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Biological control a sustainable approach for plant diseases management: A review

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Abstract
It is estimated that diseases, insects and weeds together annually interfere with the production and destroy 36.5% crop production. In general losses of crops due to disease amount to 25% of world crop production per annum (Lugtenberg, 2015). Biological disease control is an attractive strategy for the control of plant diseases. Meanwhile, it also provides practices compatible with the goal of a sustainable agricultural system. Biological control is the suppression of disease by the application of a Biocontrol Agent (BCA) usually a fungus, bacterium, or virus. Weindling (1932) reported the parasitic nature of *Trichoderma lignorum* on several plant pathogens. Biological disease control is an attractive alternative strategy for the control of plant diseases. Meanwhile, it also provides practices compatible with the goal of a sustainable agricultural system. Insofar as we know, the mechanisms of bio-control may involve and be divided into (i) antibiosis, (ii) competition, (iii) mycoparasitism (iv) cell wall degrading enzymes, and (v) induced resistance. However, these mechanisms of biological control are probably never mutually exclusive. BCAs are plays an important role in controlling plant pathogens, especially soil borne fungal pathogens. The use of BCAs based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to be done to develop stable, cost effective, easy to produce and easy to apply formulations. Effective biological control strategies in the future, critical to carry out more research studies on some less developed aspects of bio-control, including development of novel formulations, understanding the impact of environmental factors on bio-control agents, mass production of bio-control microorganisms and the use of biotechnology and nano-technology in improvement of bio-control mechanisms and strategies. Future outlooks of bio-control of plant diseases is bright and promising and with the growing demand for bio-control products among the growers, it is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

Keywords: Biological control, antibiosis, competition, mycoparasitism, induced resistance, BCAs, biotechnology, nano-technology

Introduction
Plant diseases need to be controlled to maintain the quality and abundance of food, feed and fiber produced by growers around the world. 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. Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market. Additionally, the spread of plant diseases in natural ecosystems may preclude successful application of chemicals, because of the scale to which such applications might have to be applied. Consequently, some pest management researchers have focused their efforts on developing alternative inputs to synthetic chemicals for controlling pests and diseases.

The mechanisms of biocontrol mainly include antibiosis, competition, mycoparasitism, cell wall degrading enzymes, and induced resistance. BCAs are play an important role in controlling plant pathogens, especially soil borne fungal pathogens. Muthukumar and Venkatesh (2014) [57] reported that *Trichoderma harzianum* (THA) and *Pseudomonas fluorescens* (PFM) showed the highest inhibition of mycelial growth (68.28; 74.25%) of *S. rolfsii*. Many microorganisms are identified as BCAs, such as *Trichoderma* sp., *Bacillus subtilis* and *Pseudomonas fluorescens* etc.

Colburn and Graham (2007) [14] reported naturally occurring hypovirulent isolate of *Phytophthora nicotianae* was found to effectively control citrus root rot caused by *P. nicotianae* and *P. Palmivora*. The use of BCAs based products is not only safe for the farmers and consumers but it is also good for the environment. Effective biological control strategies in the future, including development of novel formulations, understanding the impact of environmental factors on bio-control agents, mass production of bio-control microorganisms and the use of biotechnology and nano-technology in improvement of bio-control mechanisms and strategies. It is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

A variety of biological controls are available for use, but further development and effective adoption will require a greater understanding of the complex interactions among plants, people, and the environment. To that end, this article is presented as an advanced survey of the nature and practice of biological control as it is applied to the suppression of plant diseases. This survey will i) describe the various definitions and key mechanisms of bio-control, ii) explore the relationships between microbial diversity and biological control, iii) describe the current status of research and application of biological controls, and iv) briefly outline future directions that might lead to the development of more diverse and effective biological controls for plant diseases.

Definitions

The terms “biological control” and its abbreviated synonym “bio-control” have been used in different fields of biology, most notably entomology and plant pathology. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host specific pathogens. The organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA). Biological control of plant pathogens by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods.

The various definitions offered in the scientific literature have sometimes caused confusion and controversy. For example, members of the U.S. National Research Council took into account modern biotechnological developments and referred to biological control as “the use of natural or modified organisms, genes, or gene products, to reduce the effects of undesirable organisms and to favor desirable organisms such as crops, beneficial insects, and microorganisms”, but this definition spurred much subsequent debate and it was frequently considered too broad by many scientists who worked in the field (US Congress, 1995) [80]. Different definitions were proposed time to time by different workers due to disagreement amongst scientists on what constitutes biological control. However, some important definitions are the following: De Bach defined as Biological control (from the ecological viewpoint) is "the action of parasites, predators, or pathogens in maintaining another organism's population density at a lower average than would occur in their absence" Garret (1970) [18] defined biological control as “any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except man himself) with the result that there is a reduction in the incidence of the disease caused by that pathogen. Baker and Cook (1974) [5] stated that the “biological control is the reduction of inoculum 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 antagonist, or by mass introduction of one or more antagonists”.

