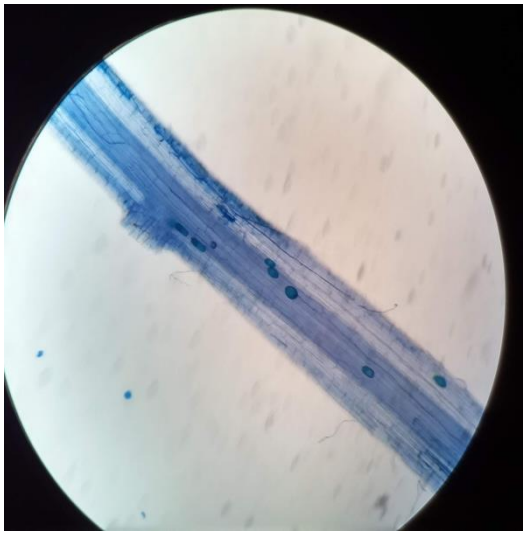
Chapter 1

Review of Literature



1. Review of Literature

1.1. Food Security and Plant Diseases

Food security is essential (Strange & Scott, 2005). Population growth is accompanied by a rise in the demand for food. Increased food production is required to meet the growing need for a growing human population's stable, nutritious, and consistent food supply. More than 800 million people lack access to sufficient food; 1.3 billion people live on less than 1 US Dollar a day, and at least 10% of global food production is lost to plant disease (Christou & Twyman, 2004; James, 1998; UNICEF, 2020). Agricultural production has nearly doubled in the last four decades due to current agricultural practices emphasizing intensive farming and the widespread synthetic fertilizers and pesticides usage. While fertilizer use has risen rapidly, yield potential for the most important food crops has stagnated, and studies have shown that this development in agricultural output is unsustainable and inconsistent.

Additionally, agricultural growth and uninterrupted cropping deplete soil organic matter, making the soil less fertile, which results in a higher need for synthetic fertilizers (Fox et al., 2007). Crop yields have declined over the last two decades, despite increasing fertilizer application. Meanwhile, modern agricultural cropping practices, which involve intensive continuous cultivation of a single crop year after year, have exacerbated plant disease episodes, nearly tripling the use of harmful chemical pesticides (Fox et al., 2007; Oerke & Dehne, 1997). Thus, there is an urgent need to boost crop yields in a scarce agricultural area and contain losses caused by plant diseases. Controlling plant diseases is one of the most effective strategies for retaining as much of a crop's current productivity as feasible (Talbot, 2010).

Plant diseases significantly threaten agricultural productivity worldwide, accounting for nearly 10% to 30% of the global harvest each year (Strange & Scott, 2005). A plant disease is any disturbance that impairs a plant's normal development and diminishes its commercial or visual worth. Plant infection impairs the wellbeing of a certain portion of the plant, resulting in decreased yield and quality. Plant diseases are typically classified into two categories according to their cause: 1) Non-infectious or abiotic plant diseases: These are caused by genetic or environmental factors such as nutrient deficiency, temperature extremes, toxic chemicals (air pollution, pesticides, or excessive fertiliser use), mechanical injury, or drought. These diseases

cannot be spread to healthy plants, and their control is entirely dependent on resolving the underlying cause; 2) Infectious or biotic plant diseases are caused by living organisms that feed on the plants as parasites. Fungi, bacteria, nematodes, and protozoa are the most aggressive plant pathogens (Agrios, 2005).

Plant infections caused by fungi account for most disease cases in plants. Most plant diseases are caused primarily by fungi. Damage is done to plants by the destruction of cells and/or the induction of stress. There are numerous sources of fungal infections, including infected seeds, agricultural waste, weeds, and neighbouring crops (AUSVEG, 2022). Various fungi can cause foliar diseases, including Downy and Powdery mildews, as well as White blister. Many other fungi, such as *Clubroot, Pythium, Fusarium, Rhizoctonia, and Sclerotinia spp*., are responsible for soil-borne diseases. Fungi can reproduce both sexually and asexually, making them an excellent source of genetic diversity. Plant tissue, living or dead, can support their growth and they can even persist in the soil as latent organisms until favorable conditions allow them to flourish. These pathogens are capable of penetrating or growing on the plant's surface (Morton, 2021).

Plant diseases are difficult to control because fungal spores, which are like seeds and can be spread through the wind and water, as well as soil, animals, and agricultural equipment (Talbot, 2010). For instance, in the 1840s, potato blight, caused by *Phytophthora infestans*, struck Europe likes “a bolt from the blue.” Around a million people perished from malnutrition in Ireland, and more than a million people made unsuccessful attempts to leave the country (Large, 1940; Strange, 2003). Similarly, the rice brown spot disease *Bipolaris oryzae* caused millions of fatalities and displaced families and social institutions during the Bengali famine (Padmanabhan, 1973). Chestnut blight caused by *Cryphonectria parasitica* (Rigling & Prospero, 2018) and Dutch elm disease caused by *Ophiostoma novo-ulma* took off a significant percentage of primary and secondary forestry in North America and Europe, causing ecological devastation (Brasier, 1991). Additionally, numerous plant pathogens produce mycotoxins that endanger the health of humans and animals directly or indirectly (Awuchi et al., 2021).

Complex interactions among plants, pathogens, and the environment result in plant diseases (He et al., 2021). The disease also made it necessary to find ways to control it so farming could

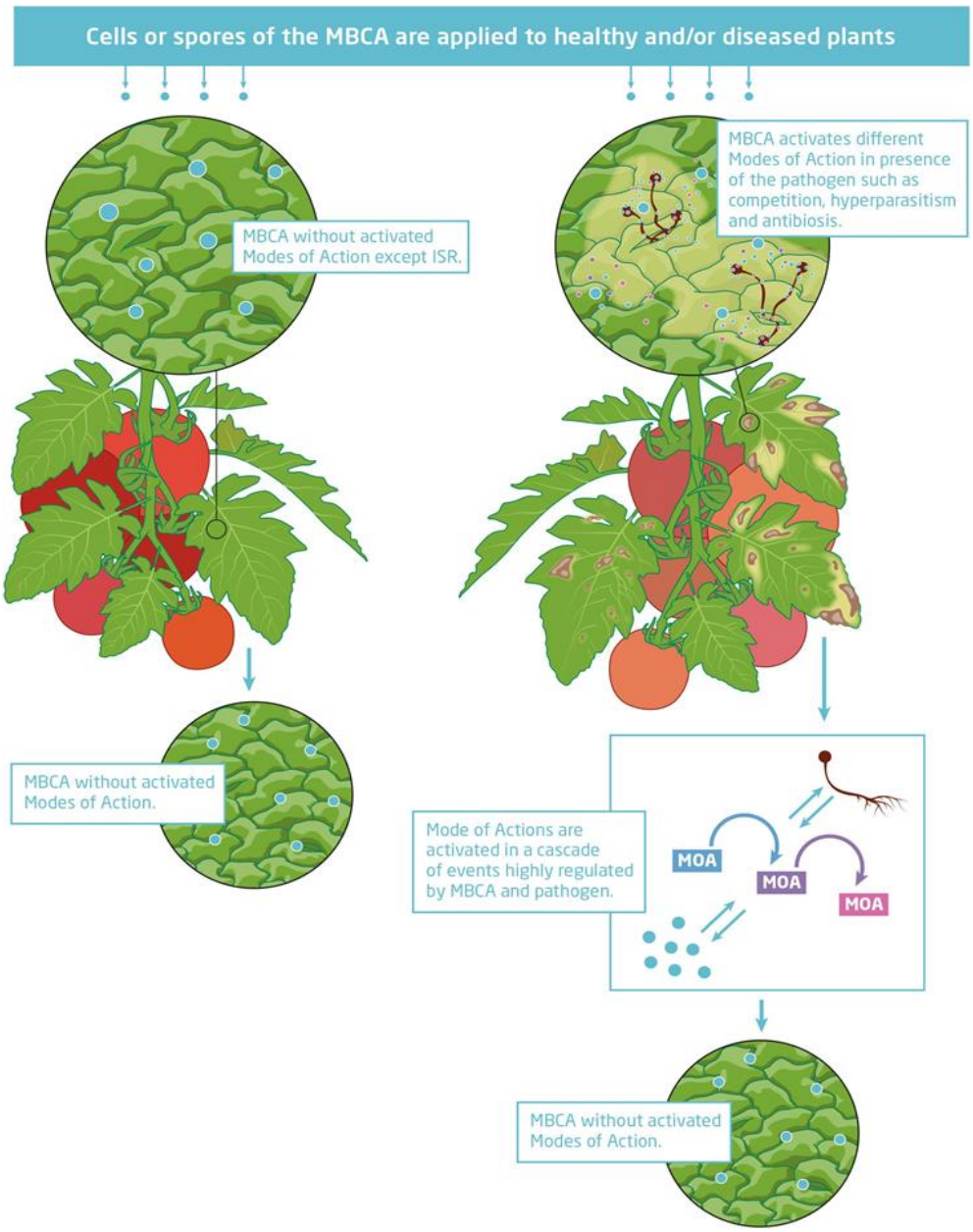
progress. The main focus of the thesis is the control of fungal infections in plants due to their important involvement in plant diseases.

1.2. Control Strategies of Fungal Disease in Plants

1.2.1. Biological control of plant diseases

The concept of biological control may be traced back over 4,000 years to Egypt, where it was first conceived (Jones, 1975). However, biological control was not seriously researched at a high level until the 19th century (Waage & Greathead, 1988).Plant infections can be suppressed through introduced or resident living organisms, such as bacteria and fungi, rather than disease-resistant host plants (Pal & Gardener, 2006). It is common in entomology to refer to biological control and its abbreviated form "biocontrol" as "the application of live predatory insects, entomopathogenic nematodes, or microbial diseases to suppress populations of a wide range of insect pests." Plant pathologists also use the word to denote the use of microbial inhibitors to avoid disease outbreaks. Biocontrol agents (BCAs) and microbial biocontrol agents (MBCAs) have been identified in a variety of microorganisms, including bacteria and fungus (Pal & Gardener, 2006).

Plant pathogens can also be controlled by living organisms, known as biological control (Heimpel& Mills, 2017). Crops are protected from disease damage by microbial biological control agents (MBCA). They interact with the targeted pathogen in real-time; activating multiple modes of action in a series of events (Figure 1.1).They may confer or improve resistance to pathogen infections in plant tissues without interacting directly with the pathogen (Conrath et al., 2015; Pieterse et al., 2014). It is possible that using BCAs on a crop will provide disease control that is on par or even better than using fungicides. Fungicide treatment of *Phytophthora cactorum*-infected apples resulted in complete disease suppression, whereas the application of various BCAs separately resulted in degrees of disease suppression varying from 79% to 98%, depending on the BCAs employed (Alexander & Stewart, 2001).



