapu, 2017). Likewise, pesticides by having impact on microbial and enzymatic activities can affect soil biochemical processes and nutrient dynamics (Monkiedje et al., 2002; Antonious, 2003; Demanou et al., 2004; Niewiadomska, 2004; Kinney et al., 2005; Mahía et al., 2008). Saramanda and Kaparapu (2017) demonstrated the destructive impact of fungicides and herbicides on *Aspergillus* species compared to insecticides. However, some pesticides such as Glyphosate herbicide have a positive effect on soil health and some fungicides can cause increase in organic matter which leads to increase in microbial activity (Araújo et al., 2003). Similarly, some microorganisms degrade pesticides and increase their population size and activity by consuming the degradation products (Das and Mukherjee, 2000; Kumar and Philip, 2006; Hussain et al., 2007).

2.9 Biological control

2.9.1 Definition of biological control

Biological control or sometimes shortened to biocontrol is a cost-effective and environmentally safe means which can be defined as employing exotic natural enemies (biocontrol agents) such as insects, pathogens, predators or parasitoids to suppress undesirable invasive populations of pests below damaging levels or make it less abundant than it would be. This suppression can be done in many ways. It is a kind of a long-term solution for management which helps mitigate the economic losses and cost of control through chemicals and their side effects on human, environment and ecological balance in the future (Bhardwaj et al., 2014).

2.9.2 History of biological control

There are historical records that can trace the evolution and development of biological control, which shows the remarkable insights about. Without these discoveries and conceptualizations, modern biological control would have been delayed. These concepts include symbioses between different species, natural control and natural enemies, role of natural enemies in abundant determination.

The recorded history of applied biological control may be referred to Egyptian dates, which were recorded almost 4000 years ago where house trained cats were portrayed as useful mean for controlling rodents. Learning more about small natural enemies became possible with the microscope invention by van Leeuwenhoek in the 1600s (Bosch et al., 2013). In the seventeenth century in Italy Vallisnieri for the first time recognized insect parasitism between *Apanteles glomeratus* (parasitic wasp) and *Pieris rapae* (cabbage butterfly) (Bosch et al., 2013). Destruction of cabbage caterpillar infestation through ichneumon fly was noted by Erasmus Darwin in 1800. Hartig proposed the idea of collecting and storing of parasitized caterpillar for later release (Bosch et al., 2013). Agostino Bassi in 1835 showed that the cause of disease of silkworm larvae (*Bombyx mori*) was a fungal pathogen (*Beauveria bassiana*) (Hajek, 2004). Inhibition between plant pathogenic microorganisms was demonstrated by Potter (Potter, 1908).

2.9.3 Types of biocontrol agents

Biocontrol agents are competent fungi and bacteria from rhizosphere that are capable of exhibiting antagonistic activity to reduce damage by plant pathogens and producing growth stimulators (Stewart et al., 2010; Kashyap et al., 2017; Pagano et al., 2017). Microbial community can be found in every kind of habitat, rhizosphere, phyllosphere or occurring on specific plant surfaces such as roots, leaves, flowers, etc. Isolation and evaluation of theses microbes and their antagonistic activity against diseases may provide a source of biocontrol agents (Saleh et al., 2013; Raza et al., 2017; Zhang et al., 2017). Phylloplane antagonists such as bacteria or epiphytic filamentous fungi were found to effective against several fungal diseases. Several studies reported the successful control of plant diseases using biocontrol agents, including soil-borne pathogens and foliar diseases (Al-Mazroui and Al-Sadi, 2015; Lopes et al., 2015; On et al., 2015; Wang et al., 2015; Alaniz Zanon et al., 2016; Arroyave-Toro et al., 2017; Yuan et al., 2017; Zhao et al., 2017). Some of the fungal species used as biocontrol gents include *Trichoderma harzianum, T. viride, Pythium oligandrum, Aspergillus* spp., *Talaromyces* spp. and others (Madi et al., 1997; Abdelzaher et al., 2000; Benítez et al., 2004; Mbarga et al., 2012; Naraghi et al., 2012; Vongphachanh et al., 2016; Manjunath et al., 2017; Vasumathi et al., 2017) (Fig. 2.6).

