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MICROBIAL BIOCONTROL BY *TRICHODERMA*, ITS BIOLOGICAL INTERACTIONS AND MODE OF ACTIONS

Mesele Admassie*, Tesefaye Alemu, Aferasa Mulatu

Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, Ethiopia.

A B S T R A C T

Biological control is the suppression of damaging activities of one organism by one or more other organisms. They are many environmental benefits of biological control including safety for humans and other non-target organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems, their advantages are numerous. microorganism–microorganism or microorganism–host interactions involve all ecological aspects, including physiochemical changes, metabolite exchange, metabolite conversion, signaling, chemotaxis and genetic exchange resulting in genotype selection. Microbial interactions occur by the transference of molecular and genetic information, and many mechanisms can be involved in this exchange, such as secondary metabolites, siderophores, quorum sensing system, bio-film formation, and cellular transduction signaling, among others. The agricultural importance of the biocontrol is that possess good antagonistic abilities against plant pathogenic microbes. Antagonism is based on different mechanisms, like the production of antifungal metabolites, competition for space and nutrients and myco-parasitism. Some of microbes like Trichoderma strains with effective antagonistic abilities are potential candidates for the biological control of plant diseases. Biotic and biotic environmental parameters may have negative influence on the bio-control efficacy of biocontrol strains.

Keywords: Biological control; physiochemical; Microbial interactions; biofilm formation; antagonistic

INTRODUCTION

Biological control is the suppression of damaging activities of one organism by one or more other organisms. Bio-control microorganisms can be used as an alternative to conventional chemical pesticides to control plant diseases (Ahmad, 1987). They are many environmental benefits of biological control, including safety for humans and other non-target organisms, reduction of pesticide residues in food, increased activity of most other natural enemies, and increased biodiversity in managed ecosystems, their advantages are numerous. Biological control of plant pathogens was applied and it can result from many different types of interactions between organisms. In addition, in the field of biological control of insects, there are varieties of the bacteria, fungi and viruses are currently used for control of a broad range of crop and forestry pests and larvae of several blood-sucking pests of humans and domestic animals. Nematodes in soil are subject to infections by bacteria and fungi; this creates the possibility of using soil microorganisms to control plant-parasitic nematodes (Ahn *et al*., 2002). The potential of using living organisms, like insects, fungi, and bacteria were tested as biological control agents for weed management, and there is a novel approach offered by living organisms as agents for biological weed control, this weed management tool is evolving as an alternative to herbicides. Recently, nuclear techniques have a significant role to play in facilitating the use and increasing the cost-effectiveness and safety of biological control agents, nuclear techniques can improve the efficiency of biological control by decreasing the cost of production of bio-agent, increasing host suitability and stimulation of biological process (Ahn *et al*., 2002).

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* Corresponding Author:
Email: mesele2006@gmail.com

Microbial interactions are crucial for a successful establishment and maintenance of a microbial population. These interactions occur by the environmental recognition followed by transference of molecular and genetic information that include many mechanisms and classes of molecules. These mechanisms allow microorganisms to establish in a community, which depending on the multi-trophic interaction could result in high diversity (Al-Rawahi *et al.,* 1998). The result of this multiple interaction is frequently related to the pathogenic or beneficial effect to a host. In humans, for example, the microbial community plays an important role in protection against diseases, caused by microbial pathogens or physiological disturbances. Soil microbial communities also play a major role in protecting plants from diseases and abiotic stresses or increasing nutrient uptake (Apel and Hirt 2004). This review is focused on the microbial interactions, mechanism of action in the host and **c**ompatibility of different bio-agents with fungicides and bactericides and its relevance in biological control.

Types of biological interactions: The microorganism–microorganism or microorganism–host interactions are the key strategy to colonize and establish a variety of different environments. These interactions involve all ecological aspects, including physiochemical changes, metabolite exchange, metabolite conversion, signaling, chemotaxis and genetic exchange resulting in genotype selection. In addition, the establishment in the environment depends on the species diversity, since high functional redundancy in the microbial community increases the competitive ability of the community, decreasing the possibility of an invader to establish in this environment (Azevedo *et al*., 2016). Therefore, these associations are the result of a co-evolution process that leads to the adaptation and specialization, allowing the occupation of different niches, by reducing biotic and abiotic stress or exchanging growth factors and signaling (Azevedo *et al*., 2016). Microbial interactions occur by the transference of molecular and genetic information, and many mechanisms can be involved in this exchange, such as secondary metabolites, siderophores, quorum sensing system, biofilm formation, and cellular transduction signaling, among others. The ultimate unit of interaction is the gene expression of each organism in response to an environmental (biotic or abiotic) stimulus, which is responsible for the production of molecules involved in these interactions (Benhamou *et al*., 2000). There are different microbial interactions:

Bacterial-bacterial pathogen interactions: In the current studies of bacteria applied to seeds and roots for the purpose of controlling bacterial diseases. One example, is the application of non-pathogenic strains of *Streptomyces* to control scab of potato (*Solanum tuberosum* L.) caused by *Streptomyces scabies* (Bhat, 2017). Here bio-control may operate through antibiosis or competition for space or nutrients in the rhizosphere. In contrast, *Pseudomonas fluorescens* (Trevisan) Migula F113 was shown to control the soft rot potato pathogen *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye by production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) and, through the use of co-inoculation experiments with mutants lacking DAPG production, that competition was not a feature of biocontrol in this system. Some evidence was also obtained that siderophore production by *P. fluorescens* F113 may play a role in bio-control of potato soft rot under iron-limiting conditions, but DAPG appears to be the major biocontrol determinant. *Pseudomonas* species may also control crown gall disease in many dicotyledonous plants caused by *Agrobacterium tumefaciens* (Braga *et al*., 2016). However, the classic and still commercially successful, bacterial-based biocontrol system is the use of non-pathogenic *Agrobacterium* strains to control *Agrobacterium tumefaciens*. Long-term molecular and ecological studies of this control system have identified how the biocontrol works and have also allowed potential problems associated with its use in the field to be overcome. The most widely used non-pathogenic *Agrobacterium* strain K84 produces a highly specific antibiotic agrocin 84, which is encoded by plasmid pAgK84. In undative inoculation of *Agrobacterium* strain K84 to roots by dipping in cell suspensions prior to exposure to the pathogen effectively controls those strains of pathogen susceptibility to agrocin84 (Cardon *et al*., 2006). However, because there is a risk that plasmid pAgK84 could be transferred to pathogenic strains and reduce effectiveness of control, a transfer deletion mutant of K84, K1026 has been constructed. Strain K1026 is as efficient as K84 in biocontrol of both strains susceptible to agrocin 84 and those resistant to agrocin 84 and so, clearly, production of agrocin 84 is not the only mechanism of biocontrol. Production of other antibiotics such as agrocin 434 or ALS 84 may play a part, but the ability to survive and compete on roots may also be important. Studies where pathogenic cells were co-inoculated with K84 or K1026 resulted in survival of the pathogen on roots up to 8 months later, although no symptoms were present, providing evidence that the non-pathogenic strains prevented disease expression rather than killing pathogen cells directly (Cardon *et al*., 2006).

Table 1. Examples of bacterial fungal pathogen interactions applied to seeds or roots providing biocontrol of fungal plant pathogens.