Source: Köhl et al., (2019)

Figure 1.1: Microbial biological control agent (MBCA) in Action**.**

1.3. Types and Mechanisms of biological control

1.3.1. Types of Biological Control

Bacillus subtilis, *Ampelomyces quisqualis*, and other antagonistic bacteria reduced the severity of various soil-borne diseases, which prompted researchers to consider utilizing BCAs to manage plant diseases (Miljaković et al., 2020; Su et al., 2020). There has been a paradigm shift in the study of biological control since that time. The three types of BCAs that He et al., (2021) identified based on the mechanisms through which they function are as follows:

1.3.1.1. Suppressing Pathogens

Several bacteria are parasitic, producing chemicals or antibiotics to compete for nutrition and niche, while others are hyperparasites that create antibiosis to kill pathogens directly or rely on pathogens for energy (Alvindia, 2018; Hou & Kolodkin-Gal, 2020; Raaijmakers & Mazzola, 2012).These characteristics are shared by several fungi, mycoviruses, and bacteriophages. In some cases, they may be BCAs that have been modified to combat plant diseases and applied once or numerous times in a field (Abbas et al., 2019; van Lenteren et al., 2018).To manage plant disease, secondary metabolites and chemicals can be produced by non-microbial or microbial organisms. Plants can produce pathogen-killing or helpful microbe-promoting substances to defend themselves from infection (Vorholt, 2012). These chemicals can be extracted from plants and coupled with the metabolism of antimicrobials or helpful microbes, such as BCAs (Brescia et al., 2021; Kim et al., 2019). For instance, numerous bacterial and fungal endophytes create a plethora of secondary metabolites with antagonistic, inhibitory, and deterrent qualities that act as deterrents against plant diseases (Card et al., 2016; Köhl et al., 2019). Antibiosis is activated in endophytic BCAs by various secondary metabolites they generate (Brader et al., 2014).

Pseudozyma flocculosa releases a chemical that causes the pathogen's cells to collapse rapidly, making it an efficient BCA for controlling powdery mildew (Bélanger et al., 2012). *Pseudomonas chlororaphis* produce phenazines, pyrrolnitrine, 2-hexyl, 5-propyl resorcinol, hydrogen cyanide, siderophores, and a complex mixture of volatile chemical compounds that are effective against a variety of plant diseases and nematodes (Raio& Puopolo, 2021). *Fluorescent pseudomonads* have been utilized to combat various harmful microbes (Haas & Défago, 2005). Avirulent strains of pathogen species can also be used for biological control. Intra-pathogen

competition is well illustrated by developing resistant *Aspergillus flavus*-linked genotypes that reduce aflatoxin contamination of cotton and other crops (Cotty & Bhatnagar, 1994).

1.3.1.2 Compounds Priming, Inducing, or Strengthening Plant Defense Responses

It is possible for beneficial microorganisms to enhance plant defence and immunity without directly encountering pathogens (Conrath et al., 2015; Renseigne, 2006). In addition to naturally occurring substances, these agents can be derived from a variety of synthetic and natural sources, such as plant extracts, microbial metabolites, synthetic compounds, and genetic material (Pal & Gardener, 2006). Some of the most important secondary metabolites for plant defence and immunity are salicylic acid and acetylsalicylic acid, as well as nitric oxide (Pusztahelyi et al., 2015). Hashem et al. (2019) found that these compounds are what cause systemic acquired resistance in plants that have been infected by pathogens. Many other non-pathogenic microbes, like rhizobacteria, can also produce these compounds (Contreras-Cornejo et al., 2016). Several of these inducer compounds have positively affected plant health and vigor, possibly due to increased hormone production (Berg et al., 2017). *Sclerotina sclerotiorum* disease is reduced by 16–30% when harzianolide, produced by *Trichoderma harzianum*, is applied to tomato plants. Harzianolide increases tomato plants' growth and defensive mechanisms (Cai et al., 2013). Similarly, field treatments with chitosan salicylic acid and humic acid substantially reduced *Fusarium solani* and *Rhizoctonia solani*-caused root rot disease of green beans by 60–80 %(El-Mohamedy et al., 2017).

1.3.1.3 Regulating the Ecosystem to Protect and Promote Natural Enemies orcompetitors of Pathogens

The only way biological control works is if there are predators, competitors, promoters, and other species in a healthy ecosystem. The genetics, composition, and structure of local plant and microbial communities determine where and when these helpful species move in crop fields (Kremen et al., 2007).The beneficial interaction between the microbiome and other soil organisms is critical to maintain a functional environment that supports plant growth and immune development. Methanol inhibits the growth of methanotrophs that cohabit with *Hyphomicrobium* sp. to form a microbial association in the rhizosphere, capable of enhancing

nutrient consumption efficiency and eliminating toxic methanol from the rhizosphere (Liechty et al., 2020). Biological control seeks to improve the quality of the environment by increasing the multitude of beneficial microorganisms in farmlands. This can be done with techniques like crop rotation, intercropping, and cultivar variation, which are all forms of agricultural diversification.There is abundant evidence that crop diversification can aid in the prevention of plant diseases (Heet al., 2021; Zheng et al., 2019). Disease suppression through crop diversification is aided by inoculum dilution, physical barriers to prevent pathogen transmission, Pathogenicity improvement, fungicide resistance, and evolutionary changes (Guzman et al., 2021; Zhu et al., 2000).

1.3.2. Mechanisms of Biocontrol

Biocontrol agents (BCAs) can directly and indirectly affect plant pathogens. The general biological control mechanism can be broken down into these two types of effects. Antibiotics and lytic enzymes are produced, the pathogen enzymes are inactivated, and parasitism occurs. These are just a few examples of the direct impacts. Changes in the host plant's structure and biochemistry can indirectly affect its ability to handle stress, such as the solubilization or sequestration of inorganic nutrients or an increase in resistance (Viterbo et al., 2002).

1.3.2.1. Direct Mechanisms of Biocontrol

1.3.2.1.1. Competition

Compared to non-rhizospheric soil, the rhizosphere of plants is nutrient-dense because it works as a carbon sink (Degenhardt et al., 2003). Phytopathogens and other microbes are drawn to nutrient-rich niches along root surfaces and in the surrounding rhizosphere, producing competition for nutrients and space (Welbaum et al., 2004). In order to preserve equilibrium, biocontrol frequently employs competition for resources and habitats. Biocontrol agents must be rhizospheric competent to be effective. In order to be effective, biocontrol agents must be able to colonize plant roots and survive and proliferate along them for a long time in the presence of their native microflora. The O antigens of lipopolysaccharides (LPS), prototrophy for amino acids and vitamin B1, growth rate, ability to use root exudates, NADH dehydrogenase and type IV pili are all implicated in bacterial root colonization. It has been discovered that *Fluorescent pseudomonads*, an effective root coloniser, reduce the number of major and mild infections (Van

Peer et al., 1990). *Pseudomonas aeruginosa* PNA1 isolated from Chicken pea plant rhizosphere has been proven efficient against various pathogens, including phytopathogens (Anjaiah et al., 2003).

Once colonized, the BCA's survival and reproduction in the rhizosphere depend on its capacity to utilize root exudates efficiently and compete for available nutrients (Canarini et al., 2019).

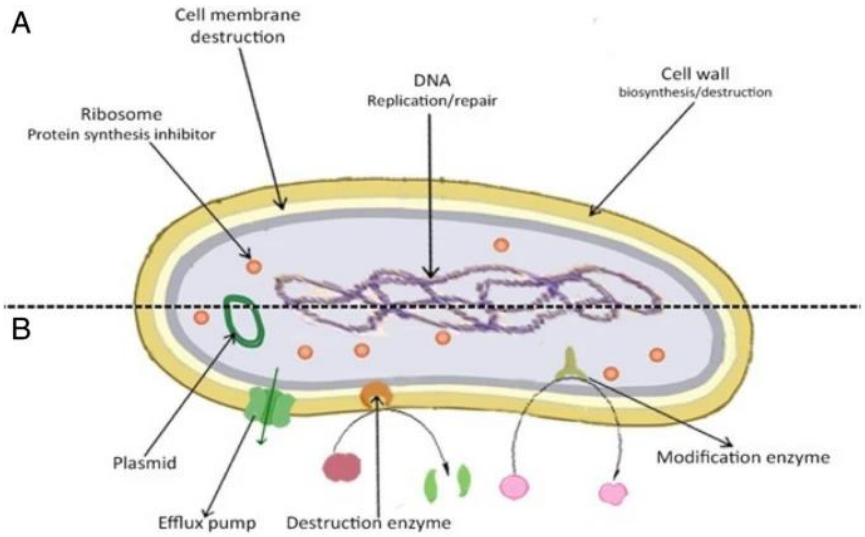
A siderophore (an iron-binding ligand) and an uptake protein are typically used when it comes to iron uptake. The fact that different organisms can use the same siderophore type is an intriguing aspect of siderophore biology. If a microorganism has the necessary absorption protein, it can use the siderophores of another microorganism (Joshi et al., 2008). The growth of other bacteria can be inhibited by iron shortage by a more adaptive strain of bacteria that can absorb siderophores generated by different other bacteria.Competition for iron has been identified as a critical mechanism for *Trichoderma sp*. biocontrol of *Pythium sp*. and *Botrytis cinerea*. Pseudobactins are a family of siderophores produced by *fluorescent pseudomonads,* structurally complicated iron-binding molecules. Competition is a widely used strategy for fungus control in which the antagonist and pathogen are intimately connected (Islam et al., 2005). Numerous studies have been conducted employing non-pathogenic Fusarium to control the fungal wilt caused by *Fusarium sp.* (Lemanceau & Alabouvette, 1991; Ogawa & Komada, 1984; Sajeena et al., 2020). Competition for nutrients and infection will arise between the two because of their closeness. The fact that they compete with one another for carbon sources and infection sites has been established (Islam et al., 2005; Larkin & Fravel, 1998).

1.3.2.1.2. Antibiosis

The most common way microorganisms conflict with one another is through antibiosis (Haggag & Mohamed, 2007). Antibiotics or compounds that act like antibiotics, lytic enzymes, volatile substances, Siderophores, or other dangerous substances can cause antibiosis. There is evidence that several biocontrol agents can produce antibiotics, compounds similar to antibiotics, or enzymes as secondary metabolites.