Historical Back Ground

William Roberts in 1874 demonstrated the antagonistic action of micro- organisms in action of micro-organisms in liquid culture between *Pencillium glaucum* and bacteria and introduced the term antagonism. 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), Kerr (1980) [39] first reported bio-control of crown gall disease using *Agrobacterium radiobacter* strain K-84, and others demonstrated that antibiotics were produced in soil by *Pencilium, Aspergillus, Trichoderma, Streptomyces* sp. Kloepper (1980), demonstrated the importance of siderophores produced by *Erwinia carotovora*. Howell (1993) reported P and Q strains of *Trichoderma* sp. (Junaid *et al.*, 2013) [36].

Biological control is nothing but ecological management of community of organisms. It involves harnessing disease-suppressive microorganisms to improve plant health. Disease suppression by use of biological agents is the sustained manifestation of interactions among the plant (host), the pathogen, the biocontrol agent (antagonist), the microbial community on and around the plant and the physical environment. The biological control of plant diseases differs from insect biocontrol in following ways

Differences between disease bio-control and insect bio-control

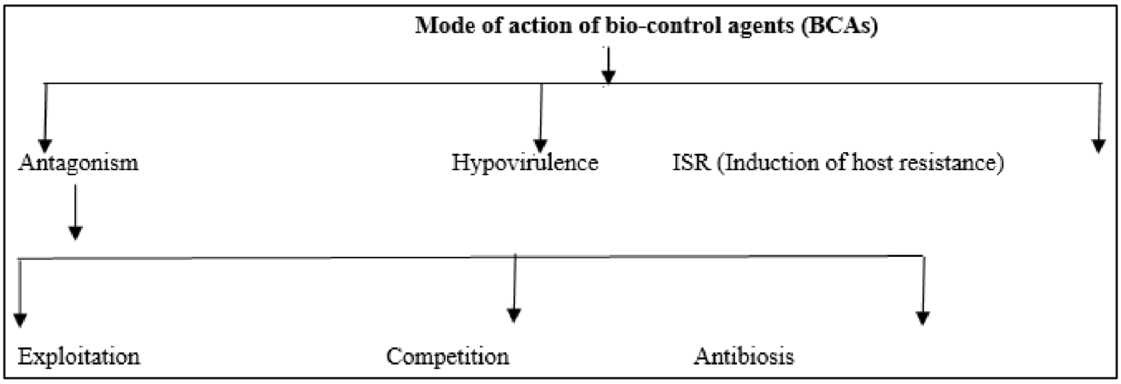
Sl. No.	Disease Bio control	Insect Bio control
1	Disease control is largely achieved by antibiosis, competition and comparatively less by hyperparasites.	Largely by parasites and predators.
2	Antagonists are largely passive and are not mobile. Contact of pathogen is accidental.	Parasites are active, mobile and seek their prey.
3	It is a mass effect. For a single species of pathogen a large number of antagonists/ competitors available.	Single predator / parasite for single prey.
4	This method relies mainly on native organisms.	Introduction of parasites / predators from other countries are normally followed.
5	Pathogen free seeds and planting materials are widely used.	Pest free seeds are not used.

Characteristics of an effective bio-control agents (BCA)

1. BCA must be able to control the pathogen by inhibiting its development.
2. BCA must have ability to compete with the pathogen for nutrient and space.
3. Production of antibiotic compounds.
4. Production of lytic enzymes effective against pathogen.
5. Ability to parasitize the pathogen.
6. Ability to interfere with reproduction of pathogen.
7. The induction of host defence.

Mode of action of bio-control agents (BCAs)

Biological control agents reduce disease of the target crop usually by one or more of the following mode of action: antagonism, hypovirulence and induction of host resistance. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. Here, we assert that the different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions. Direct antagonism results from physical contact or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, 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 nonpathogenic BCAs is the most indirect form of antagonism. However, in the context of the natural environment, most described mechanisms of pathogen suppression will be modulated by the relative occurrence of other organisms in addition to the pathogen. While many investigations have attempted to establish the importance of specific mechanisms of bio-control to particular patho-systems, all of the mechanisms described below are likely to be operating to some extent in all natural and managed ecosystems. And, 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) [30]. 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, *et al*., 2002) [68]. Various mechanisms employed by the bio control agents in controlling the plant diseases are broadly classified into.



Antagonism

Antagonism is a "type of symbiosis in which one organism is harmed by the other either by the latter being parasite or predatory on former, or through competition for food in short supply, or through secretion of certain toxic substances".

