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Concept, Principle and Application of Biological Control and their Role in Sustainable Plant Diseases Management Strategies

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Abstract: *Biological control of plant diseases has been considered a viable alternative method to chemical control. These include Augmentation, Classical and Conservation biological control. There are various naturally occurring bio-control agents that aggressively attack on plant pathogens and suppress plant disease by different mode of actions. Bio-control agents comprise of multiple beneficial characters such as rhizosphere competence, antagonistic potential, and ability to produce antibiotics, lytic enzymes, computation for nutrient and niche. These biological control activities are exerted either directly through antagonism of soil-borne pathogens or indirectly by eliciting a plant-mediated resistance response. Understanding the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective bio-control agents and to manipulate the soil environment to create conducive condition for successful bio-control. Some of the important factors that affect the efficacy of microbial bio-control agents in controlling plant diseases which should carefully be considered include biotic, a biotic, method of application, formulation of bio-control microorganisms and timing of application. Application and commercial use of biological control agents have been slow mainly due to their variable performances under different environmental conditions in the field. To overcome this problem and in order to take the bio-control technology to the field and improve the commercialization of bio-control, it is important to develop new formulations of bio-control microorganisms with higher degree of stability and survival. A significant improvement have been made in different aspects of biological control of plant diseases, but this area still need much more research for development of efficient bio-control, including development of novel formulations, understanding the impact of environmental factors on bio-control agents to improve its efficacy.*

Keywords: *Augmentation, Bio control, Inoculation, Importation, Bio pesticide*

1. INTRODUCTION

Plant pathogens causing major damages to crop plants include fungi, bacteria, viruses and nematodes. Crop losses are creating a major threat to the food production with about 27 to 42% loss in global food production attributed to plant disease caused by plant pathogens which otherwise would have been doubled if no disease management strategies are applied (Singh, 2014).

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fibre produced by growers around the world (Jan mohd *et al*., 2013). Different approaches may be used to prevent, mitigate or control plant diseases. Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality over the past 100 years. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture (Jan mohd *et al*., 2013).

Moreover, the health consciousness of the people coupled with the development of resistance to pathogens due to continuous use of the chemicals also contributes to the restricted use of chemicals in crop protection. Under such circumstances exploitation of living organisms to reduce the disease

causing activity of pathogenic micro-organisms seems to be the most appropriate alternative to chemicals (Jan mohd *et al*., 2013).

This phenomenon of reducing the amount of inoculums or the disease causing activity of plant pathogens accomplished through the use of living organisms other than the man is called as biological control. According to Baker and Cook's (1974) definition "Biological control is the reduction of inoculums or disease producing activity of a pathogen accomplished by one or more organisms other than man." In other words 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 (Pal and Gardener, 2006). The term biological control is used not only to control diseases in plants but also to control weeds and insects.

Biological control was originally defined "the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence". Biological control provided by these living organisms is especially important for reducing the numbers of pest insects, mites and pathogens. It is an environmentally sound and effective means of reducing or mitigating pests and pest effects through the use of natural enemies (Nafiu *et al*, 2014).

Interest in biological control has increased over recent decades for many reasons (Bailey *et al*., 2009). First, a greater appreciation for environmental stewardship among regulators, government, politician, growers, and the public has promoted development of more sustainable farming practices (Kogan, 1998). Second, highly economic, self propagating, no harmful effects on human, other organisms, environment, have great ability to search their prey, compatible with IPM components and permanents. Lastly, consumers increasingly demand products that are grown in a sustainable manner and are free of insecticide residue (Dabbert *et al*., 2004). The objective of this review is study of biological control including its principles and application for effective plant diseases management strategy and approach a sustainable agricultural system.

2. HISTORICAL BACKGROUND OF BIOLOGICAL CONTROL

From 200 A.D. to 1840 A.D: biological control agents were used as augmentation of biological control to control insect pests. Biological control of pest dated back to 16th century in China. They were the first to use natural enemies to control insect pests in the 3rd century, for the control of citrus insect pest such as *Tesseratoma papillosa* (Lepidoptera) using nests of the ant *Oecophylla smaragdina* was sold near Canton (http://ucanr.edu/sites/W2185/). Similarly, Date growers in Yemen went to North Africa to collect colonies of predaceous ants which they colonized in date groves to control various pests (Bellows, 1996). In 1734, de Reaumur suggested to collect the eggs of an "aphidivorousfly" (actually a lacewing) and place them in greenhouses to control aphids. In the 1800's, Darwin discussed "Ichneumonids" as natural control. During the 1840's releases of predators were used for control of the gypsy moth and garden pests in Italy factors for cabbage caterpillars. *Trichogramma* sp. (egg parasites) was shipped from the U.S. to Canada for control of lepidopterous pests in 1882.From 1930 to 1955 human interests on biological control usagehas been raised and expanded in the world and also its popularity were declined after WW-II due to the production of relatively inexpensive synthetic organic insecticides. (http://ucanr.edu/sites/W2185/).

The term biological control as a feasible preposition of plant disease management was coined for the first time by C. F. Von in 1914. Since then various bio- control products have been found to be very effective in controlling the plant disease. Sanford (1926) observed that the potato scab was suppressed by green manuring antagonistic activities. Weindling (1932) reported the parasitic nature of *Trichoderma lignorumon* several plant pathogens. Grossbard (1948-1952), Wright (1952-1957), and others demonstrated that antibiotics were produced in soil by *pencilium, Aspergillus, Trichoderma, streptomyces* ssp. Kloepper (1980), demonstrated the importance of siderophores produced by *Erwinia carotovora*. Thebio-control effect of AMF has been observed in a wide range of plant species and against many pathogens, most of them soil-borne fungal pathogens causing root rotor wilting, though successful bio-control has also been observed against above ground pathogens such as *Alternariasolani* in tomato (Jung *et al*., 2012). Both necrotrophic and biotrophic pathogens have been reported to be suppressed by AMF, either directly or indirectly (Veresoglou and Rillig, 2012).

In the second half of the 20th Century, following the Green Revolution biological control has resurfaced with renewed force in recent years, especially by the adoption of integrated pest

management (IPM) programs. These programs were implemented as a consequence of the indiscriminate use of agrochemicals, which led to a number of problems, such as insect and mite resistance to insecticides as well as environmental contamination (Guillon, 2008).

3. STRATEGY AND ITS PRINCIPLES OF BIOLOGICAL CONTROL

There are 3 basics strategies in biological control of pests, viz; Classical Biological Control (Importation), Augmentation and Conservation.

