ISBN: 978-93-90847-17-4

4. Bio-Control Approaches for Plant Disease Management

S. K. Tripathi

Professor and Head,
Department of Plant pathology,
JNKVV, College of Agriculture Rewa,
M.P., India.

Radha Singh

Assistant Professor,
Department of Plant Physiology,
JNKVV, College of Agriculture Rewa,
M.P., India.

4.1 Introduction:

Biotic stress is a major cause of limiting the agricultural productivity. It is caused by living organisms such as viruses, bacteria, fungi, nematodes, insects, arachnids, and weeds. Biotic stress agents directly take up their host of its nutrients leading to reduced plant survival rate and, in extreme cases, death of the host plant.

The term "bio-control" is abbreviated form of "biological control" mostly used in field of entomology and plant pathology. In entomology, it has been used to describe the use of live predatory insects, entomopathogenic nematodes, or microbial pathogens to suppress populations of different pest insects. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host specific pathogens to control weed populations. In both fields, the organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA). More broadly, biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens.

Biological control is basically natural control, as system by which nature maintains the biological equilibrium and during the process checks the populations of plant pathogenic organisms also in this system, nature employs interactions between microorganisms and environment.

Biological control is defined as the reduction of inoculums density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonists, or by mass introduction of one or more antagonists. Biological control is the reduction of the amount of inoculums or disease producing activity of a pathogen accomplished by one or more organisms other than man".

The bio control agents produce antibiotics or toxic compounds also and act through antibiosis. In addition, most of them have been found to induce systemic resistance in the plant against one or more pathogens of the same plant species. Even in treatment of fruits for prevention of post- harvest decay they have been found to induce resistance by triggering defense reactions.

4.1.1 Types of Bio-Control Agents

Nematodes (phylum Nematoda) -- There are over 300 species of nematodes (in 19 families) that are known to attack insects. Most of the research in biological control, however, has focused on only two genera, *Steinernema* and *Heterorhabditis*. These nematodes are unique because they harbor symbiotic bacteria that are pathogenic to the nematode's insect host.

4.2 Pathogens:

4.2.1 Fungi:

Although natural populations of insects are commonly attacked by fungal pathogens, there has been only limited success in using these organisms as biocontrol agents. In general, fungi are slow to kill their hosts.

The fungal mycelium usually invades all body tissues and may eventually cause suffocation by blocking the tracheal system. Some fungal pathogens have a relatively broad host range (e.g., *Beauveria bassiana, Metarhizium anisopliae,* and *Cordyceps* spp.) while others are more narrowly adapted to specific hosts like aphids (e.g., *Erynia radicans* and *Aschersonia* spp.), muscoid flies (e.g., *Entomophthora muscae*), mosquito larvae (e.g., *Lagenidium giganteum*, *Coelomomyces* spp. and *Tolypocladium* spp.), or Lepidoptera (e.g., *Nomuraea rileyi* and *Paecilomyces* spp.).

4.2.2 Protozoa:

Most species of entomopathic protozoa cause chronic infections that weaken, but do not kill their host. For this reason, there is little interest in these organisms as biocontrol agents. One notable exception is *Nosema locustae,* a microsporidian that has been mass-produced and marketed for control of grasshoppers under the trade name "Hopper Stopper".

4.2.3 Bacteria:

Most of the bacteria that are pathogenic to insects belong to the coccobacilli group. Members of the genus *Bacillus* are especially important as biological control agents. Some of these bacteria cause turbidity of body fluids (e.g., *Bacillus popillae*) and the diseases they cause have, therefore, come to be known as "milky" diseases. Other species form toxic protein crystals in conjunction with spore formation (e.g., *Bacillus thuringiensis*). Several strains of *B. thuringiensis* have been isolated and are now mass-produced and sold as pest control agents. Each strain has slightly different host specificity:

B. thuringiensis kurstaki -- lepidopterous larvae

B. thuringiensis israelensis-- mosquitoes and black flies

B. thuringiensis san diego-- some coleopteran larvae

4.2.4 Viruses:

The use of entomopathic viruses for insect control is still in its infancy. Many of these pathogens appear to have good potential as biocontrol agents because they are relatively host specific. Viral-induced mortality is usually caused by toxic proteins that accumulate during the reproductive cycle of the virus. After death, the integument and the internal tissues typically "melt away" into a liquified blob. Most entomopathic viruses are grouped according to the type of "inclusions" found within infected cells:

a. NPV or CPV (Nuclear or Cytoplasmic Polyhedrosis Virus): Clusters of virus particles are embedded within polyhedral inclusion bodies (crystals) that develop inside the nucleus or cytoplasm of infected cells. These are the most common viruses. They usually attack larvae of Lepidoptera or Hymenoptera (sawflies). The U.S. Forest Service has used NPVs as biocontrol agents for pine sawflies, tussock moths, and gypsy moths. There is also commercial interest in developing NPVs for use against corn earworms, cotton bollworms, cabbage loopers, and alfalfa butterflies.

b. Granulosis Virus: Each virus particle is enclosed in its own protein coat, giving infected cells a "granular" appearance under high magnification. These pathogens typically infect the fat body in Lepidopteran larvae and pupae. A granulosis virus has been developed for use in apple orchards against larvae of the codling moth (*Cydia pomonella*).

c. Non-inclusion Viruses: These pathogens (entomopox virus, for example) do not produce granules or polyhedral bodies. The cause of their toxicity is not well understood, but they are usually less virulent than other types of viruses.

Table 4.a: Inhomogeneous fungi as biocontrol agent.