The antagonistic action can be divided into three categories. (i) direct parasitism or predation of organisms over pathogenic ones (exploitation), (ii) active demand of nutrient over supply, a situation which results primarily from quicker and greater utilization of available nutrients by saprophyte microorganisms with the result that pathogens faces lysis or suppression due to starvation (competition), and (iii) suppression of pathogenic organisms due to toxic or inhibitory compounds by other microorganisms (antibiosis).

Exploitation (Parasitism/ Predation)

Exploitation is an antagonistic condition wherein an organism directly harms another organism to get benefit out of the harm done to the organism. This phenomenon operated through parasitism and predation. A parasite develops some sort of etiological relationship with its host and the latter is exploited slowly, whereas a predator physically eliminates its prey (host) by direct feeding on it without establishing any etiological relationship. 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* (Chet 1987 and Benhamou and Chet 1997) [13, 47].

Most of the plant diseases are due to fungi, and large number of fungi parasitizing fungi. When one fungus parasitizes another, the phenomenon is called mycoparasitism, hyperparasitism, direct parasitism or inter fungus parasitism. 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) [40]. Hyper-parasitism is the most considered and the most direct form of antagonism (Pal *et al*., 2006) [63]. Hyper-parasitism involves tropic growth of bio control agent towards the target organism, coiling, final attack and dissolution of target pathogens cell wall or membrane by the activity of enzymes (Tewari, 1996) [83]. It is one of the main mechanisms involved in *Tricoderma* (Sharma, 1996) [73]. *Trichoderma harzianum* exhibits excellent mycoparasitic activity against *Rhizoctonia solsni* hyphae (Altomare *et al*., 1999) [3]. Other hyperparasites attack plant-pathogenic nematodes during different stages of their life cycles (e.g. *Paecilomyces lilacinus* and *Dactylella oviparasitica*). Melo *et* *al.*, (2011) [32, 55] reported the Parasitism of *S. Sclerotiorum* by a mutant of *Coniothyrium minitans* CM4b.

Mycoparasitism is under the control of enzymes. Gupta *et al*. (1995) [20] found that a strain of *Trichoderma* deficient in the ability to produce endo chitinase had reduced ability to control *Botrytris cineria* but shows increased ability to control *Rhizoctonia solani*. Since enzymes are the products of genes, slight change in the structure of gene can lead to the production of different enzyme. Harman (2000) [22] observed the involvement of chitinase and β-1, 3 glucanase in the *Trichoderma* mediated biological control.

Competition

Both the bio control agents and the pathogens compete with one another for the nutrients and space to get established in the environment. This process of competition is considered to be an indirect interaction between the pathogen and the bio control agent whereby the pathogens are excluded by the depletion of food base and by physical occupation of site (Lorito *et al*., 1994) [50]. An ability to compete successfully with a pathogen is an important property of biological control organisms. Often successful competition occurs at the infection court, preventing the ingress of the pathogen. The fungus, *Idriella bolleyi,* controls take all of wheat, caused by *Gaeumannomyces graminis f. sp. Tritici,* by competition for both nutrients and infection sites. While difficult to prove directly, much indirect evidence suggests that competition between pathogens and non-pathogens for nutrient resources is important for limiting disease incidence and severity.

In general, soil borne pathogens, such as species of *Fusarium* and *Pythium*, that infect through mycelial contact are more susceptible to competition from other soil- and plant-associated microbes than those pathogens that germinate directly on plant surfaces and infect through appressoria and infection pegs. Strain of *Pseudomonas flurorescens* GL20 was isolated by Lim and Kin in 1997 [47] from the rhizosphere of ginseng. This strain inhibit spore germination and germ tube elongation of *Fusarium solani* (Lim *et al.*, 2002) [48]. The most abundant nonpathogenic plant-associated microbes are generally thought to protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow. For example, effective catabolism of nutrients in the spermosphere has been identified as a mechanism contributing to the suppression of *Pythium ultimum* by *Enterobacter cloacae* (van Dijk and Nelson 2000, Kageyama and Nelson 2003) [81, 37].

Biocontrol based on competition for rare but essential micronutrients, such as iron, has also been examined. Iron is extremely limited in the rhizosphere, depending on soil pH. In highly oxidized and aerated soil, iron is present in ferric form (Lindsay 1979) [49], which is insoluble in water (pH 7.4) and the concentration may be as low as 10-18 M. This concentration is too low to support the growth of microorganisms, which generally need concentrations approaching 10-6 M. To survive in such an environment, organisms were found to secrete iron-binding ligands called siderophores, having high affinity to sequester iron from the micro-environment. Almost all microorganisms produce siderophores, of either the catechol type or hydroxamate type (Neilands 1981) [59].