Bacteria	Fungal pathogen	Plant	Environment	Reference
Actinoplanes spp.	*Pythium ultimum*	Table beet	*Soil*	Cartwright and Spurr 1998
Bacillus spp.	*Rhizoctoniasolani*; *Gaeumannaomycesgraminis* var. *tritici*	Wheat	Soil	Chen and Nuss 1999
Bacillus subtilis GB03	*Fusarium oxysporum* f. sp. *Cicero*	Chickpea	Sterile soil	Chen *et al.*, 1998
B. subtilis BACT-D	*Pythium aphanidermatum*	Tomato	Soil	Chin-A-Woeng *et al.*, 1998
Burkholderiacepacia A3R	Fusarium graminearum	Wheat	Soil	Constantinescu *et al.*, 2014
B. cepacia PHQM 100	*Fusarium* spp.	Maize	Soil	Da Luz *et al.*, 1998
	*Pythium*spp	Maize	Soil	Da Luz *et al.*, 1998
Comamon asacidovorans HF42	*Magnaporthepoae*	Kentucky bluegrass	Soil	Davanlou *et al.*, 1999
Enterobacter sp. BF14	*Magnaporthepoae*	Kentucky bluegrass	Soil	Davanlou *et al.*, 1999
Paenibacillus sp. 300	*Fusarium oxysporum* f. sp. *cucumerinum*	Cucumber	Soil- less mix	De Boer *et al.*, 1999
Pseudomonas spp	*Fusarium oxysporum* f. sp. *radicis-lycopersici*	Tomato	Rockwool	De et al., 1999
Pseudomomas aureofaciens AB244	*Pythium ultimum*	Tomato	Soil-less mix	Delgado-Jarana *et al.*, 2000
P. aureofaciens 63-28	*P. phanidermatum*	Cucumber	Vermiculite	Duczek, 1997
Pseudomonas chlororaphis MA342	*Drechslera graminea*	Barley	Soil	Braga *et al.*, 2016
	D. tere	Barley	Soil	Braga *et al.*, 2016
	D. avenae	Oat	Soil	Braga *et al.*, 2016
	Ustilago avenae	Oat	Soil	Braga *et al.*, 2016
	U. hordei	Barley	Soil	Braga *et al.*, 2016
	Tilletia caries	Wheat	Soil	Braga *et al.*, 2016
P. chlororaphis PCL 1391	*Fusarium oxysporum* f. sp. *radicis-lycopersici*	Tomato	Soil	Duijff *et al.*, 1998
P. chlororaphis RD31-3A	*Fusarium*spp	Seed	Douglas fir	Ebrahim *et al.*, 2011
*Pseudomonas corrugata*13	*Pythium aphanidermatum*	Cucumber	Vermiculite	Duczek, 1997
Pseudomonas fluorescens	*Fusarium oxysporum* f. sp. *Raphan*	Radish	Soil/sand	Ebrahim *et al.*, 2011
P. fluorescens WCS417	*F. oxysporum* f. sp. *raphani*	Radish	Rockwool	Engelkes *et al.*,

				1997
P. fluorescens WCS358	*F. oxysporum* f. sp. *lini*	Flax	Nutrient solution	Fisher *et al.*, 2016
P. fluorescens BTP7	*Pythium aphanidermatum*	Cucumber	Vermiculite	Fisher *et al.*, 2016
P. fluorescens VO61	*Pythium ultimum*	*Lotus corniculatus*	Soil mix	Fisher *et al.*, 2016
	Rhizoctonia solani	Rice	Soil	Fisher *et al.*, 2016
Pseudomonas putida	*Fusarium oxysporum* f. sp. *raphani*	Radish	Soil/sand	Ebrahim *et al.*, 2011
P. putida BTP1	*Pythium aphanidermatum*	Cucumber	Vermiculite	Fisher *et al.*, 2016
Serratia plymuthica	*Pythium ultimum*	Cucumber	Peat-perlite-vermiculite	Frey-Klett *et al.*, 2011
Stenotrophomonas maltophilia C3	*Rhizoctonia solani*	Tall fescue	Soil mix	Fuchs *et al.*, 1999
Streptomyces sp. 385	*Fusarium oxysporum* f. sp. *cucumerinum*	Cucumber	Soil-less mix	De Boer *et al.*, 1999

Bacterial -fungal pathogen interactions: The number of literature in this area continues to increase at a rapid rate, stimulated by the increasing ease with which molecular techniques can be applied to answer questions concerning distribution, and occurrence and relative importance of specific modes of action (Cardon and Gage 2006). Some examples of the different types of bacteria-fungal pathogen interactions examined in the spermosphere and rhizosphere in previous years are given in (Table 1). Although a range of different bacterial genera and species have been studied, the number of papers have involved the use of *Pseudomonas* species. Clearly, *Pseudomonas* species must have activity but it begs the question as to the features that make this genus so effective and the choice of so many workers. *Pseudomonads* are characteristically fast growing, easy to culture and manipulate genetically in the laboratory, and are able to utilize a range of easily metabolizable organic compounds, making them amenable to experimentation. But, in addition, they are common rhizosphere organisms and must be adapted to life in the rhizosphere to a large extent (Cardon and Gage 2006).

Fungal–protozoan interactions: The soilborne protozoan *Plasmodiophora brassicae* Woronin is an ecologically obligate biotroph of brassicas causing clubroot disease which is characterized by proliferation of galls on infected roots. From a large-scale screening exercise, two isolates of the rootcolonizing fungus *Heteroconium chaetospira* (Grove) Ellis were found to suppress club root on chinese cabbage (*Brassica campestris* L.) in non-sterile soil. Hyphal growth occurred in the inner parts of the cortical tissues and into the root tips without causing any external symptoms on the plant and there was no sign of infection by *P. brassicae*. Further studies demonstrated that *H. chaetospira* infected epidermal cells from appressoria via infection pegs and, subsequently, intracellular hyphal growth occurred (Giesler *et al*., 1998). However, the actual mechanism of the disease control observed in the field was unclear. *Heteroconium chaetospira* appears to form a mutualistic symbiosis with *B. campestris* in terms of disease control which is of interest as the Brassicacae family is largely non-mycorrhizal. In addition, *H. chaetospira* was found to colonize the roots of plants from eight families and may have a wide host range (Giesler *et al*., 1998).

Fungal–bacterial pathogen interactions: In the previous years there have been no clear examples of fungi used to control bacterial plant pathogens in the rhizosphere or spermosphere. The reasons for this are unclear but could perhaps indicate an area that deserves further research in the future (Haldar and Sengupta 2015).

Fungal–fungal pathogen interactions: Interactions between biocontrol fungi and fungal plant pathogens continue to be the focus of a large number of researchers, on a similarity with work on bacterial–fungal plant pathogen interactions described earlier.

However, there is an extra dimension in the quality of the interactions between fungi as biocontrol fungi have much greater potential than bacteria to grow and spread through soil and in the rhizosphere through possession of hyphal growth. Some recent examples of fungal–fungal interaction concerning biocontrol in the rhizosphere and spermosphere are given in (Table 2). There are a variety of fungal species and isolates that have been examined as biocontrol agents but *Trichoderma* species clearly dominate, perhaps reflecting their ease of growth and wide host range[28]. There has been an increase in interest in non-pathogenic *Pythium* species, particularly *P. oligandrum* Drechsler where additional modes of action have been determined recently, and a continued interest in well-established saprotrophic antagonists such as non-pathogenic *Fusarium* species, non-pathogenic binucleate *Rhizoctonia* isolates and *Phialophora* species, as well as mutualistic symbionts including mycorrhizal fungi such as *Glomus intraradices* Schenk & Smith. At least one novel biocontrol agent, *Cladorrhinum foecundissimum* Saccardo and Mardial, has been described. Numerous others are listed elsewhere. The most common pathogen targets are *Pythium* species, *Fusarium* species and *Rhizoctonia solani* reflecting their worldwide importance and perhaps their relative ease of control under protected cropping systems, although numerous other pathogens have been examined. Significantly, relatively few of the examples given in (Table 2) involve studies in non-sterile soil or field conditions, with most carried out in soilless conditions reflecting the need to keep the complexity of the system to a minimum in order to achieve reproducible control. Some specific examples of the modes of action found to occur in the rhizosphere and spermosphere during interactions between fungi and fungal plant pathogens are given below.

Table 2. Examples of fungal–fungal interactions examined in the spermosphere and rhizosphere associated with biological disease control.

Antagonist	Pathogen	Host plant	Medium	Reference
Cladorrhinumfoecundissimum	*Pythium ultimum*	Eggplant, pepper	Soilless potting mix	Haris and Nelson 1999
	Rhizoctonia solani	Eggplant, pepper	Soilless potting mix	Hebbar *et al.*, 1998
Fusarium spp. (CS-1, CS-20, Fo47) (non-pathogenic)	*Fusarium oxysporum* f. sp. *lycopersici*	Tomato	Soilless potting mix	Hebbar *et al.*, 1998
	F. oxysporum f. sp*niveum*	Watermelon	Soil-less potting mix	Hervas *et al.*, 1998
Fusarium oxysporum Fo47 (non-pathogenic	*F. oxysporum* f. sp. *lycopersici*	Tomato	Soil and rockwool	Hillman and Suzuki 2004
Fusarium oxysporum (non-pathogenic)	*F. oxysporum* f. sp. *lycopersici*	Tomato	Soil-less potting mix	Hebbar *et al.*, 1998
Glomus intraradices	*F. oxysporum* f. sp. *dianthi*	Carnation	Clay	Hillman and Suzuki 2004
Idriellabolleyi	*Bipolaris sorokiniana*	Barely	Soil	Holmberg, 2011
Penicillium oxalicum	*F. oxysporum* f. sp. *lycopersici*	Tomato	Peat/soil	Holmes *et al.*, 1998
Phialophora sp. I-52	*Gaeumannomyces gramminis* var. *tritici*	Wheat	Soil	Howell, 1999
Pythium acanthophoron	*Fusarium culmorum*	Barely	Sand	Huang and Liu 2006
Pythium oligandrum	*Fusarium culmorum*	Barely	Sand	Huang and Liu 2006
	Pythium spp.	Cucumber	Hydroponic system	Jackon *et al.*, 1991
	P. ultimum	Sugar beet	Soil-based compost	Johnsson *et al.*, 1998
	Verticillium dahliae	Pepper	Potting mix	Jackon *et al.*, 1991; Johnsson