1.3.2.1.2.1.Antibiotics

Bacteria living in the soil produce low molecular weight organic compounds, such as antibiotics, that inhibit other microorganisms growth or metabolic processes at low concentrations (Haggag& Mohamed, 2007). Microorganisms of all kinds may be killed or inhibited by antibiotics, depending on how they are administered (Leclère et al., 2005). In addition to bacterial protein biosynthesis, antibacterial drugs can also target bacterial cell wall biosynthesis, bacterial cell membrane permeabilization, DNA replication and repair, and metabolic pathway inhibition (Figure 1.2) (Khameneh et al., 2019).



Source: Khameneh et al., (2019)

Figure 1.2: Mode of Actions of Antibiotics in Bacteria (A): Proven targets for antibacterial drugs. Various antibiotic families, including macrolides, tetracyclines, and aminoglycosides, target the ribosome's protein production. Certain antibiotics, such as polymyxin B, have the ability to specifically target the cell membrane. By altering and destabilizing the bacterial outer membrane's permeability, these antibiotics reduce bacterial resistance. By trapping a DNA complex bound to the enzyme DNA Gyrase, fluoroquinolone antibiotics impede DNA replication. Antibiotics of many types hinder cell-wall biosynthesis;(B):Multiple antibiotic resistance mechanisms in bacteria; Efflux pumps remove the antibiotics from bacteria (e.g. Fluoroquinolones and trimethoprim resistance in *P. aeruginosa*). Destruction enzymes that degrade the antibiotics (β-lactams in Enterobacteriaceae). Modifying enzymes which change the antibiotic structure (e.g. chloramphenicol or fosfomycin in *P. aeruginosa*).

Even though many bacteria produce antibiotics, *Streptomyces* and *Fluorescent pseudomonads* have been investigated extensively. The Biocontrol ability of the producing strain has been attributed to many antibiotics produced by actinomycetes. Macrolide benzoquinones, aminoglycosides, polyenes, and nucleosides are examples of metabolites produced by Actinomycetes (Trejo-Estrada et al., 1998). Streptomyces has been the source of approximately 60% of the antibiotics discovered for agricultural application (Tanaka & Omura, 1993). In addition, Fluorescent pseudomonads create severalpotent disease-suppressing molecules, making this bacterial group the most intensively studied antibiotic producer in the rhizosphere (Handelsman & Stabb, 1996). Despite rising evidence that they produce antibiotics and may aid in efficient disease suppression.

Antibiotics produced by *Bacillus subtilis*, a gram-positive bacterium, can be classed as ribosomal or nonribosomal. Surfactin, iturins and fengycin are non-ribosomally synthesized circular oligopeptides with a fatty acid chain that have potent antibacterial and antifungal properties (Zuber et al., 1993).Iturins and fengycins provide compelling antifungal properties and suppress the development of numerous plant diseases. Numerous biocontrol fungi have also been found to produce antibiotics, in addition to bacteria. The two organisms with the most research have been Trichoderma and Gliocladium. Each produces antimicrobial substances and prevents disease in various ways, producing antibiotics with complex structural makeup like gliovirin and gliotoxin (Howell et al., 1993; Howell & Stipanovic, 1983).

1.3.2.1.2.2. Mycoparasitism and production of extracellular enzymes

Direct competition between two species in which one is getting resources from the other is known as parasitism. Hyperparasitism occurs when the host is also a parasite, such as a plant pathogen. Fungi frequently engage in this type of interaction. Hyperparasitism in bacteria is quite unusual. As a predatory bacterium, *Bdellovibrio bacteriovorus* uses the cytoplasm of other Gram-negative bacteria as food (McNeely et al., 2017). Biotrophic mycoparasitism is a form of mycoparasitism in which the hyperparasite is dependent on the fungal host and obtains nutrition from the host cells via haustoria without harming the host. The interaction between the host and the mycoparasitic fungus is stable and balanced (Jeffries, 1995). While these frequently species-specific interactions may contribute significantly to disease suppression in ecosystems, they are

unlikely to be harnessed for commercial augmentative biocontrol due to the hyperparasite's mass production requiring living host mycelium as substrate (Köhl et al., 2019).

Necrotrophic hyperparasites obtain nutrients from dead host cells and other readily available organic matter, allowing for mass production on artificial media, making this group of hyperparasites significantly more suitable for commercial use as microbial use biological control agents than biotrophic hyperparasites. After killing host spores or hyphal cells, necrophilic hyperparasites penetrate them. The primary method of parasitism is the excretion of cell wall degrading enzymes close to the host cell, which results in holes in the cell wall and subsequent cytoplasmic disarray. Cell walls are normally degraded by a variety of chitinases, β-1, 3-glucanases, and proteases, or, in the case of oomycota hyperparasites, cellulases (Köhl et al., 2019). It is widely recognized that bacteria, particularly actinomycetes, can parasitize and destroy the spores of fungal plant diseases (El-Tarabily et al., 1997). *Trichoderma sp*., a fungal biocontrol agent, can parasitise a wide range of fungi and exert direct biocontrol. The most significant function in this process is played by cell wall-degrading enzymes (CWDEs), which are produced by biocontrol agents (Nusaibah & Musa, 2019).

Phytophthora cinnamomi root rot of *Banksia grandis* was obtained using a cellulase-producing isolate of *Micromonospora carbonacea* (El-Tarabily et al., 1996) and control of *Phytophthora fragariae* var. *rubi* Hickman causing raspberry root rot was suppressed by the application of actinomycete isolates that were selected for the production of β-1,3-, β-1,4- and β-1,6-glucanases (Valois et al., 1996). Chitinolytic enzymes produced by both *Bacillus cereus Pantoea agglomerans* also appear to be involved in the biocontrol of *Rhizoctonia solani* (Chernin et al., 1995; Chernin et al., 1997; Pleban et al., 1997).

1.3.2.1.2.3.Volatile compounds

Soil fungistasis has been linked to many volatile compounds produced by soil microorganisms. Fungistasis is a widespread phenomenon mediated by soil microorganisms and volatile organic compounds (VOCs) (Chuankun et al., 2004). These volatile inhibitors can be organic or inorganic (Chuankun et al., 2004; Ko et al., 1974). There are several soil-derived VOCs that have been shown to reduce or inhibit the spore germination of various fungal species, including ethylene (Hora & Baker, 1970; Smith, 1973), ammonia (Ko et al., 1974; Ko & Hora, 1974), allyl

alcohol, and acrylic acid (Balis, 1976). Alkaline or neutral soil was the most common habitat for most fungistatic compounds (Liebman & Epstein, 1992, 1994; Lockwood, 1977).

1.3.2.2. Indirect mechanism of biocontrol

Numerous rhizosphere microorganisms may indirectly protect plants against diseases by encouraging their growth. These microorganisms are called Plant Growth Promoting Rhizobacteria (PGPR) (dos Santos et al., 2020). An increased plant growth makes plant healthier and more resistant to disease attacks.PGPR promotes plant growth in two ways. Each mechanism is associated with many parameters that affect plant growth (Figure 1.3). The direct mechanism includes parameters relating to the production of phytohormones (Cassán et al., 2009), such as auxins (Khalid et al., 2004); siderophores (Yu et al., 2019); phosphorus solubilization (Krey et al., 2013); and nitrogen-fixing (Riggs et al., 2001). The indirect biocontrol strategy involves antagonistic action against phytopathogenic microbes, eliciting systemic resistance responses in plants, interfering with bacterial quorum sensing (QS) systems, etc. One or more of these methods may be used to boost plant development by PGPR, according to some studies (Ahmad et al., 2006; Bashan & Holguin, 1997).

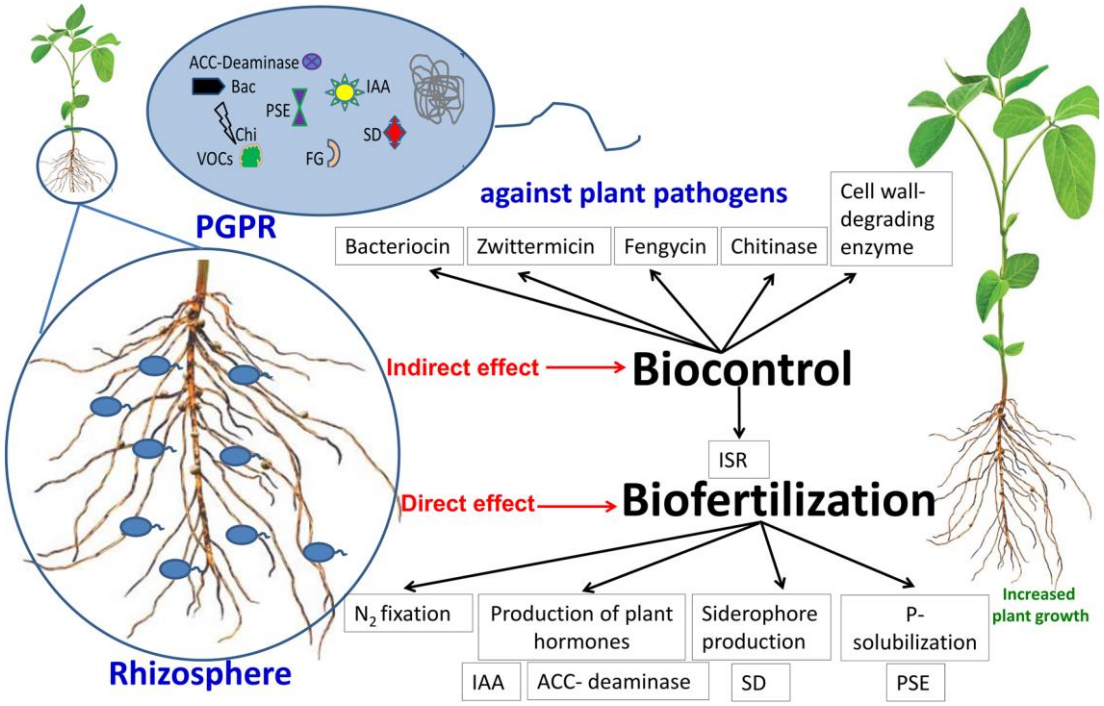


Figure 1.3: Mechanism of action of PGPR.ACC-deaminase, *Bac*bacteriocin, *Chi* chitinase, *SD* siderophore, *FG* fengycin, *ISR* induced systemic resistance, *IAA* indole-3-acetic acid, *PSE* phosphate solubilisation enzymes, *VOCs* volatile compounds, modified from Jouzani et al., (2017) and Azizoglu, (2019).