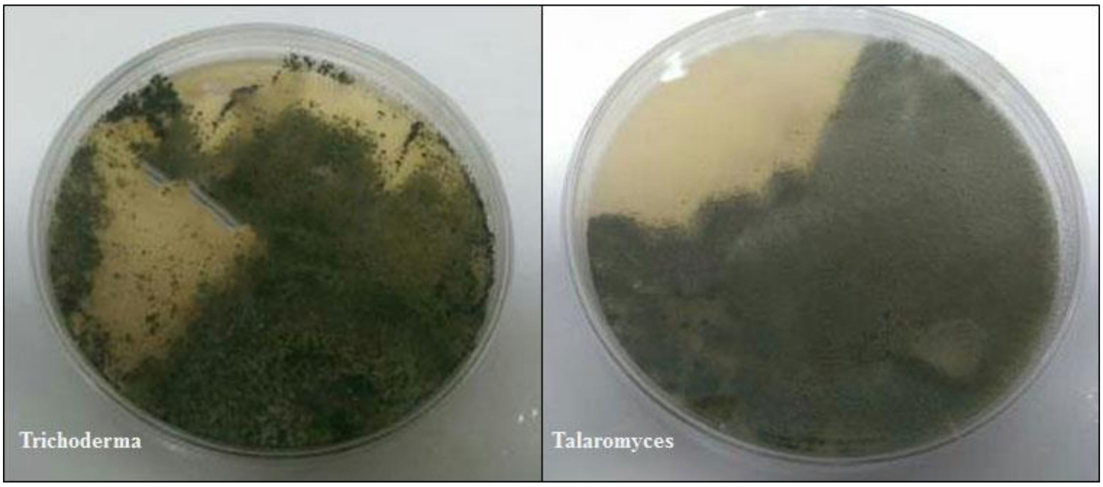

Figure 2.6 A plate of *Trichoderma asperellum* and *Talaromyces pinophilus*

Rhizosphere and rhizoplane are considered as a rich and diverse ecological niche of microorganisms. The rhizosphere is a thin layer of soil close to the root and subsequently gets influenced by root exudates, rhizodeposit nutrients and mucilages (Igiehon and Babalola, 2017; Kazerooni et al., 2017). Rhizosphere microorganisms have an effect on plant diversity directly or indirectly through various mechanisms that lead to interaction between plants, symbionts and antagonists (Cesco et al., 2012; Zhang et al., 2014a; Lenc et al., 2015; Igiehon and Babalola, 2017). Biotic agents such as arbuscular mycorrhizae fungi (for example, *Glomus intraradices* and *Rhizophagus irregularis*) have an impact on plant growth and nutrition (Igiehon and Babalola, 2017). Pear brown spot (*Stemphylium vesicarium*) was reduced by almost 57% by *Erwinia herbicola* and *Pseudomonas fluorescens* isolates, which were isolated from several plant roots and aerial parts (Montesinos and Bonaterra, 1996; Montesinos et al., 1996). Among many bacteria, actinomycetes isolated from roots of watermelon showed their ability to control watermelon Fusarium wilt (Larkin et al., 1996). Plant growth-promoting rhizobacteria (PGPR) such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas putida* which are normally isolated from disease suppressive soils besides being beneficial and growth enhancer, can also be used as biocontrol agents to suppress important diseases (Rohini et al., 2016). The other source of biocontrol agents, which has been found effective, is manure based compost extract. Fortifying biocontrol agents with compost mixture was demonstrated to be useful for controlling numerous soil-borne diseases (Zhang et al., 1998).