(Biocontrol agent)	Target Insect Pests	Name of Production
Aschersonia aleyrodis	Glass House white fly	
Beauvenia bassfianae	Green leaf hopper, Rice blackbug, Potato beetle Pinc Catterpiller	Boverin
Entompphthora	Lucern aphid	
Sphaerosperma		
Hirsutella thompsonil	Citrus rustmite	Mycar

(Biocontrol agent)	Target Insect Pests	Name of Production
Metarhizium anisopliae	Spittle bug of sugaracane blackwine weevil,	Metaquino metabiol
	Coconut pests	
Nomuraea vileyi	Soybean catter piller & Lapidoptera insect	
Verticillium lecanii	Aphids, whitefly lgreen scale	Mycotal vertelea
Bacillus thuringiensis	Mosquito, mite	Thyricde, Biocontrol

Table 4.b: Nematogeneous fungi as biocontrol agent.

Nematogeneous fungi (Biocontrol agent)	Target Nematoda
Arthrobotrys musiformis	Rotylenchus similis
A. Oligospora	Meloidogyne haple
Glomus fasciculatus	Meloidogyne haple
A. oligospora	Neoplectana sp.
A. arthobotryoides, Dactyfaria thaumasis, Dactylella oviparasitica, Gliocladium roseum, Paecilornyces lilacinus	Meloidogyne incognita
Clomus mosseae	Rotylenchus reniformis
Verticillium chalmydosporium	Heterodera sp.
Cylindrocarpon destuctains, Entomphthora etc.	
Nemtatophythora, gynophila, Catenara auxilliaris	Heterodera avenae
Catenaria auxillaris and Paecilomyces lilacinus	Globodera rostochinensis

4.2.5 Biological Control of Inoculum:

Biological control of inoculums includes (i) destruction of inoculums by parasites and predators, (ii) prevention of formation of inoculums, (iii) weakening or displacement of the pathogen from the food base (infected residue), and (iv) reduction of vigor or virulence of the pathogen by such agents as mycoviruses (ds RNA).

a. Destruction of Dormant Propagules: Natural destruction of fungal propagules in soil is common and Sclerotia are destroyed by parasitism of *Sporodesmium sclerotivorum*, *Trichoderma harzianum* and *Coniothyrium minitans* and other fungi. Oospores of P*hytophthora*, *Pythium and Aphanomyces* are parasitized by many chytridiales, hyphomycetes, actinomycetes and Pseudomonas.

Nematode trapping fungi abound in soil and are known to feed on plant parasitic nematodes including cysts by parasitization and predation.

The objective of biological control of plant pathogens is to hasten the death of pathogenic or parasitic propagules with the help of such organisms and for this several methods have been suggested to strengthen their numbers.

The incorporation of decomposable organic matter such as farm yard manure, green manures, oilcakes, sawdust etc. During the decomposition of organic matter dormant propagules of many pathogens, viz., sclerotia of *Sclerotium*, are induced to germinate (germ tubes, hyphae) and then killed by lysis through soil microbial action.

b. Prevention of Inoculum Formation: This approach to biological control can be more efficient than mass action of biocontrol agents on biomass of the pathogens. The logic behind this approach is to incapacitate the inoculum producing organs, such as females and cysts of nematodes, to prevent a pathogenic fungus from colonizing plant residue in soil where it could multiply inoculum, encouraging development of antagonists on aerial parts of the plant where they could destroy the inoculum.

The nematophagous fungus *Nematophthora gynophila* parasitizes females and cysts of *Heterodera avenae* (cereal cyst nematode), the nematode trapping fungus *Dactylella oviparasiticus* parasitizes females and eggs of Meloidogyne species and the bacterium *Bacillus penetrans* parasitizes root knot nematodes preventing production of larvae as inoculums *Verticillium chlamydosprum* parasitizes eggs, larvae and cysts of the cereal cyst nematode (Heterodera *avenae*). Many fungal pathogens such as Pythium, Phtophtora and Armillaria are unlikely to colonize host plant residues in soil and suppress the growth of nematode and other pathogens.

c. Weakening or Displacement of the Pathogen in Crop Residue: Many root pathogens (Helminthosporium, *Gaeumannomyces graminis*, *Fusarium* species that cause vascular wilt, and *Armillaria mellea*) use crop debris for short or long duration perpetuation. They are primary colonizers (pioneers) of the host residue and are difficult to displace by secondary invaders or saprophytes.

d. Reduction of Vigor or Virulence of the Pathogen: this approach involves the reduction of vigor, aggressiveness, fitness, pathogenicity, virulence or other attributes of the pathogen essential to its saprophytic or parasitic activities accomplished through factors inherent (or carried) in the pathogen itself.

4.2.6 Biological Protection Against Infection:

The approach involves establishment of an antagonist in or around the site of infection so as to provide protection of the area against attack of a pathogen. The host is not involved in the interaction between the pathogen and the antagonist. The resident antagonists on the host surface providing control of a disease, effective blological control achieved by organic treatments and the phenomenon of suppressive soils characterized by lack of propagule germination for penetration and growth in the rhizosphere fall in this category.

a. Protection of Planting Material:

There are numerous examples of biological control achieved by protective covering of seed, rhizomes, tubers, etc. with propagules of an antagonist *Bacillus subtilis*, some species of Pseudomonas, *Penicillium, Chaetomium and Trichodrma* are often as effective as seed protectant chemicals such as thiram and captan. In per-emergence seed rot of pea caused by *Pythium ultimum*, the pathogen derives nutrients for colonization of seed and subsequent invasion from seed exudates released during swelling of the seed in soil. Species of *Trichodrma* have also been used similarly to provide protection to seeds during germination against seed rot fungi. *Trichodrma hamatum and T. harzianum* are effective seeds protectants against *Pythium* spp. and *Rhizoctonia solani*. Seedling roots, corms, bulbs, tubers, etc. can also be treated with spore or cell – suspension of such antagonists. Bacillus subtilis has been used against *Fusarium* species that cause rot of cuttings and bulbs. This bacterium has been used to control plant pathogens and increase plant growth. Seed treatments with this *bacterium have been shown to control various diseases caused by R*. solani, Helminthosporium in rice and tomato damping off. It forms endospores hence can be formulated in dusts, wettable powders, etc. without losing efficacy. Similarly, control of wilt of chickpea caused by *Fusarium oxysporum f.sp ciceris* by *Pseudomons fluorescence* is effective.

b. Protection of Foliage and Flowers. Existence of epiphytic microflora on plant surfaces including leaves and flowers is a natural phenomenon. These organisms do not harm the plant. There are many studies where their presence has been cited to explain reduction of disease incidence Brown leaf spot rice (Helminthosporium *oryzea*), leaf spot of rye (Helminthosporium *sativum*), fire blight of apple and pear (Erwinia *amylovora*), *Alternaria s*pot of tobacco, and many other foliage diseases are less severe when the normal epiphytic microflora is allowed as spray of broad-spectrum fungicides.

c. Prevention of Post-Harvest decay of Fruits: Attempts to check various types of fruit rots after harvest had been mostly through heat and chemical treatment. In recent years there have been successful demonstrations of biological control of post-harvest fruit rots by using bacteria and fungi including yeasts. Application of *Penicillium capacia* to lemon fruits after harvest gives 80% control of green mold caused by *Penicilium* without any visible injury to the fruits, *Bacillus subtills* gives control of peach brown rot (*Monilinia fructicola*), *Enterobacter cloacae* reduces peach Rhizopus rot (Rhizopus *stolonifer*).

d. Inoculation of Pruning Wounds with Antagonists: This method has been successfully demonstrated in case of certain wood and stump rot causing fungi.

Table 4.c: Weed Control by Fungi as Biocontrol Agent.

Biocontrol agent	Target Weeds
Puccinla chondrillina	*Chondrilla juncea (Rush skeleton weed)*
Phragmidium violaceum	*Rubus fruticosus*
Cercosporella ageretina	*Ageratina riparia*

Biocontrol agent	Target Weeds
Colletotrichum gloeosporides (COLLEGEO)	*Aeschynomene virginicia*
Phytophthora palmivora (DEVINE)	*Monenia adoreta (Milk weed vine)*
Colletotrichum cocodes (VELGO)	*Abutilon theophrasti (Velvet leaf)*
C. gleosporiodes f. sp cuscutae f. sp malvae (BIOMOL)	*Cuscuta (Dodder)*
Alternaria cassia	*Cassia obtusifolla (Sickcepod)*
Ascochyta cypericota	*Cyperus rotundus*
Cercospora rodmani	*Water hyacinth (Eichornia Crass I pes)*
Alternaria macrospora	*Anoda cristata*

4.3 Biocontrol Agents and their Mechanism of Action:

Plant diseases are the result of interactions of the three components i.e., host, pathogen and environment. Biological control agents are the organisms that interact with three components and manage the diverse group of plant diseases. Bio control agents involve a bewildering array of mechanisms in achieving disease control. Understanding the mechanisms of biological control of plant diseases through the interactions between biocontrol agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful biocontrol or to improve biocontrol strategies (Fravel, 1988). Bio control can result from many different types of interactions between organisms. In all cases of bio control, pathogens are antagonized by the presence and activities of other organisms that they encounter. Different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions.

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 system, hyperparasitism 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. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is

Table 4.d: Types of interspecies antagonisms leading to 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*
	Antibiotics	2,4-diacetylphloroglucinol

Type	Mechanism	Examples
Mixed-path antagonism		Phenazines Cyclic lipopeptides
	Lytic enzymes	Chitinases Glucanases Proteases
	Unregulated waste products	Ammonia Carbon dioxide Hydrogen cyanide
	Physical/chemical interference	Blockage of soil pores Germination signals consumption Molecular crosstalk confused
Indirect antagonism	Competition	Exudates/ leachates consumption Siderophore scavenging Physical niche occupation
	Induction of host resistance	Contact with fungal cell walls Detection of pathogen-associated, molecular patterns Phytohormone-mediated induction

the most indirect form of antagonism. However, the most effective BCAs studied to date appear to antagonize pathogens using multiple mechanisms.

For instance, Pseudomonas known to produce the antibiotic 2,4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli et al. 2003).

Additionally, DAPG-producers can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of wheat through competition for organic nutrients (Raaijmakers and Weller 2001).

4.4 Antibiotic Mediated Suppression:

Antibiotics are microbial toxins that can, at low concentrations, poison or kill other microorganisms. Antibiotics produced by bacteria include volatile antibiotics (hydrogen cyanide, aldehydes, alcohols, ketones, and sulphides) and nonvolatile antibiotics: polyketides (diacetylphloroglucinol; DAPG and mupirocin), heterocyclic nitrogenous compounds (phenazine derivatives: pyocyanin, phenazine-1-carboxylic acid; PCA, PCN, and hydroxyphenazines) (de Souza et al. 2003), and phenylpyrrole antibiotic (pyrrolnitrin) (Ahmad et al. 2008). Bacillus strains produce a variety of lipopeptide antibiotics (Iturins, bacillomycin, surfactin, and Zwittermicin A).

Methods have been developed to ascertain when and where biocontrol agents may produce antibiotics (Notz et al. 2001) but detecting expression in the infection court is difficult because of the heterogenous distribution of plant-associated microbes and the potential sites of infection.