1.3.2.3. The increasing availability of nutrients to plants

Some plant growth promoters, such as *Rhizobium* and *Bradyrhizobium*, form root nodules of leguminous plants and fix nitrogen as ammonia, which the plant can use as a nitrogen source. Others can increase the phosphates availability to plants by dissolving phosphates bonded to organic or inorganic materials. *Azospirillum* is one bacterium that can improve plant growth by promoting root development and water and mineral absorption (Lugtenberg & Kamilova, 2009).

1.3.2.4. Phytohormone production

There are many PGPRs that are capable of producing auxins (Gupta et al., 2015; Omer et al., 2004) to exert powerful impacts on root growth (Jha & Saraf, 2015) and architecture (Vacheron et al., 2013). The most frequently researched auxin generated by PGPR is Indole-3-acetic acid (IAA). Microbe-plant interactions are facilitated by this molecule (Afzal et al., 2015; Ahemad & Kibret, 2014). The function of exogenous IAA in plants is reliant on endogenous IAA levels. Plant growth may be neutral, positive, or negative depending on the concentration of bacterial IAA used (Spaepen & Vanderleyden, 2011). Inducing longer roots, increasing root biomass and decreasing stomatal density and activating auxin response genes that boost plant growth (Ruzzi & Aroca, 2015)have been induced by auxin-producingPGPRs (Spaepen et al., 2014; Llorente et al., 2016).

Many PGPRs synthesize cytokinins and gibberellins (Gupta et al., 2015; Kumar et al., 2016), although the role of bacterial-synthesized hormones in plant and bacterial synthesis mechanisms is not yet fully known (García de Salamone et al., 2001; Kang et al., 2009). Some strains of PGPR can promote relatively large amounts of gibberellins, leading to enhanced plant shoot growth (Jha & Saraf, 2015). These hormones and auxins interact with each other to affect root structure (Vacheron et al., 2013). When plants produce cytokines, the exudate from their roots is

likely to be more abundant (Ruzzi & Aroca, 2015), leading to more PGPR associated with the plant.

1.3.2.5. Induced resistance

Induced systemic resistance (ISR) is a term used to describe how some rhizobacteria strains enhance plant defenses against a wide range of plant diseases (Ramamoorthy et al., 2001). In *Dianthus caryophyllus* (carnation), Van Peer et al. (1991) found that the leaves were protected against *Fusarium oxysporum* f.sp. dianthi when they were treated with strain WCS417, while Wei et al. (1991) found that 6 out of the 94 strains of rhizobacteria tested protected the leaves of cucumber from anthracnose caused by *Colletotrichum orbiculare*. ISR improves defensive capability in those plants (Van Loon & Bakker, 2007). Because of this improved defence, plants infected with a severe pathogen develop disease more slowly, leading to fewer sick plants or an easier to treat condition. Systemic or localized resistance can be induced (Van Loon & Bakker, 2007). ISR establishment is dependent on plant roots recognizing bacterial elicitors. Flagella, cell envelope components, including lipopolysaccharides, and released metabolites like antibiotics, volatiles, and siderophores have all been shown to activate ISR in bacteria over the past decade (Bakker et al., 2007; Ongena et al., 2007).

1.4. Limitations of Biocontrol Agents

Despite offering an environmentally acceptable and cost-effective alternative to chemical control agents, many reasons hinder the development and commercial use of biocontrol agents as a primary or sole method for controlling plant diseases.The biological and environmental components of control and the aspects related to economic and social goals are two components of these factors.

1.4.1. Biological and ecological factors

Main major drawback of Biocontrol agent to take longer to take effect than conventional insecticides applied conventionally. From a few months to a few years, the time it takes for BCAs to establish themselves in fields and begin to have an impact can vary. As living organisms, BCAs are susceptible to various biotic and abiotic influences, which results in a range of performance levels. In addition, the cost of producing some biocontrol agents that necessitate

recurrent administration is a significant impediment. There are additional reported BCAs limitations biopesticides appear to be the more favorable, mainly proof another assumptions are relatively non-existent (Van Lenteren, 1993). A common criticism of biological control is that it controls while pesticides "eradicate." However, this is a false claim because biological control regulates pest populations, not eradicates them. It is preferable to reduce pest species over the long run rather than eradicate them through biological control. Even if a pest population is eliminated through chemical treatment, the ecosystem remains vulnerable to conquering, generally at the hand of considerably decreased antagonist, whether treatment is successful (Bale et al., 2008).

When compared to pesticides, biological control has been regarded as "unreliable," however the research supporting this view is ambiguous. Some biological control programs have had mixed results, and in some cases, the level of control has fluctuated over time (Bellows & Fisher, 1999; DeBach, 1964). Additionally, pesticides vary in their efficacy, and the development of resistance over time may result in the demise of a previously effective chemical (Bale et al., 2008).

1.4.2. Social and economic aspects

The use of agrochemicals and their negative consequences on the environment and human health is becoming more and more controversial. Agriculture and food-related industries now face increasing pressure to adopt more environmentally friendly production methods. Replacing chemical controls with biological ones will help safeguard natural resources and reduce environmental contamination. As a result, non-target organisms will be shielded from exposure to synthetic and harmful pesticides, increasing agricultural sustainability and enhancing biodiversity (Moosavi & Zare, 2015). Estimating the harm these externalities do to the ecosystem and society is a challenging and time-consuming (Menzler-Hokkanen, 2006). Pimentel & Greiner (1997) summarized a series of publications that quantify pesticide use's environmental and socioeconomic costs in the United States, including bird losses, groundwater contamination, pesticide resistance, public health implications, and biodiversity loss.

There are major barriers to the commercialization of biocontrol agents due to regulatory requirements that are overly tight and complicated, as well as a lack of acceptable techniques for mass manufacture and formulation of biopesticides. An emerging BCA must undergo significant

and expensive ecological and biological research before being put to the test in massive field trials. It costs up to $200,000 to do comprehensive toxicological testing required by the Environmental Protection Agency (EPA) for the EPA to classify biopesticides as the same as chemical pesticides. Scale-up and formulation research expenditures, as well as a lack of commercial potential, are the main impediments to their manufacture.

1.4.3. Strategies to overcome the limitation of BCAs

Several attempts have been undertaken to alleviate the drawbacks associated with biocontrol. Much study has concentrated on finding and developing desiccation-resistant spore-forming agents that can live in fields for longer periods. This also overcomes the issue of formulation and viability of BCAs in formulas.

1.4.4. Combination of BCAs

Several studies have effectively exploited the combination of biocontrol agents with disparate physiologies, eco-friendly requirements, modes of action to inhibit fungal phytopathogens. The ability of BCAs to adapt to various environmental conditions (temperature, pH, host genotype, etc.) is enhanced by their diverse physiology and method of action. Guetsky et al. (2002) demonstrated that the combined application of yeast, *Picha guilermondii* and *Bacillus mycoides* isolate B16 was more effective at controlling *Botrytis cinerea* on strawberry leaves across various environmental conditions than each organism was when used alone. The control coefficient of variability was highly high when evaluated in a narrow range of environmental variables (from 1°C to 30°C and 58 to 78% relative humidity), ranging from 9.7 to 75 percent when these BCAs were used alone and from 0.4 to 9.0 percent when both BCAs were combined.

Additionally, pathologists and others face the challenge of dealing with many diseases on the same crop or cropping system simultaneously. Leibinger et al., (1997), for example, found that while Bacillus subtilis isolates AG704 and HG77 had good colonization of apples in the field compared to two yeast species, they had poor colonization and apple fruit rot control in storage compared to the two yeast species. It was also found that compatibility between BCAs is important, as the yeast *Aureobasidium pullans* was reduced by the *Bacillus* BCAs with a resultant decrease in efficacy when these BCAs were combined. Similarly, Jetiyanon et al.,

(2003) found that seed treatments with mixtures of *Bacillus spp*. had similar advantages. Anthracnose disease control and growth promotion on long cayenne pepper were greater in the winter when *Bacillus amyloliquefaciens* isolate IN937A was combined with *Bacillus pumilis* strains SE34, SE49, T4, and INR7. The combinations did not improve the control of southern blight on tomato, tomato growth promotion, or cucumber mosaic virus control and cucumber growth promotion. This means that not all crops or pathosystems will respond well to mixtures (Jacobsen et al., 2004).

1.4.5. Integration of BCAs with chemical control measures

Combining BCAs with pesticides is an additional promising technique for effective disease control. Carter & Price (1974) were the first to employ this technique to manage *Eutypaarmeniacae* with *Fusarium lateritium* and Benzimidazole. Since then, numerous studies on BCAs as chemical additives have been published. Using BCAs in conjunction with chemical pesticides has become an essential part of integrated pest management (IPM). The primary need for BCAs is resistance to the combined fungicides. Combining BCAs and chemical control agents has two advantages: it lowers the inconsistency in protection efficacy associated with BCAs and minimizes the harmful agrochemicals sprayed into the fields.

Combining biocontrol with chemical pesticides, on the other hand, may yield advantages that neither method could deliver on its own (Jacobsen et al., 2004). Fungicides provide control in combination with the BCA when the BCA has not yet established itself in the rhizosphere. For a long time after the fungicides have degraded, the BCA can still have an impact. Kodiak (*Bacillus subtilis* isolate GB03) and fungicides are used on every cotton cultivated (Brannen & Kenney, 1997). Their research revealed that combining Kodiak with fungicides is superior to utilising fungicides or biocontrol agents alone for disease control. *Bacillus sp*. L324-92 and fungicides (difenoconazolemefozam) were shown to suppress *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia solani* AG8, *R. oryzae*, and *Pythium spp*. more effectively than biocontrol agents. Yobo et al. (2010) observed that combining *Trichoderma-Bacillus* and the fungicide Tolclofos methyl provided superior control of *Rhizoctonia solani.*

1.4.6. Mass production of BCAs

The high cost of producing the majority of biocontrol agents and their products is one of the most significant factors limiting the commercial appeal of biocontrol. This can be because the substrate is expensive, the biomass yield is low, there is no adequate production medium, little knowledge of the ideal the prerequisites for mass production, or there are only modest scale economies.To make BCAs economically feasible, it must be generated in large quantities in the shortest possible time using inexpensive substrates for the active principle. A lot is already known about manipulating the production medium for some biocontrol agents to produce the desired products. Many variables are typically considered, including the carbon supply, the osmotic potential, the temperature, and the pH. For instance, altering carbon levels might result in conidiation in Trichoderma (Agosin & Aguilera, 1998). The conidia with the longest shelf life always had a C: N ratio of 14:1. (Engelkes et al., 1997).