				et al., 1998
Pythium periplocum	*Fusarium culmorum*	Barely	Sand	Huang anf Liu 2006
Rhizoctonia solani (binucleate, non-pathogenic)	*Phytophthora parasitica* var. *nicotianae*	Tobacco	Soil-less mix	Khan *et al.*, 1997
	Rhizoctonia solani	Cabbage	Soil	Khmei *et al.*, 1998
	R. solani; *Pythium ultimum*	Pepper	Potting mix	Knoester *et al.*, 1999
Talaromyces flavus	*Verticillium dahliae*	Eggplant	Soil-less potting mix	Koch, 1999
Trichoderma hamatum TRI-4	*Rhizoctonia solani*	Eggplant	Soil-less potting mix	Harris and Nelson 1999
Trichoderma harzianum 2413	*Phytophthora capsici*	Pepper	Pea-sand mix	Hillman and Suzuki 2004
T. harzianum T-22	*Pyrenophoratriticis-repentis*	Wheat	Soil	Kredics *et al.*, 2003
T. harzianum T-1	*Pythium ultimum*	Bean	Soil	Kubicek *et al.*, 2001
T. harzianum 1295-22	*R. solani*	Creeping bent grass	Peat	Kwak *et al.*, 2007
T. harzianum Th-87	*R. solani*	Egg plant	Soil-less potting mix	Harris and Nelson 1999
T. harzianum BAFC 742	*Sclerotinia sclerotiorum*	Soybean	Soil	Larkin and Fravel 1998
Trichoderma (*Gliocladium*) *virens* GL-21	*Pythium ultimum*	Cucumber	Potting mix	Leon-Kloosterziel *et al.*, 2005

Endophyte – phytopathogen plant interaction: Endophytic fungi are known to produce a large variety of bioactive secondary metabolites that are probably related to the endophyte complex interactions with the host and the phytopathogens and can perform important ecological functions, for example, in the plant development (as growth promoters) and in defense, acting against phytopathogens (Lewis and Larkin 1998). The metabolites and mechanisms involved in the interactions between endophyte and phytopathogen and host plantare still very unclear and are predicted to involve many secondary metabolites. This interaction has been studied in co-cultures of the phytopathogen Moniliophthororeri and the endophyte *Trichoderma harzianum*that cohabit in cacao plants. *T. harzianum*is extensively used as a bio-control agent and has known ability to antagonize *M. roreri.* They identified four secondary metabolites (T39 butenolide, harzianolide, sorbicillinol, and an unknown substance) which production was dependent onthe phytopathogen presence and was spatially localized in the interaction zone. T39 but enolide and harzianolide have been reported to have antifungal activity. Sorbicillinol is an intermediate in the biosynthesis of bisorbicillinoids, a family of secondary metabolites which present diverse activities. *Trichoderma atroviride*, commonly used as a biocontrol agent, produces acetic acid related indoles compounds that may stimulate plant growth. Colonization of *Arabidopsis roots* by *T. atroviride* promotes growth and enhances systemic disease resistance conferring resistance against hemibiotrophic and necrotrophic phytopathogens (Lewis and Larkin 1998). Other co-cultured studies were performed with bacteria. Isolated a great number of *Methylobacterium* strains from asymptomatic citrus plants (with *Xylellafastidiosa*but without disease), then showed that *Methylobacterium mesophilicum* SR1.6/6 and *Curtobacterium* sp (Lima, 2002). ER1.6/6 isolated from health and asymptomatic plants inhibited the growth of the phytopathogen *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis (Lima, 2002). Moreover, transcriptional profile of *Xylella fastidiosa* was evaluated during *in vitro* co-cultivation with a citrus

endophytic strain of *Methylo bacterium mesophilicum*. It was shown that genes relatedto growth, such as genes involved in DNA replication and protein synthesis, were down regulated. While genes related to energy production, stress, transport and motility, such as fumarate hydratase, dihydrolipoamide dehydrogenase (Krebscycle), *pil*Y transporter, clpP peptidase, acriflavin resistance, and toluene tolerance genes, were up regulated. Another approach to study endophyte-phytopathogen plant interaction is based on the genome sequencing and transposon mutagenesis of an endophyte strain of *Burkholderia seminalis*, which suppress orchid leaf necrosis by *Burkholderia gladioli*, revealed eight loci related to biological control (Lima, 2002). A *wcb* cluster related to the synthesis of extracellular polysaccharides of the bacterial capsule was identified. Extracellular polysaccharides are known to be key factors in bacterial–host interactions. In addition, genes clusters putatively related to indole-acetic acid and ethylene biosynthesis were identified in the sequenced genome of the endophyte strain, suggesting that this strain might interact with the plant by altering hormone metabolism (MacDonald, 1991).

Mode of actions of microbial biocontrol

Competition: Competition for nutrients, space or infection sites between fungi in the rhizosphere and spermosphere. Competition between antagonist and pathogens as a mechanism of biocontrol may occur at different levels. For example, competition for space or specific infection sites on roots or seeds has been proposed as a mechanism of biocontrol of pathogenic *F. oxysporum* by nonpathogenic strains of *F. oxysporum* and of pathogenic strains of *R. solani* by nonpathogenic *Rhizoctonia* spp. In both instances the pathogen is excluded by the more rapid and extensive colonization of the root surface by the biocontrol strain. Competition between microorganisms for carbon, nitrogen, and other nutrients in the rhizosphere is another well researched mechanism of biocontrol. Competition for iron, mediated by production of ironchelating siderophores, has been conclusively demonstrated as a mechanism of biocontrol by several species of bacteria in soils where iron is limiting. This is a widely recognized mechanism of biocontrol by *fluorescent Pseudomonas* spp., which produce a range of siderophores including pseudobactins and pyoverdines. The siderophores are thought to sequester the limited supply of iron that is available in the rhizosphere to a form that is unavailable to pathogenic fungi and other deleterious microorganisms, thereby restricting their growth (Lo *et al*., 1998).

Mycoparasitism: The mycoparasitism phenomenon was first reported by[51], who observed the parasitic nature of *Trichoderma lignorum* on several plant pathogens, including *Rhizoctnonia solani, Phytophthora parasitica, Pythium* sp., and *Sclerotium rolfsii*. Mycoparasitism or hyperparasitism occurs when the antagonist invades the pathogens by secreting enzymes such as chitinases, celluloses, glucanases and other lytic enzymes.

Mycoparasitism consists of a direct attack by a fungus to another and leads to the destruction of some of the structures of the host (e.g. mycelium, spores and sclerotia) with the consequent harnessing of their components as a nutrient source. The process of mycoparasitism exerted by a mycoparasite *e.g Trichoderma* sp. occurs in several successive stages. Starts with the chemo trophic growth of *Trichoderma*to the host stimulated by molecules from the same (Mathre *et l.,* 1999). Mycoparasitism by *Trichoderma sp.* is one of the important modes of action exhibited by biocontrol agents against host fungi including plant pathogenic ones. Its study *in-vitro* constitutes considerable importance while studying bio-efficacy of an antagonist against a fungal pathogen. At present there is no standard, rapid, easy and reliable method to study mycelial interaction between a possible mycoparasitic fungi and host. An easy, cheap, and rapid agar plate assisted slide culture technique is developed which facilitates the study of *in-vitro* mycoparasitism with ease. With the help of this technique mycoparasitism by a strain of *Trichoderma* sp. was studied against pathogenic *Rhizoctonia solani* and *Fusarium oxysporium*. A drastic mycoparasitism in the form of coiling and tightly sticking with occasional formation of appressoria like structures was observed while studying mycoparasitism with the help of this method Mathre *et al.,* 1999).