2.9.4 Mechanisms of interactions with pathogens

Biocontrol of plant pathogens can be accomplished through direct and indirect strategies. Direct biocontrol can be achieved by employing a specific microbe that interferes with growth and survival of a pathogen (Stewart et al., 2010). Various mechanisms are employed by biocontrol agents for controlling plant diseases. These different modes of interactions are classified into: mutualism, protocooperation, commensalism, neutralism, competition, parasitism, predation, induced resistance and antibiosis.

Direct antagonistic activities result from activities that involve physical contact or high degree selectivity of targeted pathogen. On the other hand, an indirect antagonistic activity does not involve physical contact and stimulating plant defense is the good example of this type of activity. The most effective biocontrol agents are those that use multiple mechanisms to antagonize targeted pathogens. *Pseudomonas* spp. are good examples for multiple mechanism biocontrol agents and are well known to produce antibiotics, induce host defense, colonize roots aggressively and suppress pathogens by competition for nutrients (Al-Hinai et al., 2010; Rohini et al., 2016; Collazo et al., 2017; Raza et al., 2017).

Mutualism is a kind of a life-long interaction between two organisms in where both of them get benefit from this association (Hawksworth, 1991; Lipson and Kelley, 2014). Many studies have proven that in this symbiotic relationship, by physical and chemical contact and exchanging mineral and the other organic resources between them, they increase their chance of survival in spite of difficult conditions (Powell and Klironomos, 2006; Robb, 2007; White Jr and Torres, 2010; Hamilton and Bauerle, 2012; Mandyam and Jumpponen, 2014). A well-known example of this type of relationship are plant and mycorrhizal fungi (Kageyama et al., 2008; Barea et al., 2011; Kohler et al., 2015; Igiehon and Babalola, 2017). This mutualistic collaboration can lead to biological control by activating host defense mechanisms and improving plant strength by exchanging nutrients. Rhizobium bacterium (nitrogen fixing bacteria) is another well-known example of this type of relationship (Esechie et al., 1998;

Naluyange et al., 2014; Suman et al., 2016). It increases and reproduces by mutualistic association with legume plants and can lead to biological control by providing nutrients for plant or stimulating its defense mechanisms.

In protocooperation, survival and growth of both organisms can occur in the absence of each other (Fowler and Garcia, 1989; Liu et al., 2009; Maslova and Dochevoy, 2016). In other words, their survival doesn't completely depend on each other. A good example of this ecological interaction is soil fungi, bacteria and plants that are growing in soil. Soil bacteria and fungi cooperate with each other and produce essential nutrients for plants' survival.

Commensalism is another type of symbiotic relationship between two organisms, where one of them derives benefits from this association and the other one remains unaffected by it or in other words the other one will not be harmed or benefit from this relationship (Tseng and Phillips, 1981; Hirsch, 2004; Hofstetter et al., 2006; Leung and Poulin, 2008). Mainly plant-associated microorganisms are assumed to be commensals in relation to their host.

Neutralism describes the biological interaction between two organisms that in the same microhabitat, they grow individually in different niches without affecting each other (Ayala, 2000; Nei, 2005). In other words, it is better to say no interaction between them and the population density of one of them absolutely will not have any effect on the other one.

Competition can be defined as an interaction among organisms that results in reduction in growth rate, fecundity or activity of one of them. It occurs when a non-pathogen competes with a pathogen for water, nutrient, oxygen and space in and around the host plant for limiting disease incidence and severity (Essarioui et al., 2017; Guijarro et al., 2017; Jabed A. Choudhury et al., 2018). By rapid colonization of the host where water and nutrients are most available such as damaged epidermal cells and exhausting available substrates, nothing will remain for pathogen to grow. Furthermore, these microbes produce metabolites, which can also suppress pathogens. *Trichoderma* and *Gliocladium* are very efficient soil borne saprophytic fungi. They are able to colonize nutrient aggressively and also survive as chlamydospores and conidia when nutrients are not sufficient (Stewart et al., 2010).