Classical biological control is defined by Eilenberg *et al.* (200 l) as: The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control. It is the importation of pest natural enemies from other countries, to a new locale where they do not occur naturally (Pickerel, 2004). The main principle of c1assical biological control is shown in figure (1). When an organism is introduced either intentionally oraccidentally into an area in which it did not occur previously, it can sometimes increase to a high population density and become a serious pest. This population increase is mostly due to the fact that the pest was introduced without its natural enemies. The goal of classical biological control is to find useful natural enemies, introduce them into the area of the target pest, and permanently establish them so that they will provide continuing pest control with the result that the pest population decreases in population density, hopefully below the economic injury level of the pest. The time scale on figure 1 can be years (Nafiu *et al.*, 2014)

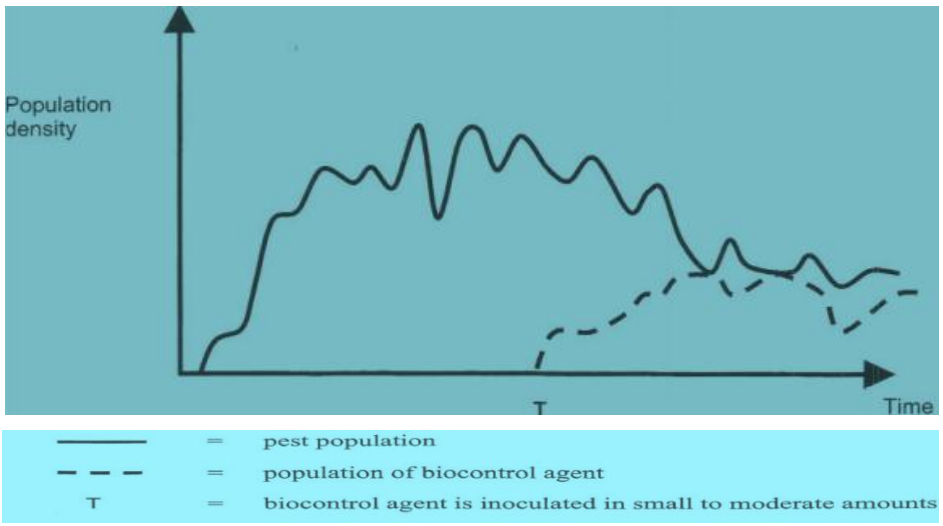

Figure1. *Classical biological control*

The process of classical biological control involves; (1) Determining the origin of the introduced pest. (2) Collecting appropriate natural enemies associated with the pest or closely related species. (3) Selected natural enemies are then passed through a rigorous assessment, testing and quarantine process, to ensure that they will work and that no unwanted organisms (such as hyper parasitoids) are introduced. (4) Mass production and release of selected natural enemies. (5) Follow-up studies are conducted to determine if the natural enemy becomes successfully established at the site of release, and to assess the long-term benefit of its presence (http://edis.ifas.ufl.edu, 2013).

In 1795 India was introduced Cochineal insect (*Dactylopius ceylonicus)* from Brazil against carmine dye producing insect (*D. coccus*). In 2010 three exotic encyrtid parasitoids viz., *Acerophagus papayae*, *Anagyrus loecki* and *Pseudleptomastix Mexicana* were introduced to India against papaya mealybug (*Paracoccus marginatus)* (http: // nptel.ac.in /courses /).

Augmentation isthe other strategy of biological control is also defined by Eilenberg *et al.* (2001) as: The intentional release of a living organism asa biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently. It includes the periodic release of a natural enemy that does not occur naturally in sufficient numbers to keep a pest below damaging levels (van Lenteren, 2000). The practice of augmentation is based on the knowledge or assumption that in some situations there are not adequate numbers or species of natural enemies to provide optimal biological control, but that the numbers can be increased by releases (http://entomolo

gy.ifas.ufl.edu). This relies on an ability to mass-produce large numbers of the natural enemy in a laboratory or by companies to produce and sell them. There are two general approaches to augmentation (Inoculative releases and Inundative releases).

Inoculation biological control is a type of augmented biocontrol strategy and defined by Eilenberg *et al*.(2001) as: intentional release of a living organism as a biological control agent with the expectation that it will multiply and control the pest for an extended period, but not permanently. Its control involves releasing small numbers of natural enemies at prescribed intervals throughout the pest period, starting when the pest population is very low. The natural enemies are expected to reproduce themselves to provide more long-term control. However, the expected outcome of inoculative releases is to keep the pest at low numbers, never allowing it to approach an economic injury level; therefore, it is more of a preventive measure (http:// www.entomology.wisc.edu).

The main principle of inoculation biological control is shown on figure (2). A pest population increases in size but in due course, before this population density has reached the potential maximum, a biocontrol agent is inoculated in small to moderate amounts (Time T on figure 2). Its goal is to increase population size of the natural enemy in order to control the pest over a period of time. The inoculated bio-control organisms will, however, not establish permanently at a sufficient high population density. The pest will therefore increase in population size after a period of time and a new inoculation would then be needed. The events in inoculation biological control are often limited to one cropping season, so the time scale on figure (2) is weeks or months (Jorgen, 2007).

Inoculation biological control has much in common with other inoculation practices, as seen from figure 2. The major factor is that the bio control organism is expected to proliferate, at least temporarily. Conceptually it is therefore comparable to c1assicalbiological control but with the main differences that l) inoculation biological control uses mostly organisms which a1ready occur in the area of application and 2) on1y temporary establishment is achieved. Typica1 examp1es are the re1eases of *Encarsia formosa* and other parasitoids in glasshouses (Lenteren, 2000) and: the inocu1ation of soil with the insect pathogenic fungus-*Beauveria brongniartii* (Enkerl*et al*., 2004).

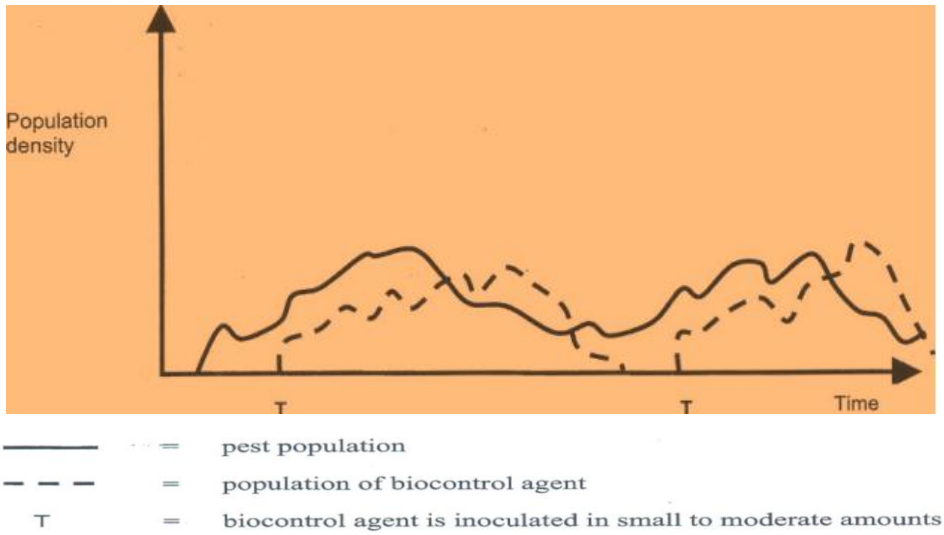

Figure2. *Inoculation biological control*

It can also be postulated that inoculation biological control represents a reestablishment of a natural balance, temporarl1ydistorted by man. Soil for cropping is for examp1einoculated with other additives to enhance growth (mycorrhiza) and inocu1ation with a bio-control agent can be seen as a moderate he1pto speed up a natural process. Inocu1ation bio control has a1ways, however, the advantage of being closely linked to monitoring pest popu1ations and thus understanding population interactions. In glasshouses, a successful inocu1ation of bio control agents requires proper diagnosis of the pests present and in due course, re1easeof the correct agents at the optimum density and time (Jorgen, 2007).

Inundation biological control is other type of augmentation bio-control and defined by Ei1enberg*et al.* (2001) as: The use of living organisms to control the pests when control is achieved exclusively by the

released organisms themselves. The main principle of inundation biological control is shown on figure (3). A pest population increases in size, but at a certain time (Time T on figure 3, for example when the economic injury level has been passed) a bio-control organism is applied in large amounts (inundated). The pest is quickly controlled and the population density of both the pest and the bio-control agent decrease over time.