Kloepper *et al.* (1980) [41] 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. And, a direct correlation was established *in vitro* between siderophore synthesis in *fluorescent pseudomonads* and their capacity to inhibit germination of chlamydospores of *F. Oxysporum* (Elad and Baker, 1985, Sneh *et al.*, 1984) [76]. The increased efficiency in iron uptake of the commensal microorganisms is thought to be a contributing factor to their ability to aggressively colonize plant roots and an aid to the displacement of the deleterious organisms from potential sites of infection. Biocontrol species are able to sequester iron for their own use by the production of iron binding siderophores. This reduces the availability of iron to other organisms such as pathogens (Santoyo *et al*., 2012) [71]. Because bacterial siderophores have a higher affinity for iron than fungal siderophores, they are effective at depriving fungi of iron (Jan *et al*., 2011) [33].

Some plant pathogens depend on growth substances or stimulants to overcome their dormancy before they can cause infection and bio control agents are known to exert competition for these stimulants there by reducing their disease causing ability. These substances include fatty acids or peroxidation products of fatty acids (Harman and Nelson, 1994) [23], volatile compounds such as ethanol and acetyldehyde (Paulitz, 1991) [64]

Antibiosis

Antibiosis is that antagonistic condition in which there is suppression of pathogenic microorganisms due to toxic or inhibitory compounds (antibiotics) by other organisms. Such compounds range from hydrogen cyanide to enzymes and microorganisms involved after often species of *Trichoderma* and *Gliocladium* among fungi, and *Bacillus* and *Psuedomonas* among bacteria. *Bacillus subtilis* effectively controls *Rhizoctonia solani* in many crops by producing bacilysin and fengymycin. Bacilysin inhibits yeasts and bacteria and fengymycin inhibits filamentous fungi (Bakker *et al*. 2003) [6]

BCAs are produce most diverse range of antimicrobial compounds. *Bacillus subtilis* effectively controls *Rhizoctonia solani* in many crops by producing bacilysin and fengimycin. Bacilysin inhibits yeast and bacteria fengimycin inhibits filamentous fungi. However, zwittermicin A antibiotic has been obtained from *Bacillus cereus* strain UW 85, another successful BCA of damping off and root rot of syabean (*Phytophthora sojae*). The first commercially BCA was probably strain K84 of *Agrobacterium* which has been used successfully to control crown gall disease by *Agrobacterium tumefaciens* by agrocin 84 antibiotic. *P. fluorescens* CHA0 produces antibiotics, siderophores and HCN, but suppression of black rot of tobacco caused by *Thielaviopsis basicola* appeared to be due primarily to HCN production (Voisard *et al*. 1989) [83]. *Pseudomonas putid*a WCS358r strains genetically engineered to produce phenazine and DAPG displayed improved capacities to suppress plant diseases in field-grown wheat (Glandorf *et al*. 2001) [19]. Yuncheng Wu *et al.* (2014) [91] were demonstrated the inhibitory effects of *B. amyloliquefaciens* NJZJSB3 on phytopathogens: *F. oxysporum* f. sp. *cucumerinum*, *Verticillium dahlia*, *F. oxysporum* f. sp. *niveum, Rhizoctonia solani*, *F. oxysporum* f. sp. *cubense*, and *F. Oxysporum* f. sp. *dioscoreae*. Inactivation of antibiotic synthesis genes in various species of *Pseudomonas*, or *Bacillus* has provided strong evidence for the role of antibiotics in biocontrol by these species (Wu *et al.* 2015). Experiments in which the darA and darB genes responsible for the biosynthesis of the antibiotic 2-hexyl, 5 propyl resorcinol (HPR) in *Pseudomonas chlororaphis* were inactivated confirmed the role of the antibiotic in antagonism (Calderon *et al.* 2013) [10]. Similarly, gene disruption was used to provide evidence for roles for fengycin (Yanez-Mendizabal *et al*. 2012) [90] and iturin in biocontrol of peach and curcubit diseases respectively by strains of *Bacillus subtilis* (Zeriouh *et al*. 2011) [93] and of iturin in biocontrol of fruit diseases by *Bacillus amyloliquefaciens* (Arrebola *et al*. 2010) [4].