In parasitism, the activity of a parasitoid as a natural enemy that parasitizes a plant pathogen can lead to biocontrol and reduction of a plant disease (Davanlou et al., 1999; Hirsch, 2004; Leung and Poulin, 2008). In this type of interaction that occurs between two unrelated organisms, one of them (parasite) benefits from the other one for growth and reproduction and the other one is harmed. Parasitism interactions are recognized from non-specific to highly specific. Mycoparasitic interactions can range from biotrophic mycoparasitism to necrotrophic mycoparasitism. Biotrophic mycoparasitism is a kind of an obligate relationship and nutrients are obtained from living cells and sometimes can cause harm to the host. On the other hand, a necrotrophic mycoparasitism leads to destruction of the host and nutrients are obtained from dead or dying host. Some of the most potential mycoparasites are *Trichoderma* spp., *Talaromyces flavus*, *Gliocladium virens*, *Coniothyrium minitans* and *Sporidesmium sclerotivorum* (Stewart et al., 2010). *Trichoderma* spp. is a good example of this type of relationship, which parasitizes fungal plant pathogens by excreting enzymes. *Trichoderma* extends hyphal branches toward *Pythium* hyphae, coils around it and digests it by exerting a variety of enzymes with antifungal activity including chitinase, glucanase and protease (da Silva et al., 2016; da Silva et al., 2017; Kashyap et al., 2017; Mayo et al., 2017; Sharma et al., 2017). Another successful example is *Pythium oligandrum* which parasites *Pythium* species (Abdelzaher et al., 1997; Al-Rawahi and Hancock, 1998; Godfrey et al., 2003; Takenaka et al., 2003). Sometimes one pathogen can be attacked and parasitized by multiple parasites. For instance, powdery mildew pathogen can be parasitized by *Ampelomyces quisqualis*, *Cladosporium oxysporum* and *Gliocladium virens* (Kiss, 2003). Some mycoparasites are capable of parasitizing hard-to-eradicate fungal resting structure like sclerotia. *Coniothyrium* is a successful biocontrol agent against sclerotia of *Sclerotinia sclerotiorum, S. minor, Botrytis cinerea* and has been commercialized under the name Contans® and Koni® (Chitrampalam et al., 2010; Öhberg and Bång, 2010; Van Beneden et al., 2010; Chitrampalam et al., 2011).

The term predation is used to describe an interaction between organisms in which one of them captures the pathogen biomass and resources for consumption, sustenance and survival (Aït Hamza et al., 2017; Al-Shorbaji et al., 2017). This term normally refers to animals that kill and feed their pray in the macroscopic world. Also this term has been applied to the action of microorganism such as fungal feeding nematodes that consume the biomass of pathogens (Steel et al., 2018).

Besides responding to different environmental stimuli such as temperature, light or stress, plants are also able to respond to chemical stimuli, which are exerted by soil or plant microbe association. The stimuli from some biocontrol agents act as elicitors to increase plant tolerance to pathogen infection by inducing and enhancing resistance in that plant. Based on different factors such as source, type or amount of stimuli, host defense can be systemic or local (Benhamou and Bélanger, 1998; Karmakar et al., 2003; Liang et al., 2005). Haas and Defago (2005) and Harman (2004) reported that certain strains of *Trichoderma* sp., which act as biocontrol agents strongly elicit host defense (Hanson and Howell, 2004; Bisen et al., 2016).

Antibiosis is mediated by the production of specific or non-specific toxic substances, volatile compounds and lytic enzymes. These active compounds can work at very low concentrations and play a role as growth inhibitor or inhibit metabolic activity of microbes. Fungal genera such as *Trichoderma* produce antimicrobial compounds (M.S.Leelavathi et al., 2014; Sharma et al., 2017).