The pest population will, after a period of time, increase again and a new application of the bio-control agent is needed. The events in inundation biological control are often limited to one cropping season, so the time scale on figure 3 is weeks or months. A typical example of inundation biological control is the widespread use of *Bacillus thuringiensis* to control epidopteran and dipteran insects.

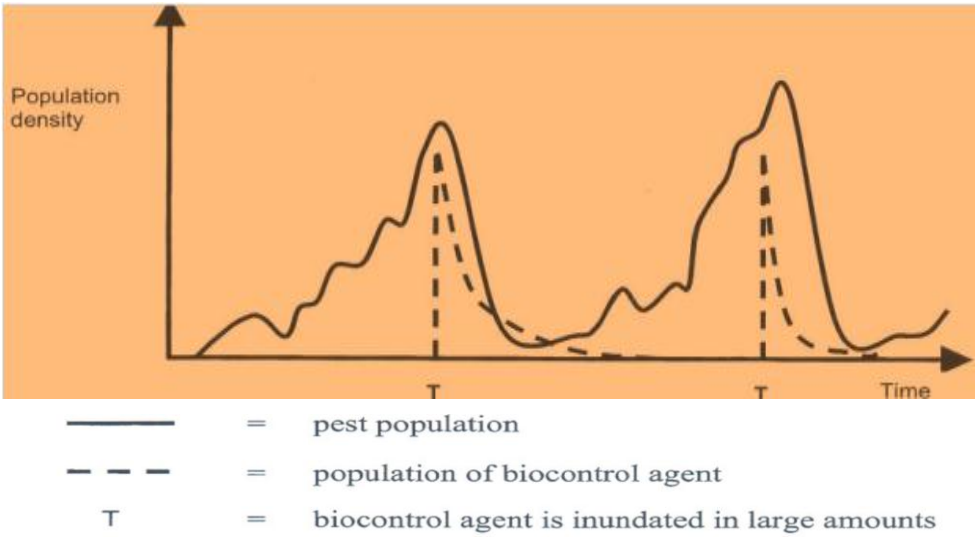

Figure3. *Inundation Biological Control*

Inundation biological control with its strong resemblance to chemical control can be perceived as 'less natural' than the other biological control strategies, especially when using a microorganism for bio-control. The amount of the control agent to be applied is often several magnitudes higher than would ever occur under so-called natural conditions. The presentation of the inundation biological control agent gives association to chemical pesticides; for microorganisms to be used in bio-control, the product label has the appearance of a chemical product with information about the concentration and application rate expressed per square unit.

Conservation biological control is also other strategy of the biological control can be defined as Modification of the environment or existing practices to protect and enhance specific enemies or other organisms to reduce the effect of pests (Eilenberg *et al.* 2001). Habitat manipulation often involves increasing the species diversity and structural complexity of agro ecosystems. Habitat manipulation approaches provide natural enemies with resources such as nectar, pollen, physical refugia, alternative prey and alternative hosts and operate to reduce pest densities via an enhancement of natural enemies (http://www.rkmp.com)**.**

The main principle of conservation biological control is shown on figure (4). A pest occurs at high population levels due to insufficient effects of the natural enemies. Natural enemies include all kinds of biological regulation: macro- and microorganisms controlling in vertebrates, weeds and plant diseases, including the antagonistic microorganisms responsible for suppressive soils. At time T on figure (4), the environment is modified or the practice is changed in order to enhance the natural enemies, which are already present. They increase in population size and their effect results in a lower pest population. The time scale on figure (4) can be years.

Conservation biological control is thus completely different from the three other biological control strategies, since no organisms are released. Only organisms, which are already present, are enhanced in order to avoid damage. It is important to keep in mind that the definition allows both passive and active conservation. An example of passive conservation is the avoidance of actions which disfavour the natural enemies, for example spraying with certain chemical pesticides. An active conservation

could be the initiation of actions to support the natural enemies actively by establishing for example 'beetle banks' (Landis *et al.*, 2000).

In Barbosa (1998) and Pickett and Bugg (1998), many other examples of habitat manipulation at different levels are found, from landscape to crop plants. Among the four biological control strategies, conservation biological control can be seen as the most tightly connected to the main principles of organic farming, which have the protection of the existing natural enemies as one of the main principles (Anonymous, 2002).

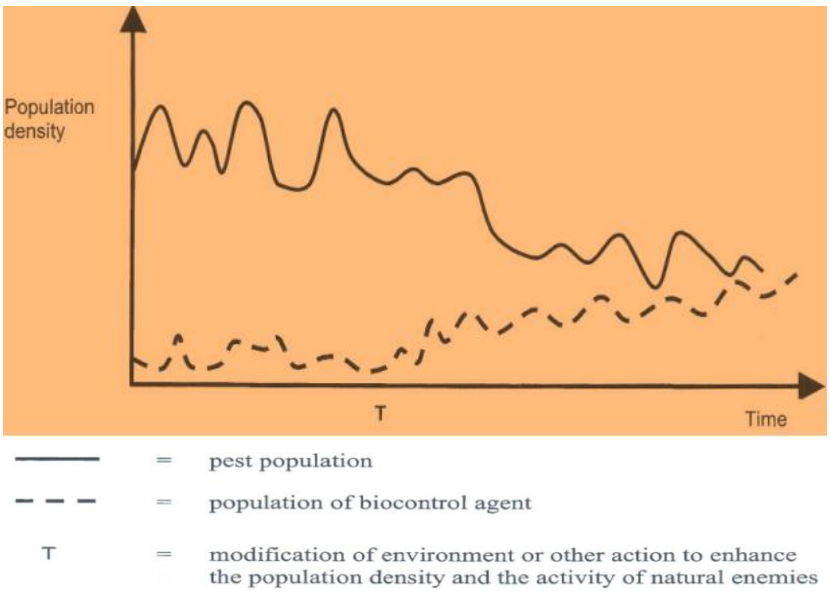

Figure4. *Conservation biological control*

There is a tight connection also to conservation biology (Letourneau, 1998), since conservation biological control to large extent builds on ecological theory about meta populations, spatial fragmentation, and fate of species in a habitat. Conservation biological control can thus be seen as an example of habitat restoration with the specific purpose of supporting natural enemies to control pests (Jorgen, 2007).

4. Biological Control Agents

Biological control agents are organisms that control pathogens and diseases through antagonists. Microbial antagonism of plant pathogens occurs in several ways, the most common mechanisms being parasitism and predation, competition for nutrients or space, production of antimicrobial substances, lytic enzymes and induced resistance. As our knowledge of these mechanisms has increased, it has become apparent that antagonism often involves the synergistic action of several mechanisms.

5. Characteristics of the Successful Biological Agents

1. Highly effective bio-control agents must be fulfill the criteria
   - Be able to compete and live longer in soil and host tissue
   - Be able to colonize and proliferate
   - Be non-pathogenic to host plant and environment
2. Inexpensive production and formulation of agent must be developed
   - Production must result in biomass with excellent shelf live
   - To be successful as agricultural agent must be
     - ✓ Inexpensive,
     - ✓ Able to produce in large quantities,
     - ✓ Maintain viability
3. Delivery and application must permit full expression of the agent
   - Must ensure agents will grow and achieve their purpose

6. MECHANISMS EMPLOYED BY BIO-CONTROL AGENTS FOR MANAGEMENT OF PLANT DISEASES

The bio-control activity is exerted either directly through antagonism of soil-borne pathogens or indirectly by eliciting a plant-mediated resistance response (Jamalizadeh *et al*., 2011). Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the bio-control agents whereas indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the bio-control agents. Stimulation of plant host defense pathways by nonpathogenic bio-control agents is the most indirect form of antagonism (Figure.5). Understanding the mechanism of action of a bio control agent may improve the consistency of control either by improving the mechanism or by using the bio- control agents under conditions where it is predicted to be more successful (Jan mohd *et al*., 2013).