In a few cases, the relative importance of antibiotic production by biocontrol bacteria has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. For example, mutant strains incapable of producing phenazines (Thomashow and Weller 1988) or phloroglucinols (Keel et al. 1992) have been shown to be equally capable of colonizing the rhizosphere but much less capable of suppressing soil borne root diseases than the corresponding wildtype and complemented mutant strains. The role of antibiotics in biocontrol has been studied by genetic analysis, e.g., mutants that do not produce antibiotics to demonstrate a correlation between antibiotic productivity and biocontrol

Table 4.e: Antibiotics Produced by Biocontrol Agents.

Antibiotic	Source	Target pathogen	Disease	Reference
2,4	*Pseudomonas*	*Pythium spp.*	Damping off	Shanahan et al.
diacetyl phloroglucinol	*fluorescens* F113			(1992),
Agrocin 84	*Agrobacterium*	*Agrobacterium*	Crown gall	Kerr (1980)
	radiobacter	*Tumefaciens*		
Bacillomycin D	*Bacillus subtilis*	*Aspergillus flavus*	Aflatoxin	Moyne et al.
	AU195		contamination	-2001
Bacillomycin,	*Bacillus*	*Fusarium*	Wilt	Koumoutsi et al.
fengycin	*amyloliquefaciens*	*Oxysporum*		-2004
	FZB42			
Xanthobaccin A	*Lysobacter* sp.	*Aphanomyces*	Damping off	Islam et al.
	strain SB-K88	*Cochlioides*		-2005
Gliotoxin	*Trichoderma*	*Rhizoctonia solani*	Root rots	Wilhite et al.
	Virens			-2001
Herbicolin	*Pantoea*	*Erwinia amylovora*	Fire blight	Sandra et al.
	Agglomerans C9-1			-2001
Iturin A	*B. subtilis* QST713	*Botrytis cinerea*	Damping off	Paulitz and
		and *R. solani*		Belanger (2001),

Antibiotic	Source	Target pathogen	Disease	Reference
				Kloepper et al.
				-2004
Mycosubtilin	*B. subtilis*	*Pythium*	Damping off	Leclere et al.
	BBG100	*Aphanidermatum*		-2005
Phenazines	*P. fluorescens*	*Gaeumannomyces*	Take-all	Thomashow et
	2-79 and 30-84	*graminis* var. *tritici*		al. (1990)
Pyoluteorin,	*P. fluorescens* Pf-5	*Pythium ultimum*	Damping off Howell and	Howell and
pyrrolnitrin		and *R. solani*	Stipanovic	Stipanovic
			-1980	-1980
Pyrrolnitrin,	*Burkholderia*	*R. solani* and	Damping off	Homma et al.
pseudane	*Cepacia*	*Pyricularia oryzae*	and rice blast	-1989
Zwittermicin A	*Bacillus cereus*	*Phytophthora*	Damping off	Smith et al.
	UW85	*medicaginis*		-1993

activity. For example, a phenazine antibiotic (Phz) produced by *Pseudomonas fluorescens* strain 2-79 has been implicated in control of take all disease of wheat caused by *Gaeumannomyces graminis* var tritici (Handelsman and Parke, 1989). Among other bacteria, antibiotic agrocin 84 produced by *Agrobacterium radiobacter* strain K84 is one of best described examples of biocontrol to control crown gall caused by virulent *A. tumefaciens* strains (Kerr, 1989). Several studies have implicated agrocin K84 in the disease control process produced by *Trichoderma virens* in the suppression of Pythium damping-off of cotton seedlings has also been confirmed recently by mutational analysis (Di Pietroet al., 1993)

4.5 Competition:

This process is considered to be an indirect interaction whereby pathogens are excluded by Biocontrol agents and their mechanism in plant disease management 51 depletion of a food base or by physical occupation of site (Lorito et al., 1993). Biocontrol by nutrient competition can occur when the biocontrol agent decreases the availability of a particular substance thereby limiting the growth of the pathogen. Particularly, the biocontrol agents have a more efficient uptake or utilizing system for the substance than do the pathogens (Handelsman and Parke, 1989). For example, iron competition in alkaline soils may be a limiting factor for microbial growth in such soils (Leongand Expert 1989). Some bacteria, especially fluorescent Pseudomonas produce siderophores that have very high affinities for iron and can sequester this limited resource from other microflora thereby preventing their growth (Loper and Buyer 1991. Some studies have found siderophores to play little or no role in disease control, particularly with Pythium species (Hamdan, *et al*., 1991). More recently, Leeman *et al*., 1996 have reported that iron-chelating salicylic acid produced by selected *P. fluorescens* strains at low iron availability may be involved in the induction of systemic resistance to Fusarium wilt of radish. Competition for specific substances or stimulants for germination of microorganisms may also occur in soil since most resting structures of microbes cannot germinate without specific stimulants due to soil fungistasis (Ko, and Lockwood 1970). Infection of plants by pathogens occurs only after dormancy is broken in the presence of stimulants from plant hosts. Consequently, microbes including pathogens may compete for specific stimulants of germination that may come from germinating seeds or growing roots. These factors may include fatty acids, or their peroxidation products (Harman and Nelson 1994), or volatile components such as ethanol and acetaldehyde (Gorecki *et al*., 1985).

4.6 Hyperparasites and Predation:

In hyperparasitism, the pathogen is directly attacked by a specific BCA that kills it or its propagules. Usually, there are four major classes of hyperparasites: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators. *Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that has been used as a BCA. Hypoviruses are hyperparasites. A classic example is the virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen. The phenomenon has controlled the chestnut blight in many places (Milgroom and Cortesi 2004).