According to the definition of the Federation of British Plant Pathologists, the term mycoparasite strictly addresses to fungi existing in close association with another fungus from which they derive some or all of their nutrients while yielding no benefit in return. Mycoparasites produce cell wall degrading enzymes which allow them to bore holes into other fungi and

extract nutrients for their own growth. Most phytopathogenic fungi have cell wall that contain chitin as a structural backbone arranged in regularly ordered layers and beta-3-1,3-glucan as a filling material arranged in an amorphic manner. Beta-3-1,3-Glucanases and chitinases have been found to be directly involved in the mycoparasitism interaction between *Trichoderma* species and its hosts (Menedez and Godeas 1998).

During mycoparasitism the synthesized hydrolytic enzymes act synergistically. Thus, understanding the induction process from these enzymes is necessary in order to select the most efficient *Trichoderma* isolates for biocontrol. *Trichoderma* species are readily isolated from Brazilian Cerrado soil by conventional methods and have been used for technological exploitation of enzyme production and biological control (Milgroom and Cortesi 2004).

Mycoparasitism or hyperparasitism occurs when the antagonist invades the pathogens by secreting enzymes such as chitinases, cellulases, glucanases and other lytic enzymes. Mycoparasitism is the phenomenon of one fungus being parasitic on another fungus. The parasiting fungus is called hyperparasite and the parasitized fungus as hypoparasite. In mycoparasitism, two mechanisms operate among involved species of fungi. This may be hyphal of inter fungus interaction i.e., fungus-fungus interaction, several events take place which leadto predation viz., coiling, penetration, branching and sporulation, resting body production, barrier formation and lyses (Muthulakshmi *et al.,* 2017).

Exploitation for hypovirulence

What is hypovirulence?: The term hypovirulence most often refers to the reduction in virulence caused by fungal viruses. The reduction of disease has been attributed to a biological control process called hypovirulence, whereby virulent strains are debilitated as a result of infection by fungal viruses (hypo-viruses). Several species of hypo-viruses now are known and each may impart unique effects on *Cryphonectria parasitica*. Lethal infections often are controlled by introducing the appropriate hypovirus into cankers (Narisawa *et al*., 2000). The first indication that virus-like agents might be involved came with the association of double-stranded (ds) RNA with the European and North American strains that were shown to be less virulent (Narisawa *et al*., 2000). These dsRNAs eventually were shown to represent a unique group of viruses, now called hypo-viruses (Milgroon and Cortesi 2004). The definitive proof of the cause and effect relationship and their infectious nature occurred through the application of molecular technology. Although fungal-virus associations have been known for decades, the hypoviruses associated with *C. parasitica*are unique; rather than being encapsulated in a protein coat, they are membrane bounded. As a result, a new virus family, the *Hypoviridae*, has been established for the four species (CHV1 though CHV4) of hypoviruses that have been discovered to date (Nautiyal, 2006). Most studies have been of the CHV1 species, as it was the first hypo virus identified and is the hypovirus associated with biological control of chestnut blight in Europe. This hypo virus also has been discovered infecting strains of *C. parasitica*in China and Japan, but it has never been identified as a natural component of *C. parasitica*in North America [58]. The CHV3 hypo virus is associated with the recovering Michigan chestnut stands but its origin remains unknown as it has not been isolated in the Orient (Neeno-Eckwall and Schottel 1999). CHV2 is uncommon and known only from a site in New Jersey. It also has been identified in *C. parasitica*populations in China (Neeno-Eckwall and Schottel 1999). CHV4 is somewhat unique; unlike CHV1-CHV3, it has little or no observable effect on the virulence or other traits of *C. parasitica*. It is widespread in its association with isolates from the central Appalachians but its origin and role remain undiscovered.

The effects of hypovirus infection on the blight fungus are variable and appear to be a function of the *C. parasitica* strain as well as the infecting hypovirus (Nuss, 1996). For those hypo viruses that reduce fungal virulence, infection often results in smaller non-lethal cankers and a corresponding reduction in the production of asexual spores and almost certainly the reduction or elimination of sexual sporulation. What is known about the molecular influence of the hypo virus on the physiological processes of the fungus has been reviewed (Oliveira *et al.,* 2016).

Mycoviruses are viruses that infect fungi and have the potential to control fungal diseases of crops when associated with hypo-virulence. Typically, mycoviruses have double stranded (ds) or single stranded (ss) RNA genomes. No mycoviruses with DNA genomes have previously been reported. Here, we describe a hypo-

virulence associated circular ssDNA mycovirus from the plant pathogenic fungus *Sclerotinia sclerotiorum*. The genome of this ssDNA virus, named *Sclerotinia sclerotiorum* hypo-virulence associated DNA virus 1 (SsHADV-1), is 2166 nt, coding for a replication initiation protein (Rep) and a coat protein (CP). Although phylogenetic analysis of Rep showed that SsHADV-1 is related to Gemini viruses, it is notably distinct from Gemini-viruses both in genome organization and particle morphology. Polyethylene glycol-mediated transfection of fungal protoplasts was successful with either purified SsHADV-1 particles or viral DNA isolated directly from infected mycelium. The discovery of an ssDNA mycovirus enhances the potential of exploring fungal viruses as valuable tools for molecular manipulation of fungi and for plant disease control and expands our knowledge of global virus ecology and evolution (Neeno-Eckwall and Schottel 1999).

Exploiting Hypovirulence: The discovery of hypovirulence and the observation of a notable level of disease control on American chestnut in Michigan brought hopes for the first time that some level of biological control was possible in North America. Procedures first employed by Grente to treat virulent infections were duplicated. Subsequently, modifications to Grente's treatment protocols and a variety of different inoculum types were used to introduce hypo-viruses into virulent cankers on American chestnut sprouts (Narisawa *et al*., 2000). The results often were very encouraging as hypo-virus transfer frequently occurred and the expansion of individual treated infections frequently was arrested as callus tissue formed at the margins of cankers. Even though many of the treatments were successful and the life of sprouts was prolonged, the sheer number of subsequent infections that developed on the same stem dramatically weakened the tree, and when some cankers were not arrested by treatment, trees died. Further, there was little evidence that natural hypo-virus spread on the same stem afforded any protection to other virulent infections that almost certainly would arise. With few exceptions, most hypo-virulent introductions were unsuccessful if measured by the number of treated sprouts that remained a-live several years after treatment (Ouda, 2014).

As a result of the early releases, several factors were discovered that may influence the effectiveness of the hypo-virulent treatments. When additional hypo-virulent strains were discovered and their infecting hypo-viruses investigated the variation in their effects on *C. parasitica* became apparent. Some virulent strains were so debilitated by hypo-virus infection that they grew poorly in bark and almost completely failed to produce hypo-virulent inoculum. Therefore, concern arose that highly debilitating hypo-viruses have such an extreme effect on their fungus host that there is little potential for the strains to grow in bark and produce inoculum to perpetuate themselves. A sense developed that hypo-viruses that do not debilitat*e C. parasitica*as significantly may be more useful biological control agents (Milgroon and Cortesi 2004). Logically, if strains are more capable of invading bark and generating hypo-virulent inoculum without killing their hosts, they may be more capable of disseminating their hypo-viruses and thus potentially better biological control agents

Rhizosphere colonization: Rhizosphere, the interface between soil and plant roots, is a chemically complex environment which supports the development and growth of diverse microbial communities. The composition of the rhizosphere microbiome is dynamic and controlled by multiple biotic and abiotic factors that include environmental parameters, physiochemical properties of the soil, biological activities of the plants and chemical signals from the plants and bacteria which inhabit the soil adherent to root-system (Pankhurst and Lynch 2005).

Inoculation of plants with beneficial microbes can be traced back for centuries. Although bacteria were not proven to exist until in 1683 von Leeuwenhoek discovered microscopic 'animals' under the lens of his microscope, their utilization to stimulate plant growth in agriculture has been exploited since ancient times. Theophrastus (372–287 BC) suggested the mixing of different soils as a means of 'remedying defects and adding heat to the soil (Pal and McSpadden 2006). Colonization of plant root system is the very first step in nearly all interactions between plants and soil borne microbes. The importance of this compartment for plant growth and soil microbiology had already been realized in the very pioneer times of microbiology in the late 19th century.

The rhizosphere of plants is one of the most fascinating microbial habitats for basic and applied studies in the field of microbiology, as it is shaped by the soil, the plant

and the microorganisms. Given the tremendous diversity of soil microbes, soil fauna, and plants, it is virtually impossible to investigate the intricacies of every potential rhizosphere interaction in every environmental circumstance. However, an understanding of controls over belowground function is becoming increasingly important as natural and agro ecosystems around the globe are exposed to anthropogenic pressures. In addition, the chemistry and development of soil present today have been strongly affected by the actions of rhizospheres over evolutionary time frames and the evolution of true plant roots and their extension deep into the substrate is hypothesized to lead to a revolution in planetary carbon and water cycling during the Devonian period Peever *et al.,* 1998).