6.1. Competition for Available Resources

Competition occurs when two or more organisms require the same resource for growth and survival. The use of this resource by one organism reduces the amount available to the other. A soil-borne pathogen which infects only certain parts of the root may therefore be limited by competition for suitable colonisable sites or space on the root surface. The rhzosphere is a region of intense microbial activity where there may also be competition for oxygen. On the leaf surface, where nutrients are in short supply, competition for nutrients is thought to play a significant role in disease suppression. Competition for the same site is sometimes called 'site exclusion' and frequently takes place amongst organisms which are closely related taxonomically (e.g. the fire blight bacterium *Ertutnia amylovora* and the saprophytic species *E. herbicola*). In the case of competition for nutrients the situation is similar. Antagonists with the same nutrient requirements as the pathogen are its most effective competitors.

Competition for the same carbon source between *Pythium ulttimum*, a common cause of seedling damping-off and rhizosphere bacteria has resulted in effective biological control of *P. uttimum* in several crops. Ethanol and acetaldehyde released from seeds of pea and soybeans following imbibition stimulate hyphal growth from sporangia of *P. uttimurn*. Treating seeds with a strain of *Pseudomonas putida* which utilises ethanol as its sole carbon source in culture reduces the concentration of volatiles released, lessens hyphal growth or sporangial germination of the pathogen and increases seedling emergence in *Pythium infested* soil. Similarly, ethanol metabolising strains of *Enterobacter cloacae* reduce the ability of cotton seed exudates and volatiles to stimulate sporangium germination of *P. ultimum*.

One of the best documented examples of nutrient competition in biological control involves competition for iron between *fluorescent pseudomonas* and soil borne fungal pathogens such as *Fusarium oxysporum*. Strains of bacteria including *Pseudomonas fluorescent* and *P. putida* produce siderophores metabolic products of micro-organisms that bind iron and facilitate it transport from the environment into the microbial cell. The siderophores pyoverdine and pseudobactin have a high affinity for the soluble ferric iron (Fe*") and inhibit the growth of pathogens by limiting the availability of iron. Evidence for involvement of siderophores in biological control has been obtained from experiments with well-characterised and genetically-manipulated bacterial strains. Pyoverdine production is correlated with biological control while pyoverdine deficient strains have reduced capabilities in biological control.

The usefulness of nutrient competition as a mechanism of biological control depends on the type of pathogen that is targeted. It may not be useful in suppressing biotrophs such as rusts and powdery mildews because they do not require exogenous nutrients to infect the host. On the other hand, a necrotrophic pathogen such as *Botrytis cinerea* is directly affected. Such pathogens require some exogenous nutrients during a definite saprophytic phase prior to infecting the host and are therefore vulnerable to nutrient competition. There are many examples of bacteria and yeasts effectively reducing spore germination or germ tube growth in necrotrophic pathogens by competing for nutrients. For instance, a *Pseudomonas* species inhibited the germination of conidia of *Botrytis cinerea* by competing for amino acids. Pollen stimulates infection of sugar beet leaves by *Phoma betae*. However, pink and white yeasts present on the leaves can reduce this effect, presumably by utilising the nutrient source before the pathogen. Active motility and chemotactic response towards

chemical attractants present in root exudates include organic acids, amino acids, and specific sugars govern arrival of bio-control agent to the root surface (Welbaum *et al*., 2004).

6.2. Active Metabolites Mediated Suppression of Pathogens

Production of active microbial metabolites including antibiotics (Table.1), iron-chelating siderophores, biocidal volatiles, lytic enzymes, and toxins play a very significant role in determining the offensive bio-control activity (Gerhardson, 2002).

6.3. Antibiotics

Antibiotics can poison or kill other microorganisms at low concentrations. To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a bio-control effect (Weller *et al.*, 2007). Some examples of antibiotics reported to be involved in plant pathogen suppression include 2, 4-diacetyl phloroglucinol against *Pythium* spp. (Weller*et al*., 2007), Agrocin 84 against *Agrobacterium tumefaciens* (Kerr, 1980), Iturin A against *Botrytis cinerea* and *Rhizoctonia solani* (Kloepper, 1992), Phenazines against *Gaeumannomyces graminis* var. *tritici* (Thomashow *et al*.,2004). Karunanithi *et al*., (2000) observed a native isolate of *P. fluorescens* producing an antibiotic compound, pyrollnitrin, which inhibited growth of *Macrophomiea phaseolina* by producing an inhibition zone of 12 mm.

Bacilluscereus strain UW85 is known to produce both zwittermycin and kanosamine (Pal and Gardener, 2006) suppress plant pathogens. The ability to produce multiple antibiotics probably helps to suppress diverse microbial competitors, some of which are likely to be plant pathogens. The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. More recently, *Pseudomonas putida* WCS358r strains genetically engineered to produce phenazine and DAPG displayed improved capacities to suppress plant diseases in field-grown wheat (Glandorf *et al.*, 2001)

Table1. *List of Antibiotics Produced by Different Bio control Agents*

SN	**Antibiotic**	**Source**	**Target pathogen**	**Disease**	**Reference**
1	2,4 - Diacetyl-pholoroglucinol	*Pseudomonas fluorescence* F113	*Pythium*	Damping off	(Shanahan *et al.*, 1992)
2	Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall	(Kerr, 1980)
3	Bacillomycin D	*Bacillus subtillus* AU195	*Aspergillus flavus*	Aflatoxin contamination	(Moyne *et al.*, 2001)
4	Bacillomycin D	*Bacillus amylo liquefaciens* strain FZB42	*Fussorium oxysporium*	Wilt	(Koumoutsi *et al.*, 2004)
5	Xanthobacin A	*Lyco*bacter sp. Strain K88	*Aphanomyces cochlioides*	Damping off	(Islam *et al.*, 2005)
6	Gliotoxin	*Trichoderma virens*	*Rhizoctonia solani*	Root rot	(Wilhite *et al.*, 2001)
7	Zwitermycin A	*Bascillus cereus* UW85	*Pythium aphanidermatum*	Damping off	(Smith *et al.*, 1993)
8	Mycostubilin	*Bascillus* BBG100	*Pythium aphanidermatum*	Damping off	(Leclere *et al.*, 2005)
9	Herbicolin	*Pantoea agglomerans* C91	*Erwinia amylovora*	Fire blight	(Sandra *et al.*, 2001)
10	Iturin	*Bascillus subtillus* QST713	*Botrytis, Rhizoctonia solani*	Damping off	(Paulitz and Blanger, 2001)

6.4. Hydrogen Cyanide (HCN) Production

Considerable numbers of free-living rhizospheric bacterial communities, mainly *Pseudomonas* sp. (Muleta *et al*., 2007), are capable of generating HCN by oxidative decarboxylation from direct precursors such as glycine, glutamate, or methionine (Castric, 1977). Other rhizobacterial genera reported to produce HCN include *Bacillus* and *Chromo bacterium* (Muleta *et al*., 2007).