2.9.5 Factors affecting biological control

2.9.5.1 Abiotic factors

Various environmental factors influence microbial activities. Hence, tolerance to these abiotic factors is a key for their successful biocontrol activity. Vänninen et al. (2000) reported that the persistence of *Metarhizium anisopliae* was poor in peat compared to clay soils. Temperature is another abiotic factor that has an impact on fungal biocontrol activity. Based on previous studies, most soil borne fungal biocontrol agents are mesophyllic and grow well normally between 15-30 °C, thus their biocontrol activity may constrain in winter (Stewart et al., 2010). For example, mycoparasitism activity of *T. harzianum* against sclerotia of *Sclerotinia sclerotiorum* was reduced at 15 °C

(Knudsen et al., 1991). On the other hand, the optimal temperature for degradation activity of *Trichoderma viride* against sclerotia of *S. cepivorum* was at 10 °C and this parasitism activity was reduced by increasing the temperature (Clarkson et al., 2004). Soil moisture has a clear impact on fungal biocontrol activity. Spore germination, mycelial growth and conidial survival do not occur in dry soils (Stewart et al., 2010). Besides water activity, pH is another important parameter that has an influence on mycoparasitism activity and efficiency (Kredics et al., 2004). Mycelial growth of *Trichoderma* spp. was optimum at pH 4 and water activity of 0.997 (Kredics et al., 2004). Numerous factors such as agricultural practices, fertilizers or organic content have an impact on soil pH. Sometimes, acidic conditions are more favorable for some fungi such as *Metarhizium anisopliae* (Padmavathi et al., 2003). Mineral nutrition such as carbon and nitrogen was reported to be essential for growth, sporulation and secondary metabolite production of microbial community as well as disease suppressiveness (Al-Azizi et al., 2013).

2.9.5.2 Biotic factors affecting biological control

Soil augmentation with a single organism would not always lead to successful establishment as the other microbial populations may show inhibitory activity against it. Soil microorganisms, plants and invertebrates are identified as the three major biotic factors that have an impact on biocontrol persistence and efficacy (Stewart et al., 2010). For example, entomopathogens persist in soil environment and they cannot produce enough inoculum in the absence of their host (Kaya et al., 1988; Padmavathi et al., 2003; Culebro-Ricaldi et al., 2017). Thus, their growth, germination and survival are affected by soil microbe metabolites. Similarly, antagonistic or competitive activity of microorganisms in the rhizosphere may lead to a decline of biocontrol agents in the soil. Inhibition of conidial germination of *T. virens* by ectomycorrhizal fungus *Laccaria laccata* has been reported (Zadworny et al., 2004). Plant species and tillage practices have an impact on persistence of fungi. Plant root exudates due to their nutrients can support microbial population development.

2.9.6 Commercial biocontrol products

Under different circumstances that have happened because of excessive usage of chemical fertilizers and pesticides (agrochemicals), as well as fear mongering by some pesticides and health consciousness of the people, exploitation of living organisms to reduce plant pathogen inoculums or activity seems to be a suitable alternative to chemicals. Many antagonists that have shown consistent results over years have been commercialized. The United States Environmental Protection agency registered *Trichoderma harzianum* ATCC 20476 as the first fungus for plant disease control. EPA has registered almost 12 fungi and 14 bacteria for plant disease control (Fravel, 2005). These commercialized biocontrol products account for only 1% of the agricultural control measures (Fravel, 2005). In this direction, some of the commercially available products have been recorded based on fungal or bacterial antagonists nationally or internationally and have entered into the world market in recent years. Some of these commercial products which utilize *Trichoderma* sp. as an active ingredient are BioGuard, Soilguard, Biocon, Fstop, or Ecofit and with *Bacillus* as an active ingredient are Mycostop, Rhizoplus and Subilex (Junaid et al., 2013). Various steps are involved in commercialization process of biocontrol products, which include searching and screening of microorganisms with desirable traits from its natural ecosystem. This step depends on the crop, cropping system and pathogen and usually a desirable isolate will be selected from leaves, roots and rhizosphere of healthy plant in infested field with target pathogen. *In vitro* and *in vivo* evaluation of the isolate will be done to examine its efficiency and compatibility under different conditions (Al-Hinai et al., 2010; Wang et al., 2015). Formulation is considered as a critical obstacle in bio-agent commercialization. A formulation must be easy to handle, stable over specific range of temperature and having desirable shelf life and viability (minimum 2 years at room temperature). At the end, registration and release of suitable isolate by considering its toxicity and residual activity will be carried out (Junaid et al., 2013).