What is the biogeochemical function of the rhizosphere on Earth today? In what major ways was rhizosphere function below ground similar across terrestrial ecosystems, and in what fundamental ways can it differ? However, since the microbial inoculations would mainly be performed in soils before the plant is grown up, the strains should also be able to survive in the soil and show a good saprophytic ability. To fulfill these requirements, progress must be made in our knowledge of which bacterial traits affect the soil and rhizosphere colonizing ability of microbes (Rajapaksha *et al.,* 2004).

Genetic Regulation of Plant–Microbe Association: Correlations have been reported between rhizosphere competence and growth rate of a plant root adhered bacteria and it has a prerequisite to multiply using organic compounds and other physiological traits which may further contribute to their rhizosphere competence and these rhizosphere competent bacteria helps in increasing the plant productivity by multiple changing in the gene expression. Auxin produced by bacteria in the rhizosphere can stimulate the activity of the1-aminocyclopropane-1-carboxylate (ACC) synthase, an enzyme normally used by plants to form ethylene and transcription of *ipdC*, an *Erwinia* IAA biosynthetic gene, is induced in response to bean and tobacco compounds and the bacterial auxin synthesis is dependent upon plant exuded tryptophan. Analyses of the more than 4000 ORFs of *Bacillus subtilis*revealed that *yqkF*are growth regulatory gene related to auxin which may manipulate hormonal processes in plants (Ross *et al.,* 1998). To control the ethylene production, increased amount of ACC is hydrolyzed by an ACC deaminase and ACC deaminase - producing rhizobacteria upregulate genes involved in cell division and proliferation but down-regulate stress genes thus reducing plant stress and induce root elongation and proliferation in plants, largely by lowering ethylene levels which bring about ISR by fortifying the physical and mechanical strength of the cell wall (Ross *et al.,* 1998). (Ryder *et al.,* 1998) by showing augmented, rapid transcript accumulation of defense related genes. including PR-1a, phenylalanine ammonia-lyase (PAL), and 3-hydroxy-3-methylglutaryl CoA reductase (HMGR) following inoculation with PGPRs– concluded that PGPRs by a typical phenomenon of potentiation using the jasmonate pathway of defense provide ISR to the plants. Phenotypically ISR in plants is similar to pathogen-induced systemic acquired resistance (SAR) known defense-related genes, i.e. the SA-responsive genes PR-1, PR-2, and PR-5, the ethylene-inducible gene (Sakuma *et al.,* 2006).

Plant-microbe coexistence is strongly influenced by abiotic stress conditions induced by drought, high salt and temperature. Stress is an unhealthy condition and prolonged stress weakens the immune system, opening the door for a variety of ailments that an otherwise healthy plant could overcome. The major consequences of drought stress is the loss of water from the protoplasm and leads to the concentration of ions in the protoplasm, higher concentration of which are toxic to plants. However, a lack of water results in the overproduction of free radicals, leading to the damage of cellular membranes and ultimately loss of solutes from the cell and organelles (Sharifi-Tehrani *et al.,* 1998). Damage to DNA under these conditions severely hinders the ability of the plant to recover, as DNA stores the genetic information that is ultimately used for the synthesis of new proteins.

Sharma *et al.,* 2013 reported that the *Arabidopsis thaliana* plants inoculated with *Paenibacillus polymyxa*plants were more resistant to drought stress and revealed that ERD 15 and RAB 18 is a drought responsive gene and gets differentially expressed in case of *P. polymyxa* treated plants in drought and its mechanism of action of the plant growth promoting was studied by (Sharma *et al.,* 2013). Using plasmid-borne *gfp* gene tagging of *P. polymyxa,* (Sharma *et al.,* 2013) concluded that it colonizes on root and form biofilm on plant root tips and enters intercellular spaces but does not spread throughout the plant. Adaptation to environmental stresses is dependent

upon the activation of cascades of molecular networks involved in stress perception, signal transduction, activation and regulation of specific stress tolerant genes. During stress conditions dehydration responsive transcription factor after binding with the cis acting elements promotes both, the stress inducible genes codes for osmo- protectants, scavengers or stress proteins such as cold responsive proteins (COR) or late embryogenesis abundant (LEA) with an undefined mechanism of action (Sharma and Singh 2018) and regulatory proteins such as transcription factors or components of signal cascades and regulate the expression of a set of genes involved in stress. Both categories of genes have been shown to impart tolerance when over expressed in plants. Significant improvement of stress tolerance in case of *A. thaliana* has been noticed by the interaction of a single transcription factor, which activates expression of downstream genes involved in drought- and salt-stress (Singh *et al.,* 1999). A novel cDNA encoding DRE-binding transcription factor, designated GhDBP3 from *Gossypium hirsutum*, showed enhanced expression by drought, NaCl, low temperature and ABA treatment. During different environmental stresses, basic cellular processes such as DNA replication, repair, recombination, transcription, ribosome biogenesis and translation initiation play essential role and all these functions are genetically controlled by helicases of DEAD-box protein superfamily. Thus, helicases might be playing an important role in regulating plant growth and development under stress conditions by regulating some stress-induced pathways (Sreenivasulu *et al.,* 2007). (Srinivasulu and Ortiz 2017) reported that *A. thaliana* genome contains eight genes of high mobility group B(HMGB) proteins, and gets up regulated by cold salt and drought stress and down regulated by drought or salt stress. Various strategies have been used to produce transgenic plants with increased tolerance to dehydration stress. These include the overproduction of enzymes responsible for biosynthesis of osmolytes, late embryogenesis-abundant proteins and detoxification enzymes. However, in case of crop improvement regulatory gene would have potential to play a broader role in stress tolerance and still a careful appraisal for the selection of the genes is expected (Suryanarayanan *et al*., 2015).

Competitive saprophytic ability: The importance of competitive colonization of organic matter and the ability of a fungus to survive in the soil and defined competitive saprophytic ability as an intrinsic characteristic of a fungal species that result in successful competitive colonization of dead organic substrates. As reported by (Srinivasulu and Ortiz 2017) the compatibility of the egg parasitic fungi with other biocontrol agents *viz*., *Trichoderma viride*, *Paecilomyces lilacinus, Pseudomonas fluorescens* and *Bacillus subtilis*, and carbofuran were studied under laboratory conditions. The result was shows the growth of both the fungi (*P. chlamydosporia* and *E. aranearum*) was observed after 5 days of incubation in the medium. The growth of *T. viride* and *P. lilacinus*was rapid when compared to *P. chlamydosporia* and *E. aranearum* isolates. Since these both fungi not exhibiting any inhibition zone it is considered that both are compatible with each other (Table. 3 and Figure. 1, 2).

Table 3. Compatibility of *P. chlamydosporia* and *E. aranearum* with other biocontrol agents and carbofuran

S. No.	Treatments	Compatibility
1.	*P. chlamydosporia*+ *T. viride*	++
2.	*P. chlamydosporia*+ P. *lilacinus*	++
3	*P. chlamydosporia*+ *P. fluorescens*	+++
4	*P. chlamydosporia*+ *B. subtilis*	+++
5	*P. chlamydosporia*+ Carbofuran	+++
6.	*E. aranearum*+ *T. viride*	++
7	*E. aranearum*+ P. *lilacinus*	++
8	*E. aranearum*+ *P. fluorescens*	+++
9	. *E. aranearum*+ *B. subtilis*	+++
10	. *E. aranearum*+ Carbofuran	+++

+ less compatible
++ Moderately compatible
+++ highly compatible
- not compatible

P. chlamydosporia+ *T. viride* | *P. chlamydosporia*+ *B. subtilis*

P. chlamydosporia+ Carbofuran | *P. chlamydosporia*+ *P.flyorescens*

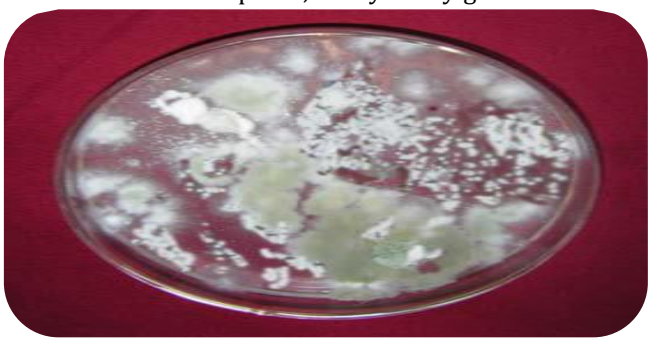
Figure 1. Compatibility of *P. chlamydosporia* with other bio-control agents

Antibiosis*:* Antibiosis is defined as antagonism mediated by specific or non–specific metabolites of microbial origin, by lytic agents, enzymes, volatile compounds or other toxic substances or through the production of specific or non-specific metabolites with antibacterial, antifungal and anti-nematode activity. Antibiosis plays an important role in biological control. Anti biosis is a situation where the metabolites are secreted by underground parts of plants, soil microorganism, plant residues etc. It occurs when the pathogen is inhibited or killed by metabolic products of the antagonists. The products include the lyric agents, enzymes, volatile compounds and other toxic substances (Nautiyal, 2006).