HCN secreted by *Pseudomonas fluorescent* strain CHAO has been demonstrated to stimulate root hair formation and suppress back root rot caused by *Thielaviopsis basicola* in tobacco plant (Voisard *et al.*,

1989). Cyanogenesis in bacteria accounts in part for the bio-control capacity of the strains that suppress fungal diseases of some economically important plants (Voisard *et al*., 1989). For instance, for many *Pseudomonades*, production of metabolites such as HCN is the primary mechanism in the suppression of root fungal pathogens. HCN effectively blocks the cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms at picomolar concentrations. However, producer microbes, mainly *Pseudomonades*, are reported to be resistant (Bashan and de-Bashan, 2005).

6.5. Iron Chelating Siderophores

Biocontrol agents are able to decrease the availability of particular substance/nutrients to the pathogens because of their efficient uptake or utilizing capacity. This phenomenon of competition for nutrients can limit the growth of pathogens (Harman and Nelson, 1994). Iron is an essential growth element for all living organisms and scarcity of its bioavailability in soil habitats and on plant surfaces creates a furious competition (Loper and Henkels, 1997). Under iron-limiting conditions, bio-control agents produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion. Kloepper *et al*. (1980) were the first to demonstrate the importance of siderophore production as a mechanism of biological control of *Erwinia carotovora* by several plant growth promoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1 and B10. Some bacteria, especially *fluorescent pseudomonas*, produce siderophores that have very high affinities for iron as compared to fungal siderophores (Sullivan and Gara, 1992) and can sequester this limited resource from other micro flora thereby preventing their growth (Loper and Buyer, 1991). Earlier reports have demonstrated the importance of *P. fluorescent* siderophores in disease suppression (Loper and Buyer, 1989).

6.6. Lytic Enzymes

Various extracellular hydrolytic enzymes produced by microbes play important role in suppression of plant pathogens. Chitinase and -1, 3-glucanase attack on chitin and -1, 3-glucan, major constituents of many fungal cell walls (Lam and Gaffney, 1993) resulting in its degradation which further kills the pathogens (Chernin and Chet, 2002). Chitinase produced by *Serratia plymuthica*, *Serratia marcescens*, *Paenibacillus* sp. and *Streptomyces* sp. was found to be inhibitory against *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*. Similarly, laminarinase (beta-glucanase) produced by *Pseudomonas stutzeri* digest and lyse mycelia of *F. solani* (Lim et *al*., 1991). 1, 3-glucanase synthesized by *Paenibacillus*, *B. cepacia* destroys*F. oxysporum, R. solani, sclerotium. rolfsii, and Pythium ultimum* cell walls (Fridlender, 1993). Recently, genetic evidence for the role of these enzymes in bio-control has been obtained where *ChiA* from*Serratia. marcescens* was inserted into the non biocontrol agent *Escherichia coli* and the resulting transgenic bacterium reduced disease incidence of Southern blight of bean caused by *Sclerotium rolfsii* (Shapira *et al*., 1989). Similarly, transformed *Trichoderma harzianum* with *ChiA* from *S. marcescens* was more capable of suppressing *Sclerotium rolfsii* than the original strain. More recently, a trademark in the history of bio-control was established by generating transgenic plants containing the gene for endochitinase from *T. harzianum* with increased resistance against plant pathogenic fungi (Lorito *et al*., 1993). These results indicate that these enzymes play an important role in bio-control and the bio-control ability of some microbes may be improved by transformation with chitinolytic enzymes.

6.7. Root Colonization and Protection of Infection Sites

Root colonization ability of bio-control agents and potential to survive and proliferate along growing plant roots over a considerable period, in the presence of the indigenous micro flora results in intimate associations that directly provide a selective adaptation to plants towards specific ecological niches (Parke, 1991). Also, the ability of bio-control agent to colonize specific substrates or sites, whether a seed, root, shoot area, stump or fruit surface (Parke, 1991), provides protection to infection site from pathogen attack (Table.2). However, they are effective only when provided with an additional competitive advantage, such as high initial cell numbers (Nautiyal, 1997), earlier establishment than the pest or pathogen, or the production of antibiotic substances (Thomashow *et al*., 1990).

Therefore, rhizosphere competence is considered as a prerequisite of effective biological control. Understanding root-microbe communication (Bais *et al*., 2004), as affected by genetic (Okubara, 2004) and environmental (Pettersson and Baath, 2004) determinants in spatial (Bais *et al*., 2004) and temporal (Chatterton *et al*., 2004) contexts, will significantly contribute to improve the efficacy of

these bio-control agents. Once bio-control agents establish on the site, the mechanism of antagonism might be competition for nutrients, space, siderophore production (Raaijmakers, *et al*., 1995), antibiosis (Dowling and Gara, 1994), production of hydrolytic enzymes or other active substances.

6.8. Detoxification of Virulence Factors

The detoxification mechanisms involve production of a protein that binds with pathogen toxin reversibly or irreversibly which ultimately results in decrease in the virulence potential of pathogen toxin. For example, role of certain bio-control agents such as *Alcaligenes denitrificans* and *P. dispersa* in detoxifying albicidin toxin produced by *Xanthomonas albilineans* has been reported previously (Walke *et al*., 1991). Similarly, strains like *B. cepacia* and *Ralstonia solanacearum* hydrolyze fusaric acid, a phytotoxin produced by various *Fusarium* species (Toyoda *et al*., 1988).

6.9. Parasitism and Predation

The parasitism of one fungus by another (hyperparasitism or mycoparasitism) is well documented and is manifested as morphological disturbance, direct penetration of hyphae and hyphal lysis. The degree of hyper parasitism is affected by environmental factors such as temperature, light and pH, but nutrient status, especially the C: N ratio is also important. The genus *Trichoderrna* contains some of the most studied mycoparasites. Formulations of some species are available commercially and are used to control fungal pathogens in the soil and on aerial plant surfaces. This mycoparasite penetrates resting structures such as sclerotia or may parasitise growing hyphae by coiling round them. *Trtchoderma harztanum* degrades fungal cell walls by the lytic action of glucanases and chitinases, while other species also produce cellulase (Chaur, 1998)

Trtchoderma species are amenable to genetic manipulation and their usefulness in integrated disease management programs has been enhanced by the production of pesticide-tolerant strains with enhanced hyper parasitic and lytic capabilities. The attributes of the partially successful mycoparasite *Ampelomyces quisqualis*, which can control powdery mildew in glasshouse grown cucumber, are discussed later in this chapter. Other widely studied mycoparasites include *Coniothyrium minitans* and *Sporidesmi.um sclerotiorum*, which are antagonists of sclerotial fungi, and *Gliocladium* spp which parasitize a range of soil-borne pathogens. Mycoparasitic Pythium spp appear to have some affinity for plant-parasitic members of the same genus. On the phylloplane, several fungi, including *Verticillium lecanii*, *Sphaerellopsis filum* and *Cladosporium* sp are known to attack rust fungi. Bacteria which occur on the phylloplane and in the rhizosphere are also known to parasitize plant pathogens (Chaur, 1998).

6.10. Induction of Systemic Resistance

Certain biocontrol agents show indirect mode of antagonism against the pathogens by inducing a state of plant resistance (Kuc, 1995). This induced resistance is of two types representing two distinct pathway responses: systemic acquired resistance (SAR) and induced systemic resistance (ISR). Typically, SAR is induced by pathogens while ISR is salicylic acid in dependent and is induced by non-pathogenic bacteria (Van Loon *et al*., 1998). SAR is mediated by a compound called salicylic acid which is frequently produced following pathogen infection that leads to expression of pathogenesis related (PR) proteins such as PR-1, PR- 2, chitinases, and some peroxidases (Ramamoorthy *et al*., 2001). These PR proteins can cause lysis of invading cells, reinforcement of cell membranes to resist infections, or induce localized cell death. In contrary, certain biocontrol agents do not induce PR proteins but increase accumulation of peroxidase, phenylalanine ammonia lyase, phytoalexins, polyphenol oxidase, and/or chalcone synthase (Ongena *et al*., 2000).