However, the medium must be optimized for increased biomass or bioproduct output while using affordable substrates for new biocontrol agents. Optimizing the medium components and growth conditions for the large-scale synthesis of specific biocontrol agents has not been attempted very often (Gohel et al., 2006). The researchers examined numerous low-cost sources of chitin in an effort to reduce the cost of producing chitinase for agricultural uses.

1.4.7. Formulation of Biocontrol agents

Another crucial factor in affecting the efficacy, durability, and safety of biocontrol drug is the formulation of BCAs.The bacterial agent needs to develop as a long-lasting product (Patiño-Vera et al., 2005). Numerous BCAs have been synthesized as wettable powders, solids, and liquids to combat certain plant diseases (Saravanakumar et al., 2007). Like any biological system, BCA formulation relies heavily on water, food, and the environment. Biocontrol agents' longevity in formulations can be significantly impacted by water activity (Patiño-Vera et al., 2005). Dry powder formulations, such as solid formulations, which can resist environmental conditions, have a lower risk of contamination, and they are simple to export, are preferred for BCAs (Streptomyces and fungi). When it comes to non-spore-forming BCAs, liquid formulations are more commonly used. Many solid or powdered compositions can be converted into liquid or water-based suspensions for drenching, spraying, or dipping (Melin et al., 2007).

1.5. Future of biocontrol

As the excessive use of synthetic fungicides becomes more apparent due to the growing danger of resistant diseases and the detrimental impacts on soil production and human and animal health, it is necessary to study alternative methods (F. Fan et al., 2017; Juntarawijit & Juntarawijit, 2018; Piel et al., 2019; Silva et al., 2020). In this context, research has concentrated on BCAs due to their low toxicity to humans and the environment. As a result, disease management via BCAs leads in highly controlled interactions involving numerous metabolites between pathogens and plants. These processes are pervasive in natural ecosystems, and people and other creatures have been exposed to them for years without knowledge of their detrimental effects (Köhl et al., 2019; Liljeroth et al., 2010; Palou et al., 2016; Wilingham et al., 2002). Their disease-fighting ability, however, is frequently shown to be insufficient and highly dependent on environmental factors (Tarique Hassan Askary, 2015; Droby et al., 2009; Gerbore et al., 2014; Walters et al., 2005). As a result, integrated pest control strategies involving the use of systemic or non-systemic fungicides in combination with antagonists or inducers of resistance are advocated (Chand-Goyal & Spotts, 1997).

The properties of BCAs vary according to their source, which can be chemical or biological, and their mode of action, which can be direct or indirect. For example, screening for BCAs that promote resistance requires more complex experiments on plants than screening for direct BCAs, which can typically be performed *in vitro* (Raymaekers et al., 2020). Additionally, BCAs that boost fungicide action should be compatible. When used in conjunction, the fungicide interacts not just with infections but also with BCAs. Given that BCAs are designed to enhance plants' defense mechanisms or to influence plant pathogens directly, it is improbable that such combinations would have a detrimental effect on the action of fungicides. Due to the non-living nature of chemical BCAs, they are unaffected by synthetic fungicides unless physical incompatibility develops. However, the danger that fungicides will have a detrimental effect on the growth or survival of living BCAs is substantially greater.

Natural pesticides and fertilizers are becoming increasingly popular and in demand as people become more aware of the environmental potential and health risks linked with chemical pesticides and the benefits afforded by BCAs. By 2010, The market share of biopesticides is

expected to increase to 4.2 percent from its 2000 level of 0.2 percent of all pesticide sales.When it comes to pesticide use, biopesticides represent only a fraction of total pesticide sales (e.g., about 15 percent), but their contributions to plant health management are nonetheless significant because they are still in their infancy and yet under development. 65% of EPA-registered organisms were registered in the last ten years, with 35% registered in the previous five years as at 2005 (Fravel, 2005). In 1979, the United States Environmental Protection Agency registered the first bacterium, *Agrobacterium radiobacter* K84, to control gall. The EPA registered the first fungus, *Trichoderma harzianum* ATCC 20476, ten years later to control plant diseases. Numerous research institutions worldwide are focusing their efforts on enhancing and applying biopesticides to boost commercial biopesticide production and use. The EPA has registered 14 bacteria and 12 fungi to control plant diseases (Höfte & Altier, 2010).

The global biopesticides market is expected to grow at a CAGR of 14.7 % annual growth rate from an estimated USD 4.3 billion in 2020 to USD 8.5 billion in 2025. The use of synthetic chemicals can contaminate and pollute the soil and negatively influence the food chain. As a result of this worry, there has been an increase in awareness of residue-free food, which places a premium on biological goods (Market and market, 2021).

1.6. Pigeon pea (*Cajanus cajan*)

Cajanus cajan (L. Millsp.) is among the most important tropical and subtropical legume crops. Pigeon pea belongs to the *Cajanus* genus, the Cajaninae subtribe, the Phaseoleae tribe, and the Fabaceae family. It is also known as red gramme, tur, arhar. The term 'Pigeon pea' was first used to describe plants grown in Barbados because the crop's seeds were seen as vital as pigeon food. India is the original origin of pigeon pea due to the observed crop's extensive genetic diversity.

The pigeon pea shrub is an annual crop that can reach up to two meters. Drought resistance is made possible by the plant's extensive taproot system. Pigeon pea has a wide range of maturation periods, is tolerant of each kind of soil, and it withstand salinities of 6–12 mmhos/cm (90-300 days). These qualities enable its cultivation across a range of environments and agricultural systems. Pigeon pea is a superior crop for sustainable agriculture in India's tropical and subtropical climates due to release of soil-bound materials like phosphorus, nitrogen fixation in the atmosphere, nutrient recycling in the soil,adding organic matter, and absorption of additional

nutrients. Pigeon pea is grown on 4.92 million hectares and produces 3.65 metric tonnes and 892 kg ha-1 per year (http://faostat.fao.org). The largest producer of pigeon peas in Asia is India, which grows the crop on 3.63 million hectares and produces an average of 2.12 million tonnes annually—nearly 90% of the world's pigeon pea production and pigeon pea cultivated area.

However, despite an increase in pigeon pea production area over the previous 50 years, productivity has remained constant at approximately 700 kg/ha, significantly below the global average productivity (Saxena & Nadarajan, 2010). This is a cause for worry because pulses are an essential plant-based protein in the human diet. Domestic demand has increased and it led to the importation of pigeon pea from other countries, resulting in a more than twofold increase in the price of pulses in the last year alone. In addition to natural disasters such as droughts and Floods, losses from pest infestations and plant diseases are some of the main reasons why India's pigeon pea crop produces so little.

1.6.1. Fungal diseases of Pigeon pea

Numerous fungi-related illnesses can affect pigeon peas. The pigeon pea is known to be infected by more than 45 different fungal infections, including *Cercospora* spp., *Colletotrichum cajani, Corticiumsolani, Diploidiacajani, Leveillulataurica, Macrophomina phaseoli, Phaeolusmanihotis, Phorrtacajani, Phyllostictacajani,Phytophthora* sp. *Fusarium udum*, a fungus that causes wilt, is the most damaging fungal pathogen to its productivity (Saxena, 2008). A soil-borne disease called *Fusarium udum* can exist unnoticed in agriculture fields for years. There is no limit to the number of crops affected by this disease. Soil temperatures between 17°C and 20°C promote the growth of pests. The spores can reach flowers and pods through roots. Since the fungus grows slowly, the symptoms typically manifest during blooming and podding, although they can also manifest in plants as young as one to two months old. The first sign of wilt is patches of dead plants. The most distinctive symptom is an upward-extending purple band from the main stem's base (Saxena et al., 2010).

There is evidence that *Fusarium udum* has been treated chemically. The pathogen's capacities to survive in various conditions and the challenge of chemically treating large amounts of soil or plants to reduce or eradicate the pathogen have hampered its efficacy.

Furthermore, a study by Fox et al. (2007) discovered that even a single pesticide application has a negative impact on the relationship between Rhizobium, a helpful nitrogen-fixing bacterium associated with root nodules, and *Cajanus cajan*. The creation of resistant cultivars is the only practical control strategy. However, the absence of wilt-resistant germplasm and the innate difficulties of breeding resistant variants in *Cajanus cajan* necessitate the development of alternate approaches for disease control (Podile & Kishore, 2007; Saxena & Nadarajan, 2010). Only a few studies on the BCAs of *Fusarium udum* for pigeon pea wilt have produced promising outcomes (Maisuria et al., 2008; Siddiqui et al., 2008; Vaidya et al., 2003). The Bacterial cultures used in the experiments; however, they were gram-negative isolates, making it difficult to synthesize them for commercial use. In order to resist *Fusarium udum*, there is a persistent need for biocontrol agents.

1.7 Stress Ethylene and Plants

In 1901, the Russian scientist Neljubov discovered that the gaseous hormone-ethylene acts as a growth regulator in etiolated pea seedlings. Decades of diligent research have shown many plant responses to this ethylene (Abeles et al., 2012). Ethylene is responsible for the growth of leaves, flowers, and fruits. Additionally, depending on the optimal or suboptimal ethylene levels, it may promote, prevent, or induce senescence (Khan, 2005; Pierik et al., 2006).

This gaseous hormone is a two-carbon-atom molecule that regulates various vegetative plant growth processes. Additionally, ethylene regulates other processes such as fruit ripening, leaf and floral senescence, and abscission, where its synthesis increases significantly. The triple response of ethylene on seedlings, which includes 1) a short hypocotyl and root, 2) hypocotyl swelling, and 3) apical hook embellishment, was first described by Neljubow, (1901) and later validated by Knight, (1910) and Crocker, (1913). Nearly a decade has been invested in understanding ethylene biosynthesis (Adams & Yang, 1979; Adams & Yang, 1977; Lieberman & Mapson, 1964; Murr & Yang, 1975). A basic concept of the pathway is demonstrated in higher plants; the amino acid methionine is required for the production of ethylene (Lieberman & Mapson, 1964), which SAM-synthetase converts to S-adenosyl-L-methionine (SAM) (Adams & Yang, 1979). Figure 1.4 depicts a streamlined representation of the overall process. ATP is required for the conversion of methionine to S-adenosyl-l -methionine (SAM) by the enzyme

SAM-synthase (SAMS). ACC synthase (ACS) then changes SAM into ACC in a reaction that cuts off a 5′methylthioadenosine (MTA). The Yang Cycle or Methionine Salvage Pathway is a sequence of intermediate stages by which MTA is recycled back to methionine. ACC oxidase (ACO) converts ACC to ethylene in the presence of oxygen. Ethylene synthesis is triggered by a variety of signals, both internal and external, shown by the ovals (Vanderstraten and Van Der Straten, 2017).