Antibiosis is typically studied in host plant populations and extends to the insects which feed upon them. "Antibiosis resistance affects the biology of the insect so pest abundance and subsequent damage are reduced compared to that which would have occurred if the insect was on a susceptible crop variety. Antibiosis resistance often results in increased mortality or reduced longevity and reproduction of the insect (Teetes, 1996).

Antibiotic production by fungi exhibiting biocontrol activity has most commonly been reported for isolates of *Trichoderma*/*Gliocladium* and *Talaromyces flavus* (Klöcker) although in the last few years antibiotics have been at least partially characterized in *Chaetomium globosum* (Kunze). *Mini medusa polyspora* and *Verticillium biguttatum* (*Thompson* et al*.,* 1998). Of particular interest are those studies where antibiotic production has a definite link to biocontrol. For example, *Trichoderma* (*Gliocladium*) *virens*comprises P and Q group strains, based on their antibiotic profiles (*Thompson* et al*.,* 1998). Strains of P group produce the antibiotic gliovirin which is active against *Pythium ultimum* but not against *Rhizoctonia solani* AG-4. Strains of the Q group produce the antibiotic gliotoxin which is very active against *R. solani* but less so against *P. ultimum*. In seedling bioassay tests, strains of the P group are more effective biocontrol agents of damping-off on cotton caused by *Pythium*, while those from the Q group are more effective as bio-control

agents of damping-off incited by *R. solani* (Thompson *et al.,* 1998). Thus, there is strong circumstantial evidence for a role for antibiotics in biocontrol in this experimental system. This has been confirmed in a zinnia–*Pythium* system where *T. virens* G-20 incorporated into soil and potting mix resulted in disease suppression clearly associated with a maximum accumulation of gliotoxin in the medium. Gliotoxin minus mutants displayed only 54% of the *Pythium* disease suppressive activity in zinnia compared with the wild-type. Gliotoxin production by *Trichoderma* is also thought to be responsible for cytoplasmic leakage from *R. solani* observed directly on membranes in potting mix. Production of hydrogen peroxide in the rhizosphere, catalyzed by glucose oxidase from *Talaromyces flavus* is thought to be responsible for the biocontrol of *Verticillium* wilt caused by *Verticillium dahliae*Kleb. on eggplant (*Solanum tuberosum* L.). Purified glucose oxidase significantly reduced the growth rate of *V. dahliae* in the presence, but not the absence, of eggplant roots, suggesting that a supply of glucose from the roots was of major importance. Further, a single spore variant, Tf-l-np, which produced 2% of the level of glucose oxidase activity of the wild type did not control *Verticillium* wilt on eggplant in non-sterile field soil in a glass house experiment, whilst the wild type provided significant control. Glucose oxidase also suppressed growth of *V. dahliae in vitro* and killed micro-sclerotia of *V. dahliae in vitro* and in soil (*Thompson* et al*.,* 1998).

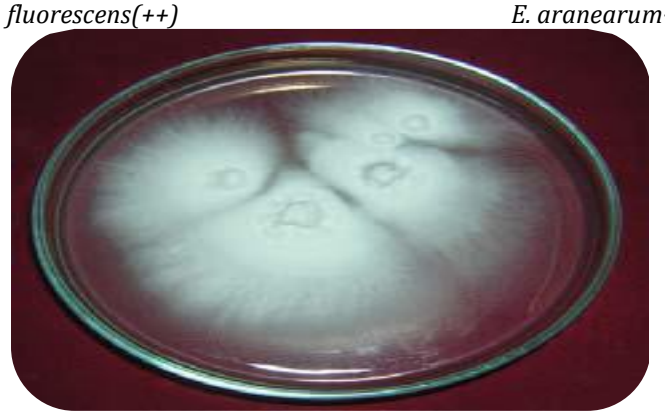


E. aranearum+ *T. viride* (+++)

E. aranearum+ *P. lilacinus* (+)

E. aranearum+ *P. fluorescens*(++)

E. aranearum+ *B. subtilis* (++)



E. aranearum+ Carbofuran (+++)

Figure 2. Compatibility of *E. aranearum* with other biocontrol agents

Induced resistance: Resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor. Disease resistance in plants is manifested by limited symptoms, reflecting the inability of the pathogen to grow or multiply and spread, and often takes the form of a hypersensitive reaction (HR), in which the pathogen remains confined to necrotic lesions near the site of infection. Induced resistance is the phenomenon that a plant, once appropriately stimulated, exhibits an enhanced resistance upon 'challenge 'inoculation with a pathogen (Thompson *et al.,* 1998).

Beneficial microbes in the microbiome of plant roots improve plant health. Induced systemic resistance (ISR) emerged as an important mechanism by which selected plant growth promoting bacteria and fungi in the rhizosphere prime the whole plant body for enhanced defense against a broad range of pathogens and insect herbivores. A wide variety of root associated mutualists, including *Pseudomonas*, *Bacillus*, *Trichoderma*, and mycorrhiza species sensitize the plant immune system for enhanced defense without directly activating costly defenses (Timmusk *et al.,* 2003). The better understanding of plant signaling pathways has led to the discovery of natural and synthetic compounds called resistance inducers that induce defense responses in plants similar as the ones induced by pathogen infection. Different types of resistance inducers have been characterized, including carbohydrate polymers, lipids, glycopeptides, and glycol proteins. In plants, a complex array of defense responses is induced after detection of microorganism via recognition of elicitor molecules released during plant-pathogen interaction. Following elicitor perception, the activation of signal transduction pathways generally leads to the production of reactive oxygen species, phytoalexin biosynthesis, reinforcement of plant cell wall associated with phenyl propanoid compounds, deposition of callose, synthesis of defense enzymes, and the accumulation of pathogenesis related (PR) proteins, some of which with antimicrobial properties (Timmusk *et al.,* 2003). When resistant plants recognize resistance inducers, intracellular signal transduction pathways are activated. These pathways ultimately result in the depression of a battery of genes called defense response genes. These latter genes encode various pathogenesis related (PR) toxic proteins such as chitinases, glucanases, lysozyme-active proteins, or cell wall strengthening proteins such as hydroxyproline rich glycoproteins. Response proteins may also be enzymes that act in the biosynthetic pathways for lignification of cell walls or production of phytoalexins, low molecular weight toxic chemicals that antagonize the invader. In the following section, the biochemical response of plant defense mechanism related to PR-proteins including chitinase and glucanase, as well as plant lignin content will be explained (Utkhede and Koch 1999).

During some mycorrhizal syntheses there is little or no induced resistance response detected. However, spatial or temporal separation experiments have indicated that increased levels of chitinases, β-1,3 glucanases, β-1,4 glucosidase, PR-1 protein, and peroxidase as well as cell wall appositions and phenolics may be associated with induced resistance due to fungi (Thompson *et al.,* 1998).

Dose response experiments involving non-pathogenic *Fusarium* species to control *F. oxysporum* on tomato have indicated that induced resistance is not an all or nothing response (Thompson *et al.,* 1998). By varying the level of inoculum of the inducing strain and the pathogenic isolate in soil, it was shown that some non-pathogenic isolates such as *Fusarium* CS-20 controlled *Fusarium* wilt effectively with antagonist levels of only 100 chlamydospores g^{-1} of soil (cgs) with pathogen densities of up to 10^5cgs. In contrast, isolate Fo47 was effective only at antagonist densities of 10^4–10^5cgs, regardless of pathogen density. Subsequent mathematical modelling provided evidence that CS-20 control was largely through induced resistance, whereas Fo47 was active primarily through competition for nutrients (Thompson *et al.,* 1998). A similar dose response effects were found with non-pathogenic isolate of *F. oxysporum* f. sp. *ciceris* (Pad w.) Matuo & Sato and non-pathogenic isolates of *F. oxysporum* to control wilt of chickpea (*Cicer arietinum* L.) caused by pathogenic *F. oxysporum* f. sp. *ciceris*. However, in addition, the plant genotype also seemed to influence the degree of resistance induced (Thompson *et al.,* 1998).