However, the interaction of virus, fungus, tree, and environment determines the success or failure of hypovirulence. There are several fungal parasites of plant pathogens, including those that attack sclerotia (e.g., *Coniothyrium minitans*) while others attack living hyphae (e.g. *Pythium oligandrum*). And a single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum*, *Acrodontium crateriforme*, *Ampelomyces quisqualis*, *Cladosporium oxysporum*, and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Kiss 2003). Other hyperparasites attack plant-pathogenic nematodes during different stages of their life cycles (e.g., *Paecilomyces lilacinus* and *Dactylella oviparasitica*). In contrast to hyperparasitism, microbial predation is more general and pathogen non-specific and generally provides less predictable levels of disease control. Some BCAs exhibit predatory behavior under nutrient-limited conditions. However, such activity generally is not expressed under typical growing conditions. For example, some species of *Trichoderma* produce a range of enzymes that are directed against cell walls of fungi. However, when fresh bark is used in composts, *Trichoderma* spp. do not directly attack the plant pathogen, *Rhizoctonia solani*.

But in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of *Trichoderma* spp., which in turn produce chitinase to parasitize *R. solani* (Benhamou and Chet 1997). This process involves the direct utilization of one organism as food by another (Handelsman and Parke 1989). Fungi that are parasitic on other fungi are usually referred to as mycoparasites (Baker and Cook 1974.). Many mycoparasites occur on a wide range of fungi and some of them have been proposed to play an important role in disease control (Adams, 1990). For example, Darlucafilum (now *Sphaerellopsis filum*) was described by Saccardo as a parasite of some rust fungi, especially *Puccinia* and *Uromyces* (Sundheim and Tronsmo 1988). *Trichoderma lignorum (T. viride)* parasitizing hyphae of *Rhizoctonia solani* and suggestion of inoculating soil with *Trichoderma* spores to control damping-off of citrus seedling was reported by Weindling and Fawcett in 1936. This and other *Trichoderma species* were observed to parasitize *Rhizoctonia bataticola* and *Armillaria mellea* (Baker and Cook. 1974). Generally, mycoparasitism can be described as a four-step process (Chet, 1987): The first stage is chemotropic growth. The biocontrol fungi grow tropistically toward the target fungi that produce chemical stimuli. For example, a volatile or water- soluble substance produced by the host fungus serves as a chemo attractant for parasites. The next step is recognition. Lectins of hosts (pathogens) and carbohydrate receptors on the surface of the biocontrol fungus may be involved in this specific interaction (Inbar and Chet 1994). The third step is attachment and cell wall degradation. Mycoparasites can usually either coil around host hyphae or grow alongside it and produce cell wall degrading enzymes to attack the target fungus (Chet, 1987). These enzymes such as chitinases and b-1,3-glucanase may be involved in degradation of host cell walls and may be components of complex mixtures of synergistic proteins that act together against pathogenic fungi (Di Pietro, et al, 1992). The final step is penetration. The biocontrol agent produces appresoria-like structures to penetrate the target fungus cell wall (Chet, 1987).

Table 4.f: List of Hyper Parasites.

Sr. No.	**Hyperparasite (S)**	**Target Pathogens**
1.	*Laetisaria arvolis (Corticum species)*	*Rhizoctnia, Pythium*
2.	*Pythium spp.*	*Phytophthora sp.*
3.	*Talaromycs flavus*	*Verticillium sp.*
4	*Coniothyrium virens (Gliogard)*	*Solerotium*
5	*Gliocladium virens (Gliogard)*	*Solerotium*
6	*Sporidesmium selerotivorum*	*Solerotinia, Sclerotium*
7	*Bacillus subtilis (Kodiak)*	*Solerotium, Phytophthora, Pythium etc.*
8	*Aphelencheus avenae (Nematode)*	*Rhizoctonia, Fusarium*
9	*Pseudomonas fluorescens (Dagger-G)*	*Pythuim, Rhizoctonia sp.*
10	*Tuberculina maxima*	*Cronartium ribicola*
11	*Verticillium lecanii*	*Rust fungi*
12	*Ampelomyces quisqualis*	*Powdery mildews*
13	*Telletiopsis sp.*	*Sphaerotheca sp.*

Sr. No.	**Hyperparasite (S)**	**Target Pathogens**
14	*Nectria inventa*	*Alternaria sp.*
15	*Trichoderma harzianum (Vinab-T), (F-stop)*	*Damping off (Rhizoctonia, Sclerotium)*
16	*Sseudomonas syrinage (Biosae)*	

4.7 Induction of Systemic Resistance

The inducible resistance in plants to a variety of pathogens is known as systemic acquired resistance (SAR). SAR may be induced by inoculating plants either with a necrogenic pathogen or nonpathogen or with certain natural or synthetic chemical compounds (Lam and Gaffney 1993). These defense responses may include the physical thickening of cell walls by lignification, deposition of callose, accumulation of antimicrobial low-molecular-weight substances (e.g., phytoalexins), and synthesis of various proteins (e.g., chitinases, glucanases, peroxidases, and other pathogenesis related (PR) proteins) (Hammerschmidt, et al, 1984). This defense system is also triggered when plants are colonized by plant growth-promoting rhizobacteria (Sticher, et al., 1997). Recently, many strains of PGPR have been shown to be effective in controlling plant diseases by inducing plant systemic resistance (Liu, et al., 1995). The chemical Biocontrol agents and their mechanism in plant disease management 53 compounds that induce resistance of plants to pathogens may include polyacrylic acid, ethylene, salicylic acid and acetyl salicylic acid, various amino acid derivatives, the herbicide phosphinotricin, and harpin produced by *Erwinia amylovora* (Sequeira, 1983). It is known that stress can induce defense mechanisms against pathogens (Maurhofer, et al., 1994). However, the hypothesis should be proved by genetic analysis such as heterologous expression, which shows that inducing ability may be transferred to other potent strains as an additional complementary mode of action, and gene mutation, which knocks out the ability and leads to less disease control.