A second pathway referred to as ISR is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some nonpathogenic rhizobacteria. ISR was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by *Fusarium* sp. (Van Peer *et al*., 1991) and on cucumber with reduced susceptibility to foliar disease caused by *Colletotrichum or biculare* (Wei *et al*., 1991). Several bacterial traits (i.e., flagellation and production of siderophores and lipopolysaccharides) have been proposed to trigger ISR (Leeman*et al*., 1995) but there is no compelling evidence for an overall ISR signal produced by bacteria (Haas *et al*., 2000). ISR results in strengthening of plant cell wall and alteration of host plant physiology and metabolic responses, leading to an enhanced synthesis of plant defence chemicals upon challenge by pathogens

and/or abiotic stress factors (Nowak and Shulaev, 2003). It has recently been reported that volatile organic compounds may play a key role in this process (Ping and Boland, 2004). For example, volatiles secreted by *B. subtilis* GBO3 and *B. amyloquefaciens* IN937a were able to activate an ISR pathway in *Arabidopsis* seedlings challenged with the soft-rot pathogen *Erwinia carotovora* subsp. *carotovora* (Ryan *et al*., 2001).

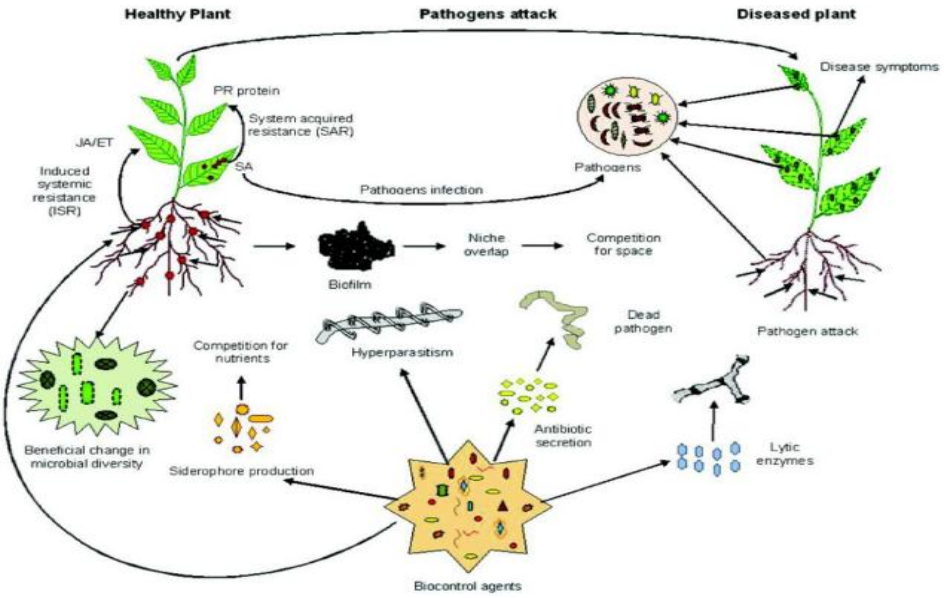

Figure5. *Mechanism of actions implemented by bio-control agents for management of plant diseases*

Table2. *Management of plant pathogens/diseases of important crops using various bio-control agents*

PGPRBs	Crops	Disease/pathogen/insect	References
Bacillus amyloliquefaciens	Tomato	Tomato mottle virus	Murply *et al*.,2000
Pseudomonas fluorescens	Tobacco necrosis virus	Tobacco necrosis virus	Park & Kloepper, 2000
Enterobacter sp.	Chickpea	*Fusarium avenaceum*	Hynes *et al*.,2008
Azospirillum brasilense	*Prunus cerasifera* L.	Rhizosphere fungi	Russo *et al*., 2008
Paenibacillus polymyxa	Sesame	Fungal disease	Ryu *et al*.,2006
Bacillus licheniformis	Pepper	*Myzus persicae*	Lucas *et al*.,2004
P. fluorescens	Rice	Blast/ *Pyricularia oryzae/*	Singh and Srivastava (2008)
T. harzianum, T. viride, T. virens, T. deliquescens	Rice	Bunt*/ Neovossia indica*	DeBach P (1964)
Rhizoctonia solani	Rice	Sheath blight/ *Rhizoctonia solani*	Singh and Srivastava (2008)
T. viride, T. harzianum, T. pseudokoningii & *T. koningii*	White	Karnal bunt/ *Neovossia indica*	Singh and Srivastava (2008)
Trichoderma spp.	Maize	Charcoal rot, Banded/ *Macrophomina phaseolina*	SINGH, 2014
A. niger AN27	Sorghum	Charcoal rot/ *Macrophomina phaseolina,*	Harman and Nelson (1994)
T. harzianum, Rhizobium	Groundnut	Stem & pod rot/ *Sclerotium rolfsii*	Singh and Srivastava (2008)

7. APPLICATION METHODS OF BIO-CONTROL AGENTS

Successful application of biological control requires more knowledge-intensive management (Heydari *et al*., 2004). Biologically based inputs such as microbial fungicides can be used to interfere with pathogen activities. Registered bio-fungicides are generally labeled with short reentry intervals and pre-harvest intervals, giving greater flexibility to growers who need to balance their operational requirements and disease management goals. When living microorganisms are introduced, they may

also augment natural beneficial populations to further reduce the damage caused by targeted pathogens (Heydari *et al*., 2004). Biological control agents can be applied using different methods such as seed treatment, root inoculation, cutting and seedling root dip, soil treatment, plant inoculation, furrow application and wound application.

7.1. Seed Treatment

Are the most effective methods. Treating seeds with bacterial cultures will improve plant production and productivity by protecting plants against phythopathogens. Seed bacterization with peat-based formulation of*P. Fluorescens* strain at the rate of 10 g kg^{-1}seed reduced rice blast and sheath blight disease (Rabindranand Vidhyasekaran, 1996). Meena *et al*. (2001) reported that seed treatment with powder formulation of *P. fluorescens* resulted in significant reduction in root rot incidence of groundnut under field conditions. Gamliel and Katan (1993) reported that tomato root when inoculated with *P. alkaligenes* reduced the wilt caused by*F. oxysporum f. sp. vasinfectum*. In cotton, seedling disease caused by *Rhizoctonia solani*and *P. ultimum* was suppressed by *Pseudomonasspp*. Under field conditions (Hagedorn *et al*.,1993). Treatment of tomato seeds with powder formulation of PGPR (*Bacillus subtilis, B. pumilus*) reduced symptom severity of tomato mosaic virus and increased the fruit yield (Murphy *et al*., 2000). Nagaraj *et al*., (2004) tested *P. fluorescens* strains isolated from the ricerhizosphere for their antagonistic effect towards rice sheath blight fungal pathogen, *R. solani*.

7.2. Seedling Dip

Application of *P. fluorescent* strain mixtures by dipping the seedling of rice in bucket of water containing talk based formulation containing mixture of 20g/l for 2h and later transplanting it in the field helps to control sheath blight of rice (Nandakumar *et al*., 2001) Weststeijn (1990) reported that tulip root rot caused by *P. ultimum* was suppressed by dipping tulip bulbs in Pseudomonas suspension. Transfer of technology for commercial use could be possible if PGPR strains are available as a product.