Mycorrhizal associations: The term mycorrhiza (meaning fungus root) was originated by Frank 1885, Mycorrhizae literally means "fungus root" and describes a mutualistic association between fungus and plant roots that exists in almost all plants. The plant supports the fungus by providing carbohydrates needed for fungal growth, while the fungus helps the plant by increasing its root surface area. More recently, mycorrhizas have been defined as associations between fungal hyphae and organs of higher plants concerned with absorption of substances from the soil. Central challenge in global

ecology is the identification of key functional processes in ecosystems that scale, but do not require, data for individual species across landscapes. Given that nearly all tree species form symbiotic relationships with one of two types of mycorrhiza fungi arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi and that AM and ECM-dominated forests often have distinct nutrient economies, the detection and mapping of mycorrhizae over large areas could provide valuable insights about fundamental ecosystem processes like nutrient cycling, species interactions, and overall forest productivity (Vashisht and Tuteja 2006).

Around 90% of higher plants are infected by fungi and form mycorrhizal associations. Fungal symbiosis with a plant host is based on the provision of carbon from plant to fungus and inorganic nutrients from fungus to plant. Benefits to the host are considerable. Without mycorrhizal associations, many higher plants would be unable to complete their life cycles. Both modern agriculture and natural ecosystems such as forests and grasslands rely strongly on mycorrhizal associations. (Source.URL: http://plantsinaction.science.uq.edu.au/edition1/?q=content/3-4-mycorrhizal-associations).

Operational mechanisms of biocontrol: Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Here, we assert that the different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions (Table 4). Direct antagonism results from physical contact and/or a high degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, hyper parasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect (Vidhyasekaran and Muthamilan 1999).

Table 4. Operational mechanisms of biological control of plant pathogens.

Type	Mechanism	Examples
Direct antagonism	Hyper-parasitism/predation	Lytic/some nonlytic mycoviruses *Ampelomyces quisqualis* *Lysobacter enzymogenes* *Pasteuria penetrans* *Trichoderma virens*
Mixed-path antagonism	Antibiotics	2,4-diacetylphloroglucinol, Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases,Glucanases, Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Physical/chemical interference
Indirect antagonism	Competition	Exudates/leachates consumption Siderophore scavenging Physical niche occupation
	Induction of host resistance	Induction of host resistance contact with fungal cell walls Detection of pathogen associated, molecular patterns Phyto hormone mediated induction

Factors affecting biological control agents: The agricultural importance of the biocontrol is that possess good antagonistic abilities against plant pathogenic microbes. Antagonism is based on different mechanisms, like the production of antifungal metabolites, competition for space and nutrients and mycoparasitism. Some of microbes like *Trchoderma* strains with effective antagonistic abilities are potential candidates for the biological control of plant diseases. Biotic and biotic environmental parameters may have negative influence on the biocontrol efficacy of biocontrol strains (Vinocur and Altman 2005).

Effects of Temperature: Studies are available on the effects of temperature on the spore germination and germ tube growth mycelial growth, competitive saprophytic abilities and on volatile and non-volatile metabolite

production of *Trichoderma* strains. The optimum temperature for growth differs among the *Trichoderma* species. Most *Trichoderma* strains are mesophilic, and cannot protect the germinating seeds from soil borne diseases caused by cold tolerant strains of plant pathogenic fungi during cold autumn and spring conditions. Previous studies of cold tolerant *Trichoderma* strains, which investigated than agonistic abilities at different temperatures, found that temperature did not have an effect on hyphal interactions with the test fungi. *T. aureoviride* and *T.viride* strains were more effective *in-vitro* antagonists against *P. debaryanum* than *T. harzianum* strains. The effect of low temperatures on the production and activities of extracellular 1,4-*N*-acetyl-glucosaminidase (NAGase), glucosidase and trypsin and chymotrypsin like proteases all enzymes thought to be involved in the mycoparasitic process were also examined and results showed that these enzymes were produced at 10 ^{0}C and remained highlyactiveevenat5^{0}Cin the cold tolerant strains (Vinocur and Altman 2005).

Water Availability: One of the most important limitations of the use of *Trichoderma* strains as bio-fungicides are their low osmo-tolerance level. Water conditions in soils are limiting parameters affecting fungal activities. Dry conditions may occur even in normally less dry soils as a result of normal drying between rains. On the other hand, biocontrol agents may be needed against plant pathogens in dry soils. Optimal water potential values for the secretion of glucosidase, cellobiohydrolase, xylosidase, NAGase and chymotrypsin like protease enzymes were different. Cello-biohydrolase and NAGase enzymes showed optimal secretion at the highest examined water potential, while the maximum activities of secreted glucosidase, xylosidase and chymotrypsin like protease enzymes occurred at lower water potential values than those optimal for growth. *In vitro* enzyme activities were affected by water potential, but significant enzyme activities were measured for most of the enzymes even at –14.8 MPa, which is below the water potential, where mycelial growth ceased. These results suggest the possibility of using mutants with improved xero-tolerance for biocontrol purposes in soils with lower water potential (Vinocur and Altman 2005).

pH: Biocontrol *Trichoderma* strains are applied in agricultural soils with certain pH characteristics. Therefore, it is important to collect information about the effects of pH on mycelial growth and on the *in vitro* activities of extra cellular enzymes involved in nutrient competition and mycoparasitism of *Trichoderma* strains with biocontrol potential. pH can also play a role in the regulation of extracellular enzyme production, as it was demonstrated by (Warren and Bennett 1999) for beta-1,6-glucanase of *Trichoderma harzianum. Trichoderma* strains were able to grow in a wide range of pH from 2.0 – 6.0 with an optimum at 4.0. However, the mycelialkhede growth of some of the examined plant pathogenic fungi had pH optima at alkalic values. Vinocur and Altman 2005 have found that optimum biomass production of three *Trichoderma* isolates occurred at pH ranges between 4.6 and 6.8. Examined the effect of pH on the *in vitro* activities of *Trichoderma* extracellular enzymes. Optimal pH values were pH=5.0 for glucosidase, cellobiohydrolase and NAGase, pH=3.0 for beta-xylosidase, pH=6.0 for trypsin-like protease and pH=6.0–7.0 for chymotryps in like protease activities (Weindling, 1932).

Effects of Pesticides: Indiscriminate use of insecticides and fungicides leads to a combined contamination of pesticide residues in the soil environment and causes a severe threat to beneficial microbial activities (Whipps, 2001). Hence, it is essential to assess the microbial populations of soils in the biosphere. (Whipps, 2001) were assessed on two insecticides, cypermethrin and chlorpyrifos alone and in combination with two fungicides, mancozeb and carbendazim, for their effects on the bacterial and fungal populations in two tomato cultivated soils. Samples of soil-1 and soil-2 were collected from tomato cultivated fields of El Quienchi, Pichincha, Ecuador. Initially the physicochemical characteristics of soils, e.g., soil pH, organic matter, total nitrogen, electrical conductivity, sand, silt and clay contents were detected, and then soil bacterial and fungal populations were determined. The influence of selected pesticides alone or in combination, on the bacterial and fungal population was concentration dependent; the populations were increased with increasing concentration of pesticides up to 5.0 kg ha^{-1} compared to the controls in 10-day incubated soils. The bacterial and fungal populations continued to increase up to 20 days, and then, gradually decreased after 30 and 40 days of incubation. The results clearly indicate that application of individual and/or mixtures of the pesticides in cultivation of tomato, at field application rates (2.5–5.0 kg ha^{-1}), significantly improved the bacterial and fungal populations in soil-1 and soil-2. However, further increase in the dose of pesticides (7.5–10 kg ha^{-1}) dramatically decreased the bacterial and fungal populations. On the other hand,

insecticides in combination with fungicides showed a negative effect on fungal populations (Whipps, 2001).