Table 2: Some of antibiotics produced by BCAs

S. no.	Antibiotic	Source	Disease	Reference
1.	2, 4 diacetylphloroglucinol	*Pseudomonas fluorescens* F113	Damping off	Shanahan *et al.* (1992) [72],
2.	Agrocin 84	*Agrobacterium radiobacter*	Crown gall	Kerr (1980) [39]
3.	Bacillomycin D	*Bacillus subtilis* AU195	Aflatoxin Contamination	Moyne *et al.* (2001) [56]
4.	Bacillomycin and Fengycin	*Bacillus amyloliquefaciens* FZB42	*Fusarium* Wilt	Koumoutsi *et al.* (2004) [43]
5.	Xanthobaccin A	*Lysobacter* sp. strain SBK88	Damping off	Islam *et al.* (2005) [31]
6.	Gliotoxin	*Trichoderma virens*	Root rots	Wilhite *et al.* (2001) [86]
7.	Herbicolin	*Pantoea Agglomerans* C9-1	Fire blight	Sandra *et al.* (2001) [70]
8.	Iturin A	*B. subtilis* QST713	Damping off	Paulitz and Belanger (2001) [65], Kloepper *et al.* (2004) [42]
9.	Phenazines	*P. fluorescens* 2-79 and 30 84	Take-all of wheat	Thomashow *et al.* (1990) [78]
10.	Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	Damping off	Howell and Stipanovic (1980) [29]
11.	Pyrrolnitrin and pseudane	*Burkholderia cepacia*	Damping off and rice blast	Homma *et al. (*1989) [27]
12.	Zwittermicin A	*Bacillus cereus* UW85	Damping off	Smith *et al.* (1993) [75]
13.	Mycosubtilin	*B. subtilis* BBG100	Damping off	Leclere *et al.* (2005) [46]

Hypovirulence

Hypovirulence is the phenomenon of reduced virulence of a pathogen strain than normal ones developed as a result of its infection by double standard RNA (dsRNA). When a hypovirulent strain was co-inoculated with highly virulent strain of a fungus, the latter become hypovirulent normally by hyphal contact (anastomosis). Some transmissible factor (ds RNA) moved from the hypovirulent stain into the more aggrasive one.

The phenomenon of hypovirulence is well established in a number of fungal pathogens. *Cryphonectria parasitica* and *Ceratocystisulmi*, the pathogens of chestnut blight and dutch elm disease, respectively, both harbour dsRNA because several different sized dsRNAs have been isolated from hypovirulent strains of these fungi (Nuss 2005) [60]. A naturally occurring hypovirulent isolate of *Phytophthora nicotianae* was found to effectively control citrus root rot caused by *P. nicotianae* and *P. palmivora* (Colburn and Graham 2007) [14].

Induction of host resistance

Plants actively respond to a variety of environmental stimuli, including gravity, light, temperature, physical stress, water and nutrient availability. Plants also respond to a variety of chemical stimuli produced by soil- and plant-associated microbes. Such stimuli can either induce or condition plant host defences through biochemical changes that enhance resistance against subsequent infection by a variety of pathogens. Disease suppression through the induction of resistance in host is an alternative, and quite different, mode of action of biological control agents this occurs as a result of the release of elicitors (proteins, antibiotics and volatiles) by the BCA that induce expression of the genes of the salicyclic acid pathway or the jasmonic acid/ethylene pathway (Nawrocka and Malolepsza 2013; Pieterse *et al*. 2014) [58, 67].

Induction of host defences can be local and systemic in nature, depending on the type, source, and amount of stimuli. Recently, phyto-pathologists have begun to characterize the determinants and pathways of induced resistance stimulated by biological control agents and other non-pathogenic microbes. The first of these pathways, termed systemic acquired resistance (SAR), is mediated by salicylic acid (SA), a compound which is frequently produced following pathogen infection and typically leads to the expression of pathogenesis-related (PR) proteins. These PR proteins include a variety of enzymes some of which may act directly to lyse invading cells, reinforce cell wall boundaries to resist infections, or induce localized cell death. A second phenotype, first referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and ethylene, which are produced following applications of some non-pathogenic rhizobacteria. For example, pathogenic strains of *Pseudomonas syringae* produce coronatine, which is similar to JA, to overcome the SA-mediated pathway (He *et al.* 2004).When *Psuedomonas flourescens* was applied to roots of carnation and the stems were inoculated one week later with *Fusarium oxysporum* f. sp. *dianthi*, the vascular wilt causing fungus, the incidence of disease was reduced as a result of increase in resistance of the host. Bacterial volatiles have also been implicated in induction of systemic resistance in the host plant via an ethylene dependent pathway (Kloepper *et al.* 2004) [42]. In addition to volatiles ISR is induced by siderophores and cyclic lipopeptide antibiotics (Jan *et al*. 2011) [33].