Various classes of compounds are released by the *Trichoderma* sp. into the zone of interaction and induce resistance in plants. The first class is proteins with enzymztic or other activity. Fungal proteins such as xylanase, cellulases and swollenins are secreted by T*richoderma* species (Martinez *et al.,* 2001). Lots of findings indicated that *Trichoderma* endochitinase can also enhance defense, probably through induction of plant defense related proteins.

Number of proteins and peptides that is active in inducing terpenoid phytoalexin biosynthesis and peroxidase activity in cotton, e.g., the small protein, SM1, which has hydrophobin-like properties, were found to be produced by strains of *T. virens* (Dreuge *et al.,* 2007. Another group of proteins that induce defense mechanisms in plants are the products of avirulence-like (Avr) genes (woo *et al.,* 2006). They usually function as race- or pathovar-specific elicitors of hypersensitive and other defense-related responses in plant species that hold the corresponding resistance (R) gene. Saksirirat *et al.*, 2009, proposed the efficacy of *Trichoderma* strains in inducing resistance in tomato and findings indicated that *Trichoderma* was effective in inducing systemic resistance in tomato plant.

4.8 Plant Growth Promotion and Competition for Nutrients

Biocontrol agents also capable to produce growth hormones like, Auxins, Cytokinin, Gibberellins etc. These hormones play vital role in suppression of deleterious pathogens and promote the growth of plants and increase in their yield. The research on mechanism of growth promotion indicated that PGPR promotes plant growth directly by production of plant growth regulators or indirectly by stimulating nutrient uptake, by producing siderophores or antibiotics to protect plant from soil borne pathogens or harmful rhizosphere organisms. Plant growth promotion and productivity stimulated by microbial endophytic communities are often associated with increased plant health, achieved by direct and/or plant-mediated control of plant pests and pathogens. Some research reported that root-associated microbes, particularly mycorrhizae and/or rhizobacteria, might influence and change plant physiology such that the aboveground parts are less prone to attack by phytophagous insects (Pangesti *et al.*, 2013). Plant defense is then achieved by priming for enhanced expression of sequences regulated by the production of jasmonic acid, ethylene, or salicylic acid. In other cases, beneficial microbes, such as root-colonizing pseudomonads, may directly act against plant-feeding insects by producing volatile organic compounds (VOCs) that have insecticidal properties (Kupferschmied *et al., 2013*). In diverse studies, most of the antagonistic relationships between beneficial microbes and pathogens have been successful in elucidating efficient biocontrol activity against various fungal diseases (Baker, R. 1991). Various studies, researchers have found that endophytic microorganisms may have a symbiotic association with their host plants. The endophytic Bacillus pumilus efficiently protected pea plants from *Fusarium oxysporum* f. sp. pisi, the causal agent of Fusarium root rot (Benhamou *et al*., 1996). in the same way, The growth-promoting activity in various plants elicited by the endophytic fungus Piriformospora indica (Varma *et al*., 1999). These endophytic microorganisms offer actual advantages to the host plants, for example, by enhancing the physiological activity of the plant or facilitating the uptake of nutrients from the soil. Thus, they may serve as biocontrol agents or plant growth promoters (Shimizu *et al*., 2009). Among other microorganisms, a variety of actinomycetes inhabits a wide range of plants as endophytes (Tian *et al*., 2004); therefore, such actinobacteria may have both the potential to serve as effective biocontrol agents and to be considered as efficient plant growth promoters (Kunoh, H. 2002). The genus Streptomyces has been extensively used for biocontrol of soil borne fungal pathogens due to its intense antagonistic activity through the production of various antifungal metabolites (El-Tarabily *et al*., 2006). In soil, most of the known actinomycetes belong to genus Streptomyces and have been used for various agricultural purposes, mainly due to their production of antifungal and antibacterial metabolites and a number of plant growth-promoting (PGP) traits (Suzuki *et al*., 2000). *Trichoderma* spp. are rapidly growing fungi that have persistent conidia and a broad spectrum of substrate utilization. They are very efficient competitors for nutrition and living space (Hjeljord *et al*., 2000). In addition, *Trichoderma* spp., are naturally resistant to many toxic compounds, including herbicides, fungicides, and phenolic compounds. Therefore, they can grow rapidly and impact pathogens by producing metabolic compounds that hamper spore germination (fungistasis), kill the cells (antibiosis), or alter the rhizosphere, (e.g., by acidifying the soil so that the pathogens cannot grow) and starvation is the most common cause of death for microorganisms, so competition for limited nutrients is mainly important in the biocontrol of phytopathogens. Iron uptake is essential for filamentous fungi and under iron starvation; fungi excrete low-molecular weight ferriciron-specific chelators, termed siderophores.

Trichoderma spp. produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Benitez et al., 2004). Therefore, soil characteristics influence Trichoderma as a biocontrol agent.