7.3. Seed Bio-Priming

Treating of seeds with bio-control agents and then incubating under warm and moist conditions until just prior to emergence of radical is referred to as bioprimming. This technique has potential advantagesover simple coating of seeds as it results in rapid and uniform seedling emergence. *Trichoderma* conidia germinate on the seed surface and form a layer around bio-primed seeds. Such seeds tolerate adverse various soil conditions better. Biopriming could also reduce the amount of biocontrol agents that is applied to the seed. Seed biopriming is successfully used in tomato, brinjal, soybean and chickpea in Tarai region of Uttaranchal. (Mishra *et al*., 2001). Three rhizosphere competent microbial strains, *viz., P. fluorescens* OKC, *Trichoderma asperellum* T42 and *Rhizobium* sp. RH4, individually and in combination in bioprimed seeds of chickpea and rajma in pots and fields showed higher germination percentage, and better plant growth in both the crops compared to non-bioprimed control plants. It was also observed that the combined application of the microbes enhanced seed germination and plant growth better than their individual application. Among the combinations, all combinations comprising *Trichoderma* showed better results comparedto the others and the triple microbial combination demonstrated best results in terms of seed germination and seedling growth in both chickpea and rajma (Yadav*et al*., 2013).

7.4. Foliar Spray Application

The efficacies of bio-control agents for foliar diseases are greatly influenced by microclimate. The concentration of nutrients like amino acids, organic acids and sugars exuded through stomata, lenticels, and wounds varies highly. It affects the efficacy and survival of antagonist in phylloplane. Application of *B. subtilis* to bean leaves reduces the incidence of bean rust. Foliar application of *P. fluorescent* reduces the severity of rust and leaf spot under field conditions. Kelly Cartwright (1995) reported that three spray applications of *P. cepacia* to cuttings during a two-week period were more effective than either one or two bacterial sprays in the control of *Rhizoctonia* stem rot of Poinsettia. Rice blast (*P. oryzae*) can be effectively controlled by foliar spray of talc based powder formulation of *P. fluorescent* strain Pf1 (1 kg ha^{-1}). The effectiveness of spraying persisted up to 15 days. When the bacterial product was sprayed on plants grown from treated seed, the effectiveness was higher than when spraying was carried out without any prior seed treatment. The dosage and frequency of

application has to be standardized based on the crop value, which could be a reliable and practical approach. Selected strains from many genera of bacteria isolated from these suppressive soils have the potential to reduce plant diseases when applied to the plant root environment (Weller *et al*., 2002).

7.5. Soil Application

soil being as the repertoire of both beneficial and pathogenic microbes, delivering of PGPR strains to soil will increase the population dynamics of augmented bacterial antagonists and thereby would suppress the establishment of pathogenic microbes onto the infection court. Weststeijn (1990) found that root rot in tulip caused by *P. ultimum* was reduced by mixing *Pseudomonas* suspensions thoroughly through the soil to a concentration of 10^8 cells g^{-1} dry soil before planting the bulbs. Wilt disease of sunflower was found to be suppressed when *P. cepacia* strainN24 was applied to the seedbeds at the rate of500 ml m_2 under greenhouse conditions (Hebber *et al*., 1991). Take-all disease of wheat was found to be suppressed by applying 120 ml of *P. aureofaciens* suspension to 13 kg of soil as atomized mist produced by use of chromatography sprayer and compressed air (Mazzola *et al*., 1992).

7.6. Applying to the Infection Site

Application directly to the infection court at a high population level to swamp the pathogen (inundate application), seed coating and treatment with antagonistic fungi and bacteria, e.g., *Trichoderma harzianum* and *Psudomonas fluorescens* (Heydari and Misaghi, 2003), antagonists applied to fruit for protection in storage, e.g., *Pseudomonas fluorescens* (Janisiewicz and Korsten, 2002). These types of applications are the most commonly used procedures which have resulted in the successful control of several fungal plant pathogens.

8. FACTORS AFFECTING EFFICACY OF BIOLOGICAL CONTROL

8.1. Plant Host Effect

Mechanisms and attributes of bio-control other than those described above are of importance under field conditions. Among these is the ability of the bio-control agent (BCA) to survive and proliferate on the plant surface under varying nutritional and microclimatic conditions, and to colonize the plant in such a way as to prevent the establishment of the pathogen. This complicates the use of BCAs because of the fact that an isolate, although effective against a certain pathogen on one crop, may be ineffective against the same pathogen on another crop. The host plant may directly influence the BCA by stimulating its establishment and survival and by stimulating antagonism towards the pathogens (Paulitz and Matta 1999; Smith *et al*., 197).

The host can provide favorable environmental niches and nutrients that influence BCA-pathogen interaction by supplying plant exudates needed for the production of antibiotics or for competition. These factors are determined by the genetic background of the host plant and by its physiological status as manipulated by the growth conditions. Soluble and volatile nutrients that arise from the root, seed, flower and leaf exudates and mucigal of the root cap can support introduced BCAs. The plant can influence microbial populations associated with its surfaces as was shown for flax and tomato that were grown in the same soil and found to select for different bacteria (Lemancheau *et al*., 1995). Strains of *Pseudomonas fluorescens* CHAO that were engineered to overproduce diacetylphloroglucinol and pyoluteorin increased in biocontrol efficacy much more on roots of cucumber than on wheat, suggesting that the plant species may affect antibiotic production (Maurhofer *et al*., 1995). Likewise, different plant cultivars may accommodate BCAs to a different degree (Smith *et al*., 1997) for example, different cultivars of wheat responded differently to PGPR (Chanway *et al*., 1998) and biocontrol strains (Weller 1986).

8.2. Microclimate, Abiotic Factors

Suppression of plant diseases by BCAs is largely affected by environmental conditions (Shtienberg and Elad, 1997). Under field conditions the plant surface is subjected to fluctuating temperature, vapour pressure deficit (VPD), surface wetness, gases and air movement (Burrage, 1971). These factors affect the indigenous microflora and BCAs directly, or may have an indirect effect by modifying the disposition of the host plant, e.g., the metabolic state and the plant surface chemistry. For example, *Trichoderma harzianum* T39 was less effective in suppression of cucumber fruit and

stem grey mould under wet conditions and temperatures below 20°C than under dry conditions at elevated temperatures (Elad *et al*., 1993). High temperature during the day and high VPD during the night were associated with reduced efficacy of *Botrytis cinerea* suppression in cucumber and tomato by T39, *Aureobasidium pullulans* and *Cryptococcus albidus* (Dik and Elad, 1999). *Tilletiopsis minor* was very effective in controlling powdery mildew on cucumber plants under controlled conditions, but under greenhouse conditions, the effect was disappointing due to elevated VPD (Hijwegen, 1992).

8.3. Physical and Chemical Nature of Plant Surfaces and the Environment

Chemical exudates on the plant surface contain macro- and micro-elements, sugars, sugar alcohols, pectic substances, amino acids, and organic acids. The quality and quantity of leachates from plants are affected by plant age and factors such as temperature, VPD and surface moisture, light, fertilization and pollen that change constantly. These changes may affect plant surface micro flora directly or have an indirect impact by modifying leaf characteristics, e.g., metabolic state, morphology and surface chemistry (Cutter, 1976). As nutrients fluctuate, there are community changes in colonization by bacteria, yeasts and filamentous fungi (Blakeman, 1985).