A number of strains of root-colonizing microbes have been identified as potential elicitors of plant host defences. Some biocontrol strains of *Pseudomonas* sp. and *Trichoderma sp*. are known to strongly induce plant host defences. In several instances, inoculations with plant-growth-promoting rhizobacteria (PGPR) were effective in controlling multiple diseases caused by different pathogens, including anthracnose (*Colletotrichum lagenarium*), angular leaf spot (*Pseudomonas syringae pv. lachrymans* and bacterial wilt (*Erwinia tracheiphila*). A number of chemical elicitors of SAR and ISR may be produced by the PGPR strains upon inoculation, including salicylic acid, siderophore, lipopolysaccharides, and 2,3-butanediol, and other volatile substances (Van Loon *et al*. 1998, Ongena *et al*. 2004, Ryu *et al*. 2004) [82, 62, 69].

Table 3: Commercially available BCAs, their trade name (s) and target pathogen

S. No.	BCA	Trade name	Target pathogen
1.	*Agrobacterium radiobacter strain k 84*	Galltrol	*Agrobacterium tumeifaciens*
2.	*Agrobacterium radiobacter strain k 1026*	Nogall	*Agrobacterium tumeifaciens*
3.	*Bacillus subtilis*	Kodiak	*Rhizoctonia* and *Fusaurium*
4.	*Bacillus subtilis*	Quantum 4000	*Alternaria* and *Fusarium*
5.	*Burkholderia cepaci*	Deny	*Pythium* and *Bacillus subtilis*
6.	*Psuedomonas fluoroscens*	Blight Ban	Frost damge and *Erwinia amylovora*
7.	*Psuedomonas fluoroscens*	Doggar G, Bioshield	*Rhizoctonia* and *Pythium*
8.	*Streptomyces griseoviridis*	Mycortop	*Pythium*
9.	*Peniophora gigantean*	P G Suspension	*Heterobasidion*
10.	*Gliocladium virens*	Gliogard	*Pythium* and *Rhizoctonia*
11.	*Ampelomyces quisqalis m 10*	A Q 10 Biofungicide	Powdery mildew fungi
12.	*Trichoderma viride*	Antagon TV, Biocon,Biogaurd	Soil borne fungi
13.	*Trichoderma harzianum*	F- stolp, Binap- T, root shield and trichodex	Soil borne fungi
14.	*Fusarium oxysporum* (Non-pathogenic)	Fusaclean	*Fusarium oxysporum*

(**Source:** Junaid *et al.*, 2013) [36]

Biological control of plant diseases

Biological control of soil borne diseases

Gliocladium virens and *Trichodrma* sp. etc. used in potting mixes, are mixed with soil, or are used as solid matrix in seed priming treatments. They are effective against damping off disease, *Sclerotinia* stem rot, *Rhizoctonia* rot and *Fusarium* wilt diseases. Crown gall disease of pome and stone fruits can be controlled by treating the seeds, seedlings and cuttings with galltrol and nogall suspension. This control is based on production by strain K 84 of bacteriocin called agrocin 84. Seed treatments with suspension of *Bacillus subtilis* strain A13 has protected the plants against root pathogens.

Biological control of diseases of aerial parts

Several foliar diseases have also been reduced significantly when the leaves were sprayed with spores of antagonistic fungi. For example, Cucumber powdery mildew with *Ampelomyces quisqalis*, the wheat rust with *Darluca filum* and the carnation rust fungus with *Verticillum lecanii*. Bacterial diseases also controlled by spraying with saprophyte bacteria or with avirulent strains of the pathogenic bacteria and fungal pathogens. For example, fire blight of apple controlled with spraying of *Erwinia herbicola* and bacterial leaf streak of rice was reduced with sraying of *Erwinia* and *Psuedomas*. Stockwell *et al.*, (2010) [77] reported that biological control agent *Pantoea vagans* C9-1R reduced the incidence of fire blight of pear or apple (*Erwinia amylovora*) by an average of 42% compared with water treatments in eight orchard trials.

Biological control of post-harvest diseases

Post harvest rots of several fruits could be reduced by spraying the fruits with spores of antagonistic fungus or bacteria and saprophytic yeast at different stages of fruit development. For example *Psuedomonas* bacteria protect the lemon fruits from Penicillium green mould and Bacillus subtilis protected from brown rot (*Monilinia fructicola*). *Botrytis* rot strawberry was reduced by spraying of *Trichoderma* spore and Post-harvest rot of peach was reduced by yeast treatments. The antibiotics iturin, produced by *B. subtilis* B-3 (70), and pyrrolnitrin, produced by *Pseudomonas cepacia* LT-4-12W (94), reduced in vitro growth and conidia germination of the stone fruit pathogen *M. fructicola*, and pome fruit pathogens *P. expansum* and *B. cinerea*, respectively (Janisiewicz and Korsten, 2002) [34].

Biological control of weeds

Microorganisms used as biocontrol agents of weeds are generally fungi pathogenic to specific weeds, to which they cause significant damge or death.