4.9 References:

1. Adams, P.B. (1990). The potential of mycoparasites for biological control of plant diseases. Annu. Rev. Phytopathol. **28**:59-72.
2. Ahmad, F., Ahmad, I. and Khan, M.S. (2008). Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. **163**:173–181.
3. Baker, K.F., and Cook, R. J. (1974). Biological Control of Plant Pathogens. Am. Phytopathol. Soc., St. Paul. MN.433.
4. Baker, R. (1991). Diversity in biological control. Crop Prot.**10**: 85–94.
5. Benhamou, N. and Chet, I. (1997). Cellular and molecular mechanisms involved in the intersection between *Trichoderma harzianum* and *Pythium ultimum*. Appl. Environ. Microbiol. **63**:2095–2099.
6. Benhamou, N., Kloepper, J.W., Quadt-Hallman, A. and Tuzun, S. (1996). Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. Plant Physiol.**112**: 919–929.
7. Benitez, T., Rincon, A.M., Limon, M.C. and Codon, A.C. (2004). Biocontrol mechanism of Trichoderma strains. Int. Microbiol. **7**: 249-260.
8. Chet, I. (1987). *Trichoderma* application, mode of action, and potential as biocontrol agent of soil-borne pathogenic fungi. in: Innovative Appro. Plant Disease Control. I. Chet,ed., John Wiley, New York. 137-160.
9. De Souza, J.T.A., Arnould, C., Deulvo,t C., Lemanceau, P., Gianinazzi-Pearson, V. and Raaijmakers, J.M. (2003). Effect of 2,4-diacetylphloroglucinol on Pythium: cellular responses and variation in sensitivity among propagules and species. *Phytopathol*ol. **93** :966–975.
10. Di Pietro, A. (1993). Chitinolytic enzymes produced by Trichoderma harzianum antifungal activity of purified endochitinase and chitobiosidase. *Phytopathol*. **83**: 302-307.
11. Druege, U., Baltruschat, H. and Franken, P. (2007). *Piriformospora indica* promotes adventitious root formation in cuttings. *Scientia Horticulturae.* **112:** 422–26.
12. Fravel, D.R. (1988). Role of antibiosis in the biocontrol of plant diseases. Annu. Rev. Phytopathol. **26**: 75-91.
13. Gorecki, R.J., Harman, G.E., and Mattick, L.R. (1985). The volatile exudates from germinating pea seeds of different viability and vigor. Can. J. Botany.**63**:1035- 1039.
14. Hamdan, H., Weller, D.M., and Thomashow, L.S. (1991). Relative importance of fluorescent sensitivity among propagules and species. Phytopatholol. **93**:966–975.
15. Handelsman Jo. and Parke, J.L. (1989). Mechanisms in biocontrol of soil borne plant pathogens. in: Plant-Microbe Interactions, Molecular and Genetic Perspectives T. Kosuge, and E. W. Nester, eds., McGraw-Hill, New York. **3**:27-61.
16. Harman, G.E. and Nelson, E.B. (1994). Mechanisms of protection of seed and seedlings by biological control treatments: Implications for practical disease control. Seed Treatment: Progress and Prospects. T. Martin, ed., BCPC, Farnham, UK. 283-292.

17. Homma, Y., Kato, Z., Hirayama, F., Konno, K., Shirahama, H., and Suzui, T. (1989). Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soil borne plant pathogens. *Soil Biol. Biochem*. **21**:723-728.
18. Howell, C. R., Beier, R.C. and Stipanovi, R.D. (1980). Production of ammonia by Enterobacter caloacae and its possible role in the biological control of phythium pre-emergence damping off by the bacterium. *Phytopathology.* **78:** 105-1078.
19. Inbar, J. and Chet, I. (1994). A newly isolated lectin from the plant pathogenic fungus *Sclerotium rolfsii*: purification, characterization and role in mycoparasitism. J. Microbiol. **140**: 651-657.
20. Islam, T.M., Hashidoko, Y., Deora, A, Ito, T. and Tahara, S. (2005). Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne peronosporomycetes. *Applied Environmental Microbiology.* **71:** 3786-3796.
21. Kerr, A. (1980). Biological control of crown gall through production of agrocin 84. *Plant Disease.* **64:** 25-30.
22. Kerr, A. (1989). Commercial release of a genetically engineered bacterium for the control of crown gall. *Agric. Sci*. **2**:41-48.
23. Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science.* **59**: 475–83.
24. Kloepper, J.W., Ryu, C.M. and Zhang, S. (2004). Induce systemic resistance and promotion plant growth by *Bacillus* spp. *Phytopathology.* **94:** 1259-1266.
25. Ko, W. H. and Lockwood, J. L. (1970). Mechanism of lysis of fungal mycelia in soil. *Phytopathol*. **60**:148-154.
26. Koumoutsi, A., Chen, X.H., Henne, A., Liesegang, H., Gabriele, H., Franke, P., Vater, J. and Borris, R. (2004). Structural and functional characterization of gene clusters directing nonribosomal synthesis of bioactive lipopeptides in *Bacillus amyloliquefaciens* strain FZB42. *Journal of Bacteriology.* **86:** 1084-1096.
27. Kunoh, H. (2002). Endophytic actinomycetes: Attractive biocontrol agents. *J. Gen. Plant Pathol*. **68**, 249–252.
28. Kupferschmied, P., Maurhofer, M. and Keel, C. (2013). Promise for plant pest control: Root-associated pseudomonads with insecticidal activities. *Front. Plant Sci*. **4**: 287.
29. Lam, S.T. and Gaffney, T.D. (1993). Biological activities of bacteria used in plant pathogen control. in: Biotechnology in Plant Disease Control.I. Chet,ed., *John Wiley*, New York . 291-320.
30. Lavicoli, A., Boutet, E., Buchala, A. and Métraux, J.P. (2003). Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Mol. Plant-Microbe Interact*. **16**:851-858.
31. Leclere, V., Bechet, M., Adam, A., Guez, J.S., Wathelet, B., Ongena, M., Thonart, P., Gancel, F., Chollet-Imbert, M. and Jacques, P. (2005). Mycosubtilin overproduction by *Bacillus subtilis* BBG100 enhances the organism's antagonistic and biocontrol activities. *Appl. Environ. Microbiol*. **71**:4577-4584.
32. Leeman, M., Den Ouden, F.M., Van Pelt, J.A., Dirkx, F.P.M., Steijl, H., PAHM, B. and Schippers, B. (1996). Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology*.**86**:149–155.
33. Leong, S.A. and Expert, D. (1989). Siderophores in plant pathogen interactions. in: Plant-Microbe Interactions, Molecular and Genetic Perspectives, T. Kosuge, and E. W. Nester, eds., McGraw-Hill, New York. **3**: 62-83.