Effects of Metal Ions: Toxic effects of heavy metals on soil microorganisms have been extensively studied in the past and almost every group of organisms has been studied in this respect. Fungi and bacteria constitute the main components of the soil microbial biomass. It has often been stated that fungi are more tolerant of heavy metals as a group than bacteria (Windling, 1932). The soil respiration rate was only slightly affected by the metal contamination, although a clear dose-response effect was seen at added metal concentrations above 4 mmol kg/1. The highest level of Cu and Zn resulted in a 30% reduction in soil respiration rate compared with the control soil. The metal effect on soil respiration rate did not differ between Zn and Cu contamination. In the pilot experiment respiration rate decreased in a similar manner as in the main experiment, with 38% at the highest level of Cu and with 34% at the highest level of Zn addition. Bacterial activity, measured as the thymidine incorporation rate, decreased linearly with the logarithm of the metal addition above a metal concentration of 2 m mol kg/1. No differences were observed between effects due to Zn and Cu contamination. The highest levels of metals decreased bacterial activity to less than 10% of that in the control samples. Fifty percent effective dose values (the ecological dose resulting in a 50% decrease in activity) were around 10 m mol of added metal kg/1. In the pilot experiment, bacterial activity decreased in 2 days to less than 20% of the control at the highest level of both Cu and Zn addition. Fungal activity, measured as acetate-in-ergosterol incorporation, increased with the added soil metal concentration above 4 m mol kg/1. The increase was most evident for the Cu-contaminated soil, where the highest level of Cu addition increased fungal activity seven times. In the Zn-contaminated soil, fungal activity in the soil with the highest level of contamination was three times higher than that in the control samples. In the pilot study, fungal activity also increased with metal levels after 2 days, with about three times at the highest level of Cu and with two times at the corresponding level with the Zn addition (Whipps *et al.,* 2001).

Effects of Antagonistic Bacteria: One of the limiting factors of the application of mycoparasitic *Trichoderma* strains as fungicides in agricultural soils is that many strains of soil bacteria suppress the activity of *Trichoderma* (Vinocur and Altman 2005). Therefore, this is advantageous if a biocontrol *Trichoderma* strain is able to antagonize and degrade bacteria present in compost or in the rhizosphere of plants. The influence of bacteria on the competitive saprophytic ability of *Trichoderma* species has been investigated by Vashisht and Tutejaand the competitive success of *Trichoderma* has been suggested to be attributable mainly to its sensitivity to the inhibitory effect of bacteria. Eighteen *Trichoderma* strains were screened for their ability to degrade bacterial cells. The specificity spectrum and the intensity of degradation were highly variable. In the case of five strains showing outstanding degrading abilities towards *Bacillus subtilis*, the NAGase, trypsin-like and chymotrypsin-like protease activities were determined under inductive and non-inductive circumstances. All strains were able to produce NAGaseand proteases constitutively at a moderate level, which could be elevated by induction with *B. subtilis* cells. In inductive media, 3–6 times more NAGase and proteases were produced. The inductive fermentation broth of an outstanding strain, *T. harzianum*T19, was fractionated on a Sephadex G 150 column. The strain produced at least 3 trypsin like proteases, 6 chymotrypsin-like proteases, and 4NAGases upon induction with *B. subtilis* cells. Muramidase-like activities were also present in the fermentation broth of this *T. harzianum*s train. These shows that bacterium degrading ability is common, but highly variable among *Trichoderma* strains. Proteases, NAGases and muramidases seem to have great importance in the degradation of bacterial cells. In addition to testing their ability to antagonize plant pathogenic fungi, the determination of their bacterium degrading capabilities may also be useful in the evaluation of bio fungicide *Trichoderma* strains, as this property can perhaps help the strains to be dominant microorganisms in the habitats where they are applied (Whipps, 2001).

Compatibility of different bio-agents with fungicides and bactericides and its relevance in biological control

Compatibility of fungal bio-agents with fungicides: The compatibility studies between the bio-control microorganisms and chemical pesticides were based on the microbial strains interaction with the active substance from the chemical insecticide and insect of fungicides (imidacloprid 600g/l and mix of imidacloprid 460 g/l with thiram 176 g/l, respectively. *Trichoderma viride* can thrive in diverse environmental conditions as aggressive colonizers of soil and the roots of plants and act as natural bio-agent to protect plants from infection by soil-born fungal pathogens. Laboratory conducts were conducted to

test the possibility of combining fungicides with *Trichoderma viride* to work out their compatibility to diverse a suitable integrated management of soil borne plant disease. Five fungicides: dithane M-45, ridomil, captaf, blue copper and Bavistin. Among these only captaf and blue copper had recorded compatibility to some extent with *T. viride.* Investigation suggests that compatible fungicide can be used with Trichoderma in an IDM package to control soil borne plant pathogen Whipps *et al.,* 2001).

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Compatibility of Bacterial bio-agents with fungicides: The bacterial compatibility with the chemical phytosanitary products (at different concentration) did not inhibit completely the bacterial growth. The insecticide based on imidacloprid 600 g/l, did notaffect the growth of any Bacillus biocontrol strains, when it was tested at 20% concentrations (Woo *et al.,* 2006). Woo *et al.,* 2006 showed that only the insect fungicides mixture, in 20% concentration, caused moderate growth inhibition (less than 5 mm) to some of the bacterial strains tested. The results revealed that none of the pesticides tested inhibited totally the bacterial growth in the tested concentration (In case of *Pseudomonas chlororaphis*ssp.*aurantiaca*Sal.c2 strain, there was no inhibition of bacterial growth in any of the tested pesticides concentrations. Likewise, in the presence of imidacloprid insecticide, at 20% concentration, all tested bacterial strains were able to grow without any inhibition.

Effect of bactericides on radial growth of fungal bio-agents: Bleaching powder even at one fourth of the recommended dosage significantly reduced the mycelial growth thus found to be moderately incompatible whereas bleaching powder at recommended dosage completely inhibited the mycelial growth of *Trichoderma harzianum*thus found to be incompatible with *T. harzianum* (Yu *et al.,* 2010).

Table 5. Effect of bactericides on radial growth of *Trichoderma harzianum*

Bactericides	Concentration (ppm)	Colony diameter (mm)	Inhibition of mycelial growth (%)
Streptomycin	64	65.80	26.88
Streptomycin	32	75.66	15.93
Streptomycin	16	80.16	10.93
Ampicillin	40	54.16	39.82
Ampicillin	20	64.33	28.52
Ampicillin	10	76	15.55
leaching Powder	15000	0.00	100.00
Bleaching Powder	7500	11.00	87.77
Bleaching Powder	3750	38.16	57.60
Copper hydroxide	2000	51.16	43.15
Copper hydroxide	1000	80.66	10.33
Copper hydroxide	500	86.83	3.52
Chloramphenicol	13	53.50	40.55
Chloramphenicol	6.5	68.50	23.88
Chloramphenicol	3.2	73.83	17.96
Control	90.00	0.00 90.00	0.00

Effect of bactericides on growth of bacterial bio-agents: *Pseudomonas fluorescens* was found compatible with copper hydroxide (Kocide 3000) even at a high concentration of 300 ppm**.** No work has been done on the compatibility of these bactericides with bacterial biocontrol agents under *in vitro* conditions except copper hydroxide. This is the first report about the compatibility of above bactericides with bacterial biocontrol agents for controlling bacterial wilt disease in tomato (Yu *et al.,* 2010).

Table 6. Effect of bactericides on growth of *Pseudomonas fluorescens*

Bactericides	Concentration(ppm)	O.D at 610 nm
Streptomycin	64	1.12
Streptomycin	32	1.27
Streptomycin	16	1.29
Ampicillin	40	1.11
Ampicillin	20	1.22
Ampicillin	10	1.24
Chloramphenicol	13	0.98
Chloramphenicol	6.5	1.11
Chloramphenicol	3.2	1.16
Bleaching Powder	15000	0.84
Bleaching Powder	7500	0.92
Bleaching Powder	3750	1.03
Copper hydroxide	2000	1.00
Copper hydroxide	1000	1.06
Copper hydroxide	500	1.38
Control	---	1.36

CONCLUSION

Biological control seems to the best alternative to disease suppression. Bio agents bring the disease suppression with no environmental hazards. Research has proved that the bio agents trigger the growth of plants. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favors the activity and survival of microbe it contains. Most of the bio agents perform well in the laboratory conditions but fail to perform to their fullest once applied to the soil. This is probably attributed to the physiological and ecological constraints that limit the efficacy of bio agents. To overcome this problem, genetic engineering and other molecular tools offer a new possibility for improving the selection and characterization of bio control agents. Various methods that can contribute to increase the efficacy of bio agent include mutation or protoplasm fusion utilizing poly ethylene glycol. There is also an urgent need to mass produce the bio agents, understand their mechanism of action and to evaluate the environmental factors that favor the rapid growth of bio control agents.

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