Plant surfaces are covered with a hydrophobic wax layer on which water is distributed as discrete droplets. Fertilization of plants affected the survival of *Trichoderma* on the foliage (Elad and Kirshner, 1993). Similar to the phyllosphere, the rhizosphere is subject to dramatic changes on a short temporal scale. Rain events and daytime drought can result in fluctuation in salt concentration, pH, osmotic potential, water potential and soil particle structure. The rhizosphere can also change due to root growth, interaction with other soil biota and weathering processes. These changes affect the establishment and activity of the introduced BCAs. Duffy *et al*., (1997) calculated that the soil chemical and physical properties of soil that are associated with suppression of take-all disease of wheat by *Trichoderma koningii* are nitrate-nitrogen, pH, copper, and soluble magnesium and suggested to amend the inoculants with such beneficial factors that will enable bio-control activity.

8.4. Indigenous Microbial Populations

On the plant surface, the introduced BCA faces not only the pathogen but also a variety of other microorganisms with which it constantly interacts. For example: cucumbers inoculated with PGPR strains interacted with the population of endophytes within the plant (Press *et al*., 1995) and the BCA *Bcillus cereus* interacted with the bacterial communities in the rhizosphere of soybean (Gilbert *et al*., 1996). The colonization of apple wounds by natural microorganisms and an introduced yeast, *Candida oleophila*, was monitored by Mercier and Wilson (1994) who found that the presence of naturally occurring micro flora was in some cases beneficial to biocontrol of storage rot. The effect of introduced *Trichoderma harzianum* T39 on other phylloplane microorganisms (Elad and Kirshner, 1992) was found to either promote or inhibit bacteria, yeasts or filamentous fungi, depending on plant nutrition and the microclimatic conditions under which the plants were incubated.

9. Commercialization of Biological Control Products

Commercialization and growers acceptance of the biological control has been slow to develop, mainly due to the variation in efficacy under the range of the environmental conditions likely to occur in the field. This problem can only be overcome by better understanding the environmental parameters that limit bio-logical control. In addition to this problem, there has been relatively little investment in the development commercially viable products for biological control, partly due to the cost of developing, testing efficacy, and risky, registering and marketing such a product. Although the number of bio control products in plant disease management is increasing, these products still represent only 1% of the agricultural control measures while fungicides account for 15% of total chemicals used in agriculture (Friavel *et al.*, 2005). In recent years many small and large entrepreneurs have entered into the commercial production of bio control agents resulting into the entry of various bio- control products into the world market. Commercialization of bio-control products is a multi-step process involving a wide range of activities: (1) isolation of micro- organism from the natural ecosystem. (2) Evaluation of bio-agent both in vitro and under glass house conditions. (3) Testing of the best isolate under field conditions. (4) Mass production (5) Formulation (6) Delivery (7) Compatibility (8) Registration and release.

The first bacterium called *Agrobacterium radiobacter* strain K 84 was registered with the United States Environmental Protection agency (EPA) for the control of crown gall in 1979. Ten years later the first fungus *Trichoderma harzianumi* ATCC 20476 was registered with the EPA for the control of plant disease. Commercial production of *Trichoderma* for the protection and yield enhancement of crops is in progress in the countries such as USA, India, Israel, New Zealand and Sweden. Under commercial conditions, Haddad and collaborators (2009) also showed that the strain B157 of *Bacillus* sp. can be considered a potential biocontrol agent for coffee leaf rust in organic crop systems in Brazil (Haddad *et al*., 2009). In 2008, more than 12,000 kg of this product were commercialized only for the treatment of potato and carrot. 5 - 10 kg/ha of the product, with 2x1010 CFU/g, were applied by irrigation and other ways.

In Brazil the product formulated with *Bacillus subtilis* and *Bacillus licheniformis*, has been used for the control of diseases caused by the nematode pathogens *Meloidogyne incognita, Meloidogyne javanica, Pratylenchus brachyurus* and *Pratylenchus coffeae* on potatoes and carrots. Currently a total of 14 bacteria and 12 fungi have been registered with the EPA for the control of plant diseases (Fravel, 2005).

Most of these are sold commercially as one or more products. The technology of commercialization is still in its initial phase. 65% of the EPA registered organisms have been registered within the past 10 years while the remaining 36% registered over the past 5 years. Many technological problems were overcome and shifts in tinking occurred for these products to reach the shelves. Some of the commercially available bio control products available in the market are shown in table (3).

Table3. *Some Commercially Exploited Microbes as Biological Control*

SN	Bio control agent	Product	Target disease/organism	Crop	Manufacturer
1	*Ageobacterium radiobacter strain 84*	Galtrol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	AgBioChem, USA
2	*Ageobacterium radiobacter strain* K 1026	Nagol	*Agrobacterium tumefaciens*	Ornamentals, Fruits, Nuts	Bio-care
3	*Bascillus subtillus* strain GB34	GB34	*Rhizoctonia, Fussarium*	Soyabean	Gustafon, USA
4	*Bascillus subtillus* strain GB03	Kodiac, companion	*Rhizoctonia, Aspergillus*	Wheat, barley, peas	Growth products,USA
5	*Pseudomonas aureofaciens* strain TX-1	Bio–jet, spot less	*Pythium, Rhizoctonia solani*	Vegetables and Ornamentals ingreen houses	EcoSoil system
6	*Pseudomonas fluorescence* strain A506	Frostban	Fire blight, bunch rot	Fruit crop, Tomato, Potato	Plant Health Technologies
7	*Streptomycine griseoviridis*	Mycostop	Soil borne pathogens	Ornamentals, Tree seedlings	Kemira Oy, Finland
8	*Trichoderma harzianum T-22*	Root shield, plant shield	Soil borne pathogens	Green house nurseries	Bio works, USA
9	*Trichoderma harzianum T-39*	Trichodex	*Botrytis cinerea*	Most of the food crops	Bio works, USA
10	*Ampelomyces quisquallis* isolate M-10	AQ10	Powdery mildew	Fruits,Vegetables Ornamental	Ecogen,USA
11	*Aspergillus flavus* AF36	Alfa guard	*Aspergillus flavus*	Cotton	Circleone globa,USA
12	*Gliocladium catenulatum* strain JI446	Prima stop soil guard	Soil borne pathogens	Vegetables, Herbs , Spices	Kemira Agro Oy, Finland
13	*Gliocladium virensGL-21*		parasitic ematodes	Food, Fibre,	-Do

10. CONCLUSIONS

Plant pathogens are among the most important factors that cause serious damages and losses to plants. Harmful impacts of the chemical pesticides on the environment and non-target organisms have clearly been documented. The need for the development of non-chemical alternative strategies to protect plants against plant diseases including fungal pathogens is therefore clear. Biological control using microbial antagonists to manage plant diseases seems to be a promising alternative strategy and have

successfully been applied to control some diseases on different plants and crops. Some of the important factors that affect the efficacy of microbial bio-control agents in controlling plant diseases which should carefully be considered include biotic and a biotic factors, method of application, formulation of bio-control microorganisms and timing of application. Understanding the mechanisms or activities for antagonist-pathogen interactions will be one of important steps because it may provide a reasonable basis for selection and construction of more effective bio-control agents. Biological control do not leave any residual effects in to the environments and not hazardous to the human being, live stocks and other beneficial living organisms so due to its important characteristics biological control is one of the best choices to sustain agricultural production and productivity without harming the environment.

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