Classical biological control, involving the importation, colonization, and establishment of exotic natural enemies (predators, parasites, and pathogens) to reduce exotic pest populations to, and maintain them at, densities that are economically insignificant, is the predominant approach to biological weed control (McFadyen,1998) [54]. This method employs an inoculative release strategy, whereby natural enemies are liberated once or over a limited period of time to establish self-perpetuating populations. Conservation, the manipulation of the environment to favor (often native) natural enemies, rarely is employed. Augmentation, the mass production and periodic release of enemies, usually involves mycoherbicides (fungi applied in inundative doses like a chemical herbicide) and some insects, but also the use of grazing animals, to control weeds. Recent work has suggested that bacteria also may hold promise as bioherbicides in augmentative weed control (Johnson, Wyse, and Jones,1996; Kremer and Kennedy, 1996) [35, 44]. Biological weed control is predicated on the thesis that introduced plants become invasive because they have escaped from the insect herbivores and other natural enemies that regulate their abundance and distribution in their native regions (Keane and Crawley, 2002; McEvoy, 2002; Hoddle, 2004) [38, 52, 26], although other factors as well are likely to contribute to the tendency for particular plant species to become invasive (McEvoy and Coombs, 1999; Hierro and Callaway, 2003; Zedler and Kercher, 2004) [53, 25, 92]. Introduction of natural enemies into the invaded habitat can reduce a weed's population density to levels similar to those that naturally occur in the home range, leading to a restoration of ecological balance and recovery of the previous floral diversity (Bellows, 2001) [7].

Natural enemies may control weeds directly, by destroying vital parts, leading to reduced reproduction or to the death of the plant, or indirectly, by making the weed more susceptible to attack from pathogenic or saprophytic organisms or by exerting sufficient stress on the weed so as to put it at a competitive disadvantage to other, valued plants (Bellows and Headrick, 1999) [8]. There are several cases of highly successful weed control using fungi (Charudattan, 2001) [12]. Taxonomic and other difficulties of demonstrating the safety of plant bacteria, viruses, and microorganisms other than fungi has meant that these organisms have rarely been selected for use in classical biological weed control (Wapshere, Delfosse, and Cullen, 1989) [84].

Table 5: Commercially available BCAs, their Product and target weed

S. No.	Product	Content	Target weed
1.	Devine	*Phytophtora palmivora*	Strangle vine
2.	Bipolaris	*Bipolaris sorghicola*	Johnson grass
3.	Collego	*Colletotrichum gloesporiodes*	*Saccharum spontanium*
4.	Tripose	*Shrimp*	*Echinochloa sp.*
5.	Dr.Biosedge	*Puccinia coriculata*	*Cyperus exculentus*
6.	Luboe-2	*Colletotrichum gloesporiodes*	Cuscuta
7.	Velgo	*Colletotrichum coccoids*	Volvet leaf in soyabean and maize
8.	Biomal	*Colletotrichum gloesporiodes f.sp. malvae*	*Cassia obtusifolia*
9.	Abg 500b	*Cercospora rodmanii*	*Abutilion theopharsti*
10.	Casst	*Alternaria cassia*	*Morrenia odorata*

Application method of bio-control agents (BCAs)

Seed treatment

Seed treatment is most effective method. *Trihoderma* formulations are used for seed treatment @ 10 g/kg seeds. (Kumar and Godika, 2011) [45].

Seedling dipping

Applications of *P. fluorscens* mixture by dipping the seedlings of rice in bucket of water containg talc based formulation containg mixture of (20g/lt.) for 2 hours and later transplanting in the field helps to control sheath blight of rice.

Soil application

Trihoderma can be applied as granules as well as drench. *Trichoderma* 2.5 kg/hac. Pre incubated in 50 kg FYM used as Soil application. Soil application of peat based formulation *Pseudomonas fluorscens* @ 2.5 kg formulation mixed with 25 kg of FYM helps in reducing chickpea wilt.

Foliar spray

Foliar spray of *Pseudomonas fluorscens* on beet leaves help in inhibiting spore germination of *Botrytis* and *Cladosporium*. Spraying with 0.2% *Trichoderma* helps in reducing *Alternaria* blight and white rust infection in mustard. (Kumar and Godika, 2011) [45].

Bio- Control agents and Pathogen Management

Ganesan and Sekar (2004) [17] worked on Screening of Biocontrol Agents Against *Rhizoctonia solani* Causing Web Blight Disease of Groundnut (*Arachis hypogaea* L.) In vitro studies revealed that among the bacterial biocontrol agents, Maximum level of growth reduction of *R. solani* was noted in *B. subtilis* (42.53%) followed by *B. polymyxa* (39.13%) and *B. licheniformis* (30.36%). Lowest level of inhibition was found with *B. pumilus* (11.84%). Percentage of inhibition varied with biocontrol agents used. Among the fungal biocontrol agents, 78.76% of inhibition was recorded with *T. virens* followed by *T. hamatum* (77.64%) and *T. harzianum* (72.24%). Minimum level of inhibition was noticed with *T. reesi* (33.06%). Volatile activity of *T. harzianum* showed 100% inhibition of *R. solani*, where as *T. viride* showed 93.44% of inhibition against *R. solani*. *B. speriacus* and *B. polymyxa* showed 87.30% and 94.08% of inhibition. *B. megaterium* and *B. licheniformis* showed 95.7% and 94.41% of inhibition and *P. putida* showed 88.5% inhibition of *R. solani* mycelial growth.