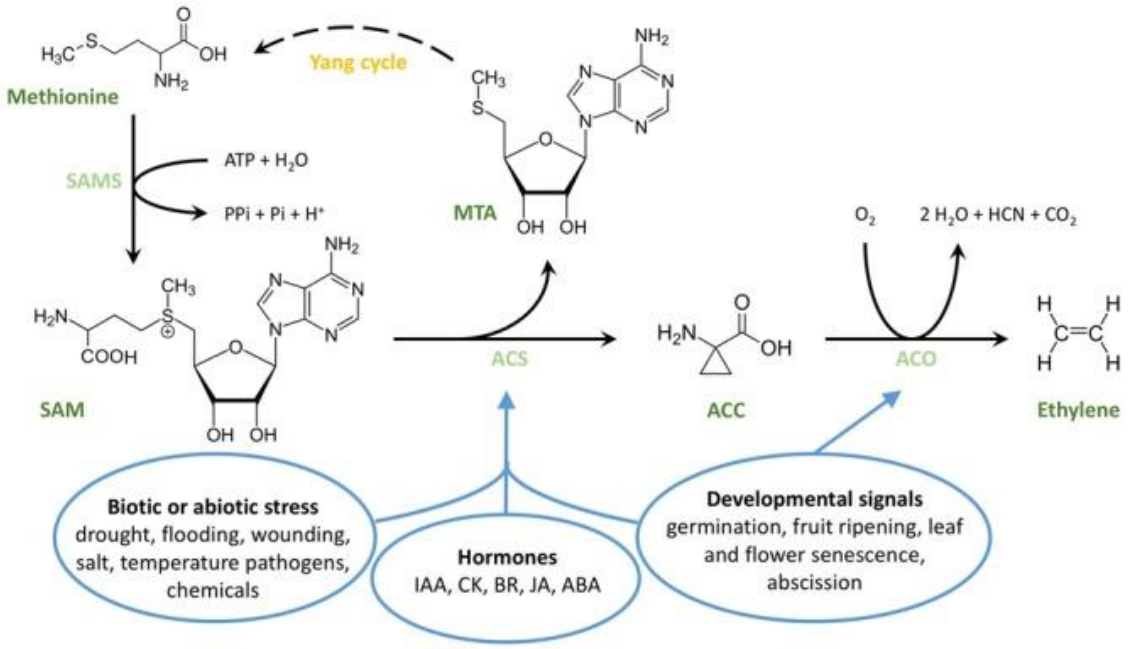


Figure 1.4: Structural overview of ethylene biosynthesis

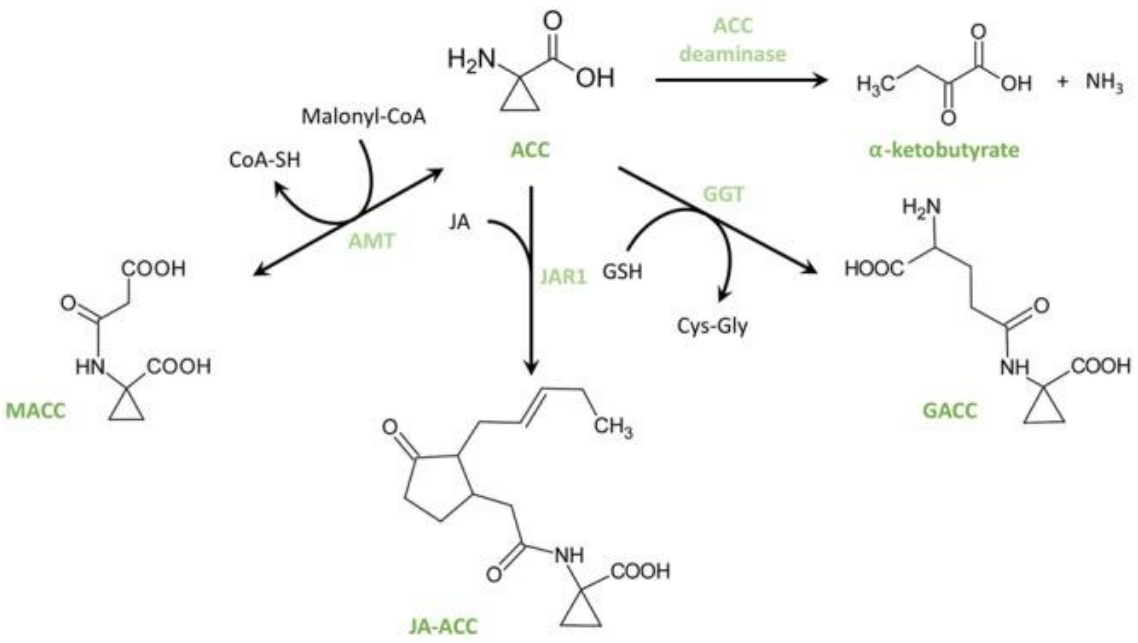
Synthesis of the three-carbon membered ring of amino acid ACC is catalysed by the enzyme ACC synthase (Boller et al., 1979). This major discovery of the presence of ACC made the methionine cycle in plants unique from that in all other organisms. The cofactor for ACS is pyridoxal-5'-phosphate (PLP), making it a member of the PLP-dependent enzyme family. Cleavage of MTA from SAM occurs once PLP is bound to its catalytic site, and this process is followed by the production of ACC (Yu et al., 1979). Methionine Salvage Pathway or Yang-cycle reactions are used to convert MTA to methionine (Bürstenbinder et al., 2010). ACC-oxidase (ACO) catalyses the final biosynthetic step, in which ACC is transformed into ethylene (Ververidis & John, 1991). However, the increase in ethylene production was related to different stress (abiotic/biotic) conditions. Yang & Hoffman (1984) reported that the constitutive

expression of ACC oxidase was due to differential expression of the ACS gene. Later, it was discovered that ACS is a multigene family, each of which is regulated by a distinct factor, some of which are influenced by stress (Morgan & Drew, 1997).

1.7.1 Role of ACC deaminase in the regulation of ethylene biosynthesis

The production of ACC from SAM catalyzed by ACS is considered a rate-limiting step of the entire ethylene biosynthesis (Yang & Hoffman, 1984). The C-terminus of the enzyme is not required for catalysis and hence plays a critical role in the stability of the enzyme in bacterial cells before proteasomal degradation. Increased phosphorylation maintains the enzyme's stability, whereas increased dephosphorylation results in proteasomal destruction (Xu & Zhang, 2014). The hormones cytokinin and brassinosteroids also positively affect the ethylene biosynthesis by stabilising the ACS (Hansen et al., 2009) and decreasing its rapid degradation (Chae et al., 2003). Ethylene biosynthesis is known to be a rate-limiting phase in ACS; however ACO can be rate-limiting under situations of substantial ethylene production (Ruduś et al., 2013).

In contrast to the ACS gene, significantly less is known about the ACO gene's transcriptional and post-transcriptional regulation. As with the ACS gene, expression of the ACO gene can be regulated by plant hormones such as salicylic acid, auxins, abscisic acid, and gibberellic acid (Zhang et al., 2009). Apart from ACS and ACO regulating ethylene biosynthesis, ACC derivatisation via the generation of conjugates such as N- malonyl ACC (MACC) (Peiser & Yang, 1998), γ-glutamyl ACC (GACC) (Martin et al., 1995), and jasmonic-ACC (Staswick & Tiryaki, 2004) also results in ethylene biosynthesis regulation (Figure 1.5). Additionally, regulation can be accomplished by lowering the accessible ACC pool, which is achieved through irreversible deamination catalyzed by an enzyme called ACC deaminase (Honma & Shimomura, 1978). Notably, plants are not the sole source of this enzyme; certain plant growth-promoting bacteria can also manufacture it (Misra et al., 2017). The PGPR obtains carbon and nitrogen from the ACC produced by plants in the rhizosphere (Glick et al., 1998; Penrose et al., 2001). As a result, the ensuing ACC pool decreases ethylene production, relieving the plant of stress (Glick et al., 2007).



Source: Vanderstraeten & Van Der Straeten (2017)

Figure 1.5: Structural overview of ACC conjugation and deamination**.**"From ACC, three known conjugates can be formed. 1-malonyl-ACC (MACC) is formed by ACC-N-malonyl transferase (AMT), a reaction that requires malonyl-CoA. Jasmonyl-ACC (JA-ACC) is formed by jasmonic acid resistance 1 (JAR1). γ-glutamyl-ACC (GACC) is formed by γ-glutamyl-transpeptidase (GGT), a reaction that requires glutathione (GSH). The deamination of ACC by ACC deaminase yields α-ketobutyrate and ammonium."

1.8 Mycorrhiza

Terrestrial fungi appear to have evolved around the same period as land plants. Along with their role as saprotrophs, some fungi developed an intimate relationship with plant roots, improving their ability to sequester nutritional components. This evolved into a symbiotic connection known as mycorrhizae, which has evolved in various directions, resulting in a variety of morphological modifications to root structure and offering a variety of ecological functions to various plant groups (Dighton, 2009). The Rhynie cherts (410–360 mya) reveal these relationships as probably primitive endomycorrhizae, while the Princeton cherts (50 mya) revealed them as ectomycorrhizae of pines. Mycorrhiza is derived from the Greek words 'mykos', which means fungus, and 'rhizos', which means roots. Thus, the term 'fungus roots' refers to a