2.9.7 Evaluations of biological and chemical controls

Biological control is considered as a part of the permanent natural control for suppressing pest population and density while chemical control can decimate localized pest population temporarily and immediately (Al-Sadi, 2012; Bosch et al., 2013). Pesticides along with their side effects can cause tremendous benefits. Improving productivity and higher yields can be expected from their use. Several factors may have effect on increases in productivity, which include high yield varieties of seed, agricultural chemicals, fertilizers and irrigation system. Pesticides are an inevitable and integral part of productivity process by reducing diseases, pests, weeds or the other factors which can reduce the amount of crop yields noticeably (Sukul and Spiteller, 2000; Saramanda and Kaparapu, 2017). The other benefit that can be achieved by chemical control is controlling vector borne diseases (Kingdom, 2013; Queiroz et al., 2016).

However, long-term use of agricultural chemicals has led to serious health effects on human and environment. Their detrimental effects on soil fertility, degradation of beneficial microbes and development of resistant pathogens are undeniable (Al-Sadi et al., 2015a; Manjunath et al., 2017). Their excessive use ends in non-sustainable agriculture, which is far from the need to develop sustainable farming, ecofriendly and long-term management. Farm workers, formulators, mixers or sprayers are categorized at high and increased risk groups for exposing to chemicals.

Biological control of damping-off disease caused by *Pythium* species gained increased popularity during the 1990s and is still a new area of research. Suppression of damping-off disease by fungal biocontrol agents has been attained through the use of *P. oligandrum* Drechsler, *P. nunn* Lifsh, Stangh. & Baker and *Trichoderma* species and a few other fungal species (Martin and Loper, 1999). When applied to tomato seedlings before transplanting, *P. oligandrum* significantly reduced damping-off caused by *P. ultimum* and *P. aphanidermatum* and was as efficacious as metalaxyl (Martin and Semer, 1992). Paulitz and Baker (1987) reported a decrease in population densities of *P. ultimum* and greater emergence of cucumber seeds when *P. nunn* was added to soil and seeds, respectively. In a greenhouse trial to evaluate the efficacy of *T. harzianum* Rifai in the management of damping-off of cucumbers, Deadman *et al*. (2002) reported *T. harzianum* to give a level of control equivalent to metalaxyl. Bacterial strains of *Pseudomonas fluorescens* and some other species are the most commonly reported fluorescent pseudomonads used to suppress damping-off (Martin and Loper, 1999). Some reports indicated their successful biocontrol in greenhouses (Rankin and Paulitz, 1994) and fields (Weller and Cook, 1986).

Although some biocontrol agents are very effective in the management of damping-off disease, their use has encountered some difficulties. A report by Fang and Tsao (1995) indicated a reduced seedling growth of sweet orange when *P. nunn* was applied at a high concentration to control *Phytophthora* root rot. Some bacterial strains have a short shelf life (Powell and Jutsum, 1993) and are affected by soil conditions (see Martin and Loper, 1999). A requirement for the effective control of damping-off disease is that the biocontrol agent should be active immediately after application. Another important consideration is for the biocontrol to be integrated with other disease and pest management strategies which are implemented in a given area.

The lack of studies in Oman on fungal diversity in conventional and organic farming systems as well as the limited amount of work, which is available in Oman, on the potential biocontrol agents that can be used for the management of soil borne diseases necessitates a detailed study to be carried out in this area.