34. Liu, L., Kloepper, J.W. and Tuzun, S. (1995). Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathol***. 85**: 843-847.
35. Lo, C.T. (1997). Biological control of turfgrass diseases using *Trichoderma harzianum*. Plant Prot. Bull. **39**: 207-225.
36. Lo, C.T., Nelson, E.B., Hayes, C.K. and Harman, G.E. (1998). Ecological studies of transformed *Trichoderma harzianum* strain 1295-22 in the rhizosphere and on thephylloplane of creeping bent grass. *Phytopathology* **88** :129 - 136.
37. Loper, J.E. and Buyer, J.S. (1991). Siderophores in microbial interactions on plant surfaces. Molec. Plant Microbe Interact. **4**:5-13.
38. Lorito, M., Harman, G.E., Hayes, C.K., Broadway, R., Tronsmo, A, Woo, S.L. and Di Pietro, A. (1993). Chitinolytic enzymes produced by *Tricboderma barxianurn*: antifungal activity of purified endochitinase and chitobiosidase. *Plytopathology.***83**: 302-307.
39. Martinez, C.,Blanc, F., Le, C.E., Besnard, O., Nicole, M. and Baccou, J.C. (2001). Salicylic acid and ethylene pathways are differentially activated in melon cotyledons by active or heat-denatured cellulase from *Trichoderma longibrachiatum*. *Plant Physiology*. **127:** 334–44.
40. Maurhofer, M., Keel, C., Schnider, U., Voisard, C., Haas, D. and Defago, G. (1992). Influence of enhanced antibiotic production in *Pseudomonas fluorescens* CHA0 on its disease suppressive capacity. Phytopathol. **82**:190-195.
41. Meguro, A., Ohmura, Y., Hasegawa, S., Shimizu, M., Nishimura, T. and Kunoh, H. (2006). An endophytic actinomycete, Streptomyces sp. MBR-52, that accelerates emergence and elongation of plant adventitious roots. *Actinomycetologica*. **20**: 1–9.
42. Moyne, A.L., Shelby, R., Cleveland, T.E. and Tuzun, S. (2001). Bacillomycin D: an Iiturin with antifungal activity against *Aspergillus flavus*. *J. Applied Microbiol*. **90:** 622-629.
43. Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D., and Defago, G. (2001). Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene phlA in Pseudomonas fluorescens biocontrol strain CHA0 in the rhizosphere. *Phytopathol*. **91**:873-881.
44. Paulitz, T C. (1991). Effect of *Pseudomonas putida* on the stimulation of *Pythium ultimum* by seed volatiles of pea and soybean. *Phytopathology*. **81:** 1282- 1287.
45. Raaijmakers, J.M., Vlami, M. and De Souza, J.T. (2002). Antibiotic production by bacterial biocontrol agents. *Anton. van Leeuw*. **81**:537-547.
46. Saksirirat, W., Chareerak, P. and Bunyatrachata, W. (2009). Induced systemic resistance of bio control fungus, *Trichoderma* sp. against bacterial and gray leaf spot in tomatoes. *Asian Journal of Food and Agro industry*. 99-104.
47. Sandra, A. I., Wright, C. H., Zumoff, L.S.and Steven, V. B. (2001). *Pantoea agglomerans* strain EH318 produces two antibiotics that inhibit *Erwinia amylovora in vitro*. *Appied Environmental Microbiology*. **67:** 282-292.
48. Shimizu, M., Yazawa, S. and Ushijima, Y.A. (2009). Promising strain of endophytic Streptomyces sp. for biological control of cucumber anthracnose. *J. Gen. Plant. Pathol*. **75**: 27–36.
49. Smith, K.P., Havey, M.J. and Handelsman, J. (1993). Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW85. *Plant Disease.* **77:** 139-142.
50. Sticher, L., Mauch-Mani and Metraux, J.P. (1997). Systemic acquired resistance. *Annu. Rev. phytopathol*. **35**: 235 - 270.

51. Sundheim, L. and Tronsmo, A. (1988). Hyperparasites in biological control. in: Biocontrol of Plant Diseases. K. G. Mukerji, and K. L. Garg, eds., CRC Press, Boca Raton, FL. 53-69.
52. Thomashow, L.S., Weller, D.M., Bonsall, R.F., and Pierson, L.S. III. (1990). Production of the antibiotic phenazine-1-carboxylic acid by fluorescent pseudomonas in the rhizosphere of wheat. *Appl. Environ*. Microbiol. **56**: 908-912.
53. Varma, A. Verma, S., Sahay, N., Bütehorn, B. and Franken, P. (1999). *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol***.** **65**: 2741–2744.
54. Woo S L, Scala F, Ruocco M, Lorito M. (2006). The molecular biology of the interaction between *Trichoderma* spp., phytopathogenic fungi, and plants, *Phytopathology,* **6**:181–185.