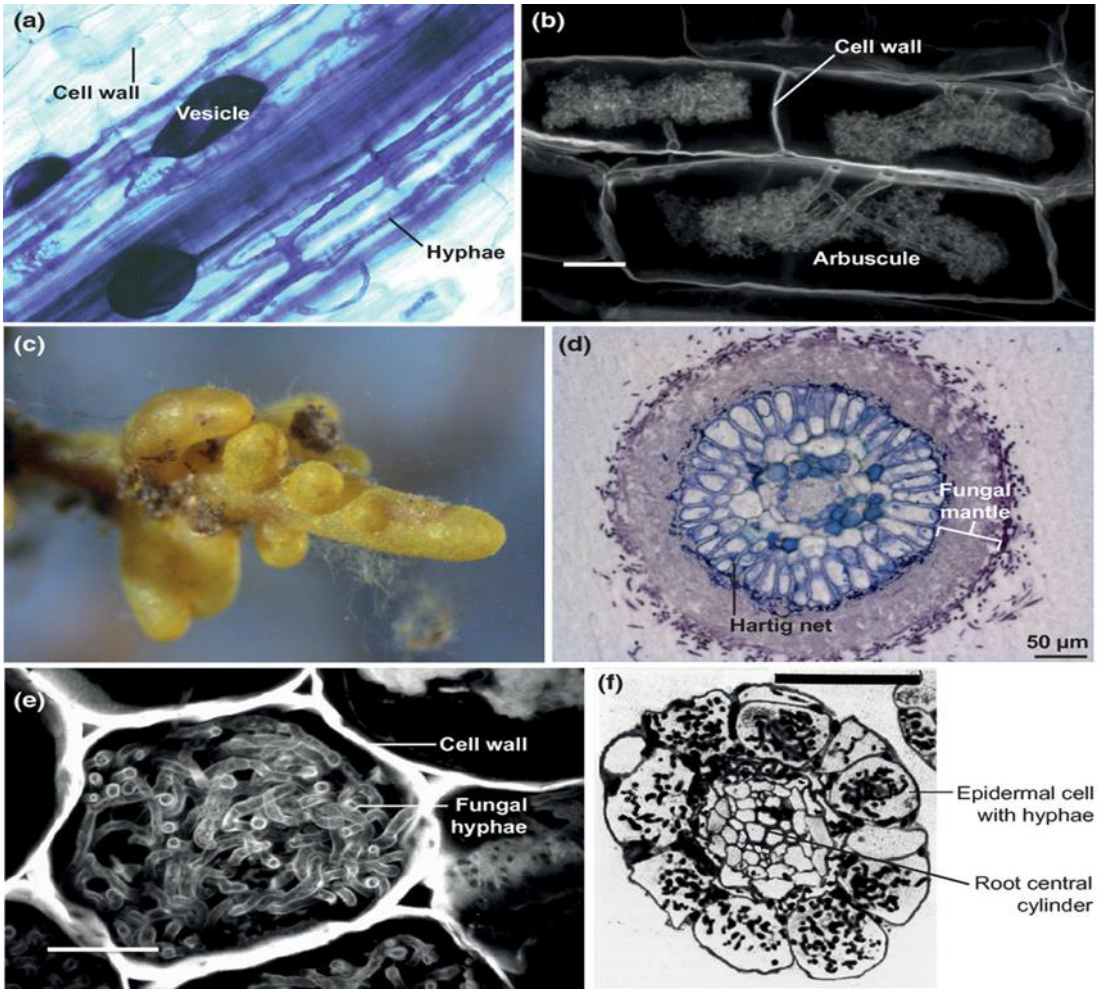
particular adaption of plant roots that occurs in around 85 percent of all plant species. According to recent estimations, about 3617 plant species belonging to 263 families are mycorrhizal. Thus, the mycorrhizal state is the most frequent symbiotic condition (Dighton, 2009; Van der Heijden & Sanders, 2002).

In 1985, Frank may have been the first to find that mycorrhizal fungal interactions between plant roots and mycorrhizal fungi are widespread (Frank, 2005). The partners and processes involved in this symbiosis have been thoroughly studied over the last century (Gardes& Bruns, 1993; Phillips & Hayman, 1970; Smith & Read, 2010), and we now know that mycorrhizal associations exist in almost every ecosystem, from deserts to tropical rainforest to arable land (Brundrett, 2009; Read, 1991). Since mycorrhizae have an impact on plant productivity and genetic diversity, they are an essential partner in symbiotic ecosystems. It is generally accepted that mycorrhizal associations boost plant productivity; however, this is not always the case and symbiotic partnerships can span a wide variety of species interactions, from mutualism to parasitic interactions (Maherali, 2014). Mycorrhizal fungi are capable of parasitic associations with plants if the net advantages of the symbiosis are less than the net cost. In order to fully appreciate the role that mycorrhizae play in a plant's ecology, one must have a thorough grasp of the rhizosphere, community, and ecosystem-level biotic and abiotic elements that influence the symbiotic relationship.

Seedling inoculation with spores or mycelial cultures is typically the first step in the commercial production of diseased plants (Grimm et al., 2005). On the basis of the structure of their hyphae, mycorrhizae can be divided into two classes. Fungal hyphae that do not penetrate individual root cells are known as ectomycorrhizal fungi, whereas hyphae that penetrate the cell wall and invade the cell membrane are known as endomycorrhizal fungi (Szabó et al., 2014). Arbuscular, ectomycorrhiza, orchid, and ericoid are four most common mycorrhizal types (As shown in Figure 1.6, a short description of each type is provided) (van der Heijden et al., 2015). Mycorrhizal fungi colonize the cortex, root surface, and root epidermal cells of plant roots. The hyphae of these fungi also extend from the roots into the soil, where they scavenge for minerals lacking in plants, mainly nitrates, and phosphates. Other mycorrhizal fungi (for example, EM and ericoid mycorrhizal fungi) absorb biologically bound nutrients as well (Read & PerezMoreno, 2003). Their host plants subsequently exchange these nutrients and other benefits

for carbs (Smith & Read, 2010). As a result, mycorrhizal symbiosis significantly impacts plant development and fitness.

Arbuscular mycorrhizal fungi are defined as vesicular-arbuscular mycorrhizal (VAM) fungi or soil fungi (Vogelsang et al., 2004). They are members of the Glomeromycota division and reproduce asexually. To reach their greatest growth potential, plants rely greatly on these fungi. Arbuscular mycorrhizal symbiosis, the most widespread non-pathogenic soil symbiosis, is found in the roots of 80 percent of vascular plants (Brundrett, 2002). Furthermore, the host plant species influences arbuscular mycorrhizae growth. Arbuscules are fungal entities that grow inside individual plant cells. Almost all Angiosperm phyla have arbuscular mycorrhizal (AM) fungus. According to Hart and Forsythe (2012), AM fungi boost plant phosphorus nutrition and the uptake of important micro and macro nutrients. They are also resistant to a variety of root diseases and can withstand drought.

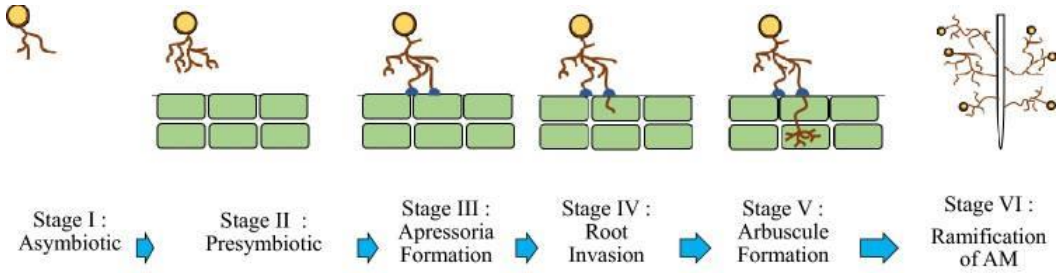


Source: van der Heijden et al. (2015)

Figure 1.6: Typical structures of mycorrhizas; arbuscular mycorrhizas (a, b), ectomycorrhizas (c, d), orchid mycorrhizas (e), and ericoid mycorrhizas (f).

1.8.2 Formation of mycorrhizae

There are various stages in the growth of mycorrhizae for arbuscular mycorrhizal fungi (Figure 1.7). Arbuscular mycorrhizal fungus spores germinate during the symbiotic stage, but their hyphal development is limited due to the lack of host plants. Presymbiotic spore growth occurs when roots exudates, and this is when they enter the presymbiotic stage after germination a second stage of appressoria formation occurs when the fungus encounters a root surface, but before the hyphae reach the epidermis of the root. Symbiotic colonization of the root cortical tissue begins with the creation of arbuscules (tree-like, densely branched structures) or hyphal coils within the root tissue and a distinct extraradical mycelium is also formed. A host plant can facilitate mycorrhizal AM infection, and similar processes may occur in the cortical cells. The plant and arbuscular mycorrhizae must communicate molecularly to carry out these functions, including the exchange and perception of signals (Huey et al., 2020).



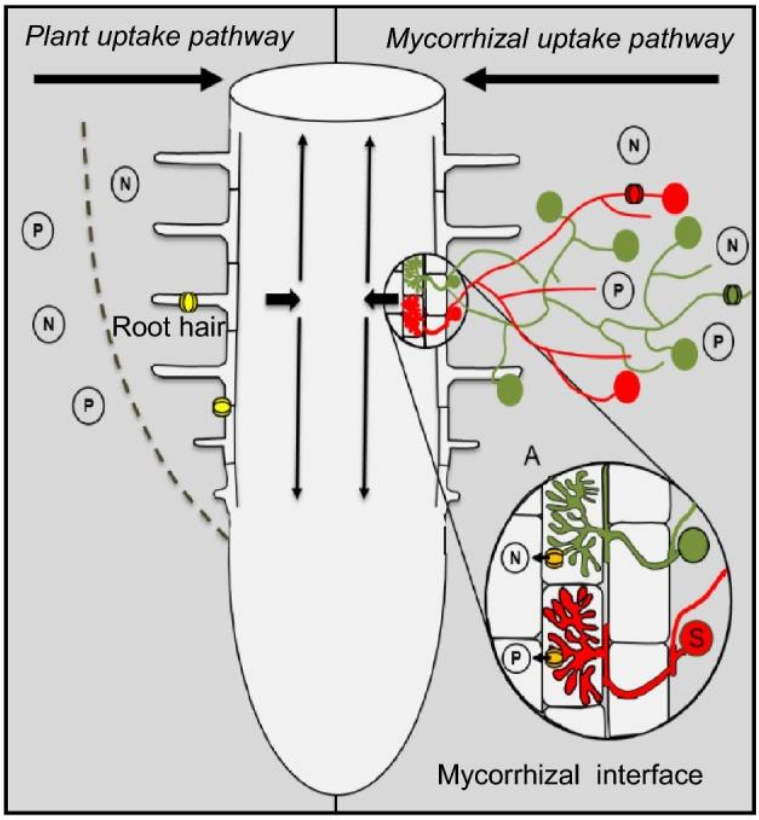
Source: Huey et al., (2020)

Figure 1.7: Developmental stages of arbuscular mycorrhizae**.**The different stages are indicated. Generally, six stages are involved in the formation of a complete association

1.8.2 Mycorrhizal plant interactions

The plant uptake pathway (PP) and the mycorrhizal uptake pathway (MP) are the two processes through which mycorrhizal-colonized roots absorb nutrients (Figure 1.8). The PP occurs in the

root epidermis and hairs, where the transporter's nutrients are absorbed directly. In the MP, the fungus' extraradical mycelium (ERM) contains fungal transporters that allow nutrients to be transmitted indirectly to the Hartig net during EM contacts or the arbuscular mycorrhizal system's intraradical mycelium (IRM) (shown in the mycorrhizal interface). Mycorrhiza-inducible plant transporters carry out the interfacial apoplast uptake in the periarbuscular membrane (Huey et al., 2020). Multiple fungal species have colonized a single host root, as shown by the fungus structures, with varying degrees of success. For example, fungus can take nutrients from the earth and transfer them to their hosts through these activities (Bücking et al., 2012).