Muthukumar and Venkatesh (2014) [57] In vitro studied on Biological inductions of systemic resistance to collar rot of peppermint caused by *Sclerotium rolfsii* and revealed that *Trichoderma harzianum* (THA) and *Pseudomonas fluorescens* (PFM) showed the highest inhibition of mycelial growth (68.28; 74.25%) of *S. rolfsii*. The antagonists *T. harzianum* and *P. fluorescens* were compatible with each other and they were tested alone and together in in vivo for the control of *S. rolfsii*. Besides, the induction of defenserelated enzymes such as peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, and the accumulation of phenolics in peppermint plants due to the application of bioagents were also studied.

Advantages and Limitations or disadvantages of bio control agents (Chandrashekara *et al*., 2012) [11]

Advantages of bio control agents

1. Avoid environmental pollution. 2. Avoid adverse effect on beneficial organisms. 3. Less expensive than pesticides and avoids problems of resistance. 4. BCAs are self-maintaining in simple application and fungicide needs repeated applications. 5. BCAs are very effective for soil borne pathogens where fungicide approach is not feasible. 6. BCAs are eco-friendly, durable, long lasting. 7. BCAs helped in induced systemic resistance among the crop species. 8. Not a water contaminant concern. 8. Biocontrol agents not only control the disease but also enhance the root and plant growth by way of encouraging the beneficial soil microflora. It increases the crop yield also. It helps in the volatilization and sequestration of certain inorganic nutrients. For example Bacillus subtilis solubilizes the element, phosphorous and makes it available to the plant. 9. Biocontrol agents can be combined with biofertilizers. 10. Biocontrol agents are very easy to handle and apply to the target.

Limitations or disadvantages of bio-control agents

Although biological control is advantageous in many aspects, it has the following disadvantages. 1. Major difficulties is the application of BCAs in getting them to the right place at the right time in sufficient density to be effective and then maintaining them there. 2. Other difficulty is the apprehension of the growers about the efficacy of BCAs. 3. Labour intensive, 4. Bio-control agents can only be used against specific diseases (Host specific), 5. Bio-control agents have slow effect in the control of plant diseases (Very slow action), 6. They are unavailable in larger quantities at present, 7. Effected by environment. 8. They are less effective than the fungicides, 9. At present, only few bio-control agents are available for use and are available only in few places, 10. Bio-control method is only a preventive measure but not a curative measure, 11. Requires skilled persons for multiplied and supplied of bio-control agents without contamination, 12. The shelf life of bio-control agents is very short. Antagonists, *Trichoderma viride* is viable for four months and *Pseudomonas fluorescens* is viable for three months only, 13. The required amount of population of bio-control agents should be checked at periodical interval and should be maintained at required level for effective use, 14.

The efficiency of biocontrol agents is mainly decided by environmental conditions, 15. A biocontrol agent under certain circumstances may become a pathogen.

Conclusion

Biological disease control is an attractive alternative strategy for the control of plant diseases. Meanwhile, it also provides practices compatible with the goal of a sustainable agricultural system. Insofar as we know, the mechanisms of biocontrol may involve and be divided into (i) antibiosis, (ii) competition, (iii) mycoparasitism, (iv) cell wall degrading enzymes, and (v) induced resistance. However, these mechanisms of biological control are probably never mutually exclusive. BCAs are play an important role in controlling plant pathogens, especially soil borne fungal pathogens. The use of BCAs based products is not only safe for the farmers and consumers but it is also good for the environment. However, much more work needs to bedone to develop stable, cost effective, easy to produce and easy to apply formulations. Effective biological control strategies in the future, critical to carry out more research studies on some less developed aspects of biocontrol, including development of novel formulations, understanding the impact of environmental factors on biocontrol agents, mass production of biocontrol microorganisms and the use of biotechnology and nanotechnology in improvement of biocontrol mechanisms and strategies. Future outlooks of biocontrol of plant diseases is bright and promising and with the growing demand for biocontrol products among the growers, it is possible to use the biological control as an effective strategy to manage plant diseases, increase yield, protect the environment and biological resources and approach a sustainable agricultural system.

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