Source: Huey et al., (2020)

Figure 1.8: Nutrient uptake pathways.The involvement of the plant uptake pathway and mycorrhizal uptake pathway is shown. The major nutrients are indicated, and the directions of the movements are displayed

Roots' quick uptake of nutrients, such as phosphorus, causes a depletion zone to emerge, and fungal hyphae penetrate and exploit a larger soil volume to uptake nutrients. Except for ectomycorrhizae and monotropoid mycorrhizae, nutrients are transported intracellularly into plant cells from the hyphal network to the fungal sheath and then intercellularly to the Hartig net. The fungal sheath can store nutrients, allowing the fungi to continue supplying the plant host with nutrients when soil nutrient levels decline. Photosynthates produced by mycorrhizal plants are lost as they take extra nutrients, which are needed by mycorrhizal fungi and their related structures for development and maintenance (Huey et al., 2020).

1.8.3 Mycorrhiza and Bacteria

Arbuscular mycorrhizal (AM) fungi and bacteria can cohabit synergistically to enhance plant growth by increasing the absorption of nutrients and preventing fungal plant diseases from thriving (Artursson et al., 2006). Mycorrhizosphere is the soil zone neartheroots and fungal hyphae where these interactions occur (Rambelli, 1973). Interactions between bacteria and AM fungi could have numerous beneficial impacts, including plant growth promotion and biocontrol (Kloepper, 1994, 1996; Meyer & Linderman, 1986; Von Alten et al., 1993) and N2-fixing bacteria (Biró et al., 2000; Secilia & Bagyaraj, 1987).

Despite the fact that little is known about how bacteria interact with AM fungus and plant roots in the mycorrhizosphere, a number of potential pathways have been suggested (Artursson et al., 2006). According to investigations by Carpenter-Boggs et al. (1995), Daniels & Trappe (1980), Mayo et al. (1986), and Mosse (1959), certain bacteria have been shown to have a direct effect on the germination and development rate of AM fungal species. This suggests that a favorable effect on plants may occur via the AM relationship. Another way bacteria might alter a plant's physiological state is by increasing the permeability of its root cells. Aside from directly interacting to boost the mycorrhizal connection and/or plant growth (Garbaye, 1994; Linderman, 1988, 1992; Vivas et al., 2003), particular bacteria in combination with AM fungi may generate an indirect synergy that enhances plant growth (Barea, 1997), such as nutrient uptake (Barea et al., 2002), suppression of plant pathogenic fungi (Budi et al., 1999), and enhancement of root branching (Gamalero et al., 2002).

In addition to these effects of bacteria on AM fungi, the AM fungi themselves have also been shown to impact the composition of bacterial communities (Artursson et al., 2005). Root exudates, which can be a source of nutrients for bacteria in the mycorrhizosphere, have been shown to change in chemical composition when mycorrhizal fungi are established (Azcón-Aguilar & Bago, 1994; Harley & Smith, 1983; Linderman, 1992; Smith et al., 1994; Barea, 1997; Barea, 2000; Gryndler, 2000; Linderman, 2000). However, more direct interactions, such as competition for inorganic nutrients, have been attributed to changes in bacterial community composition and activity caused by AM fungus (Christensen & Jakobsen, 1993). According to Andrade et al., (1997) and Artursson et al., (2005), some bacteria have been demonstrated to respond to the presence of certain AM fungal species, showing that bacteria associated with AM fungus are highly specific. The activation of certain bacterial species by specific AM fungus has been linked to the presence of fungal exudates peculiar to those fungi.

1.9 Genome-Based Taxonomic Classification of Genus *Streptomyces*

Metabolic and physiological properties of Actinobacteria members are diverse while expressing varied mycelium forms such as coccoid and fragmenting hyphal or branching mycelium (Reddy, 2011). Some Actinobacteria are soil dwellers (such as *Streptomyces*), while others are intestinal commensals (such as *Bifidobacterium*) or pathogens (example: *Mycobacterium* and *Corynebacterium*) (Sadeghi et al., 2014). *Streptomyces* is the largest genus within the Streptomycetaceae family, a member of the phylum Actinobacteria (Sadeghi et al., 2014). Streptomyces bacteria are physiologically active and capable of producing secondary metabolites with various biological functions (Berdy, 2005). About ten thousand bioactive chemicals have been isolated from Streptomyces (Berdy, 2005; Ser et al., 2017, 2018).

Taxonomic characterization of Streptomyces is certainly more complicated and challenging than that of other microbial genera, owing to the genus's large number of reported species (Labeda et al., 2012). Streptomyces classification techniques have improved over time, progressing from classical morphological classifications based on spore chain morphology, substrate color, and aerial mycelia to numerical taxonomic analyses that include phenotypic characterization using standardized sets and, more recently, molecular and phylogenetic analyses (Labeda et al., 2012; Williams et al., 1983).

Due to the advent of polymerase chain reaction (PCR), DNA-DNA hybridization (DDH), and DNA sequencing approaches, identification and characterization methods of *Streptomyces* have evolved to molecular and phylogenetic characterizations with analysis of gene sequences that target predominantly linear 16S rRNA gene sequences (Anderson & Wellington, 2001). The molecular revolution has advanced our understanding of molecular cell biology during the last three decades, but it has also significantly improved our understanding of evolution, conservation, and ecology. In other words, genetic techniques enable the taxonomic classification of bacteria that are difficult to define only based on morphological traits while also increasing the efficiency of identification through rapid and high-throughput methods (Emerson et al., 2008).

1.10 Aim and scope of the present investigation

Food security around the world is threatened by crop disease. Up to 2 billion people endure food insecurity today, and over 852 million still suffer from chronic hunger. Plant diseases are responsible for 10 to 30% of agricultural output losses (Strange & Scott, 2005). The number of people who lack enough food has significantly increased over the past forty years, while agricultural productivity has nearly increased by twofold as a result of contemporary farming methods and excessive agrochemical use (synthetic fertilizers & pesticides).This is partly because most of the world's population lives in poverty or developing countries, where expensive agrochemicals and cutting-edge farming equipment are rarely used. Overuse of agrochemicals has led to several problems with the environment, human health, and financial costs.

The increase in yields brought on by agrochemicals is also short-lived. The use of fertilizers has increased over the past 20 years, yet even that hasn't prevented a reduction in agricultural output. The rise in soil fertility reaches its maximum. Additionally, continued farming depletes the soil's organic matter, diminishing its fruitfulness (Fox et al., 2007). Thus, limiting crop disease damage becomes a crucial strategy for increasing agricultural productivity. Chemical pesticides are used in modern agriculture to control plant diseases (Talbot, 2010). Their uncontrolled usage, however it has been resulted in harmful residues in food products and the rise of bacteria that are antibiotic-resistant.As a result, contemporary agriculture constantly needs novel disease management techniques that are safe for the environment, economical, and effective. Biological

control is more effective than synthetic insecticides. When compared to toxic pesticides, they provide many advantages. Because they are very specific to the target ailment, they not only exercise their effects without harming other beneficial microorganisms or insects but are also good to the environment and the economy. Once established, the controls they provide is irreversible.There haven't been any reports of these agents developing resistance (Van Lenteren et al., 2006).

One of India's most important legume crops is the pigeon pea. In the diet of the largely vegetarian nation of India, it serves as the main source of protein. There are many plant diseases that impair pigeon pea, but Fusarium wilt, caused by *Fusarium udum*, is the most serious (Saxena, 2008). In India, pigeon pea wilt is thought to cause annual losses of US$71 million (Gwata et al., 2006; Hillocks, 1984). Despite the use of pesticides to protect pigeon pea plants from fungus, such as Thiram, Bavistin, and Benomyl, the fungus can still persist in the field and negatively impact the crop for a very long time after it has been planted following the fungicide's degradation (Saxena et al., 2010). Previous research has shown that biocontrol agents have a potential for controlling pathogens (Maisuria et al., 2008; Vaidya et al., 2003). To stop the harm that infections inflict, it is still necessary to constantly look for biological control agents. Biological control agents generally use an antibacterial approach to interact with fungal infections.

The production of antifungal metabolites and lytic enzymes, which break down pathogens' cell walls and result in cell lysis, can lead to antibiosis (Haggag& Mohamed, 2007). Numerous biological insecticides have been shown to use the mycolytic enzyme chitinase to break down the fungal cell walls (Neeraja et al., 2010). The use of chitin-degrading bacteria in agriculture to combat fungal plant diseases is justified by the great thermal stability of some biocontrol agents' chitinase. Proteases and glucanases are two additional cell wall-degrading enzymes effective against fungal phytopathogens. Several biocontrol agents have reported the presence of antifungal proteases and glucanases. In addition to cell wall-degrading enzymes, numerous biological pest control agents also create antifungal metabolites involved in managing fungal phytopathogens.

Even though biological control has been shown effective in the laboratory, field applications have had inconsistent results. The main reason for this is that numerous biotic and abiotic stimuli affect biological control agents. Recent research has shown that biological control agents and chemical fungicides can be used together to prevent inconsistent efficiency. The Biocontrol agents used in the treatment must be resistant of the fungicide applied to it, and it is the most crucial requirement. Research has been done on the susceptibility to insecticides biological control the potential of agents in the integrated treatment of plant-fungal infections because of the novelty of this method.This thesis focuses on discovering *Streptomyces* sp. as a biological control agent against *Fusarium udum*; research on pesticide tolerance on antifungal principles for prospective use purification and characterization of the isolates antifungal principles, as well as integrated pest management; Statistical optimization of the average components to enhance antifungal principles.

1.11 Objectives of the Study

1. Isolation, Identification and characterization of actinomycetes from rhizosphere soil of *Cajanus cajan*.
2. To check the antifungal activity of isolated actinomycetes.
3. To check the efficacy of Biocontrol actinomycetes against fungal phytopathogens of *Cajanus cajan.*