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# Review on Concepts in Biological Control of Plant Pathogens

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## ABSTRACT

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. 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 biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. Many factors have to be considered in deciding whether a biological system is feasible for the control of a particular pathogen. Of prime importance is the availability of a suitable antagonist capable of maintaining itself on the host plant. The environment under which the crop is grown will play a significant part in determining whether effective population levels of an antagonist can be established in competition with the existing microflora. Environment may also govern the choice of antagonist; for example, yeasts can survive on leaves more readily than non-spore-forming bacteria under adverse humidity conditions. It is essential that the primary mechanism by which antagonism is brought about should be known. 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. With people turning more health conscious Biological control seem to the best alternative to disease suppression. Bio-agents bring the disease suppression with no environmental hazards. Research has proved that the bio agents trigger the growth of plants. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favours the activity and survival of microbe it contains. Moreover, the organism that suppresses the pathogen is referred to as the biological control agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. And, while such inputs may mimic the activities of living organisms, non-living inputs should more properly be referred to as biopesticides or biofertilizers, depending on the primary benefit provided to the host plant. Over the past few years, the novel applications of molecular techniques have broadened our insight into the basis of biological control of plant diseases. New molecular approaches have been available for assessment of interaction between the antagonist and pathogen, ecological traits of antagonists in rhizosphere and improving the efficacy of bacterial, fungal and viral biocontrol agent. Currently, biological control will thus be an alternative strategy for the control of plant diseases given the history of fungicides in the near future. However, other methods in IPM for crop disease control are still necessary in various environmental conditions, because an agro-ecosystem is a variable and functioning system that includes several factors that influence disease and crop development. Consequently, for economic threshold, other control strategies of IPM besides biological control should be also considered and applied to effectively reduce the disease development and the yield loss of crops in the different crop systems.

**Keywords:** Biocontrol, biofumigation, microbial antagonism, natural compounds, pathogen.

## 1. INTRODUCTION

Bio-control of plant diseases involves the use of an organism or organisms to inhibit the pathogen and reduce disease (Chaur, 1998.). There are many definitions for biological control; however, the basic idea involves a strategy for reducing disease incidence or severity by direct or indirect manipulation of microorganisms Shurtleff and Averre (1997). Consequently, understanding the mechanisms of biological control of plant diseases through the interactions between bio-control agent and pathogen may allow us to manipulate the soil environment to create conditions conducive for successful bio-control or to improve bio-control strategies (Chaur, 1998).

Biological control of plant pathogens is considered as a potential control strategy in recent years, because chemical control results in accumulation of harmful chemical residues, which may lead to serious ecological problems. At present, effective management of plant diseases and microbial contamination in several agricultural commodities is generally achieved by the use of synthetic pesticides. However, the continual and indiscriminate application of these chemical fungicides has caused health hazards in animals and humans due to residual toxicity.

In recent years, large numbers of synthetic fungicides have been banned in the western world because of their undesirable attributes such as high and acute toxicity. Many pathogenic microorganisms have developed resistance against chemical fungicides. This seriously hinders the management of diseases of crops and agricultural plants. Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. And also, there is a need to reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on bio-control agents (BCAs) for pest and disease control alone or to integrate with reduced doses of chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment (Vinale *et al*., 2009). Biological control of plant diseases has been considered a viable alternative method to manage plant diseases (Heydari and Pessarakli, 2010). 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 or reproduction of one organism using another organism (Pal and Gardener, 2006). 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.

Although the value of eco-friendly pest (bacteria, fungi, insects, mites, nematodes, rodents, weeds, etc.,) management in sustainable agriculture has been well recognized, only very little is being adapted at field level. This eco-friendly pest management gives greater emphasis for the usage of biological control. Bio-control methods are successful in non-chemical and eco-friendly approach in the sustainable agricultural production. Fungi belonging to the genus *Trichoderma* and bacteria such as *Pseudomonas, Bacillus subtilis* are the most promising bio-control agent against a range of plant pathogens under a variety of environmental conditions (Chen, *et al*, 1995).

Moreover, the uses of microbial antagonists to suppress diseases as well as the use of host-specific pathogens to control weed populations. The organism that suppresses the pathogen is referred to as the biological control agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. And, while such inputs may mimic the activities of living organisms, non-living inputs should more properly be referred to as biopesticides or biofertilizers, depending on the primary benefit provided to the host plant. Therefore, biological control of plant pathogens has now emerged as a broad concept, evident in the accounts and encompasses several mechanisms. Hence, the aim of this review is to give an overview on concepts in biological control of plant pathogens

## 2. Over view on concepts in biological control of plant pathogens

### 2.1. Methods of Biological Control of Plant Diseases

### 2.1.1. Suppressive soils

Several soilborne pathogens, such as *Fusarium oxysporum* (the cause of vascular wilts), *Gaeumannomyces graminis* (the cause of take-all of wheat), *Phytophthora cinnamomi* (the cause of root rots of many fruit and forest trees), *Pythium* spp. (a cause of damping-off), and *Heterodera avenae* (the oat cyst nematode), develop well and cause severe diseases in some soils, known as conducive soils, whereas they develop much less and cause much milder diseases in other soils, known as suppressive soils. The mechanisms by which soils are suppressive to different pathogens are not always clear but may involve biotic and/or abiotic factors and may vary with the pathogen. In most cases, however, it appears that they operate primarily by the presence in such soils of one or several microorganisms antagonistic to the pathogen. Such antagonists, through the antibiotics they produce, through lytic enzymes, through competition for food, or through direct parasitizing of the pathogen, do not allow the pathogen to reach high enough populations to cause severe disease (Agrios, 2005).

Numerous kinds of antagonistic microorganisms have been found to increase in suppressive soils; most commonly, however, pathogen and disease suppression has been shown to be caused by fungi, such as *Trichoderma*, *Penicillium*, and *Sporidesmium*, or by bacteria of the genera *Pseudomonas*, *Bacillus*, and *Streptomyces*. Suppressive soil added to conducive soil can reduce the amount of disease by introducing microorganisms antagonistic to the pathogen. For example, soil amended with soil containing a strain of a *Streptomyces* species antagonistic to *Streptomyces scabies*, the cause of potato scab, resulted in potato tubers significantly free from potato scab. Suppressive, virgin soil has been used, for example, to control *Phytophthora* root rot of papaya by planting papaya seedlings in suppressive soil placed in holes in the orchard soil, which was infested with the root rot oomycete *Phytophthora palmivora*. However, in several diseases, continuous cultivation (monoculture) of the same crop in a conducive soil, after some years of severe disease, eventually leads to reduction in disease through increased populations of microorganisms antagonistic to the pathogen. For example, continuous cultivation of wheat or cucumber leads to reduction of take-all of wheat and of *Rhizoctonia* damping-off of cucumber, respectively. Similarly, continuous cropping of the watermelon variety ‘Crimson Sweet’ allows the buildup of antagonistic species of *Fusarium* related to that causing *Fusarium* wilt of watermelon with the result that *Fusarium* wilt is reduced rather than increased. Such soils are suppressive to future disease development. That suppressiveness is due to antagonistic microflora can be shown by pasteurization of the soil at 60°C for 30 minutes, which completely eliminates the suppressiveness.

A sort of “soil suppressiveness” develops after appropriate crops are plowed under as soil amendments. Such crops, usually in the crucifer family, provide material and the time required for biological destruction of pathogen inoculum by resident antagonists in the soil. For example, significant control of lettuce drop, caused by the fungus *Sclerotinia sclerotiorum*, occurs when broccoli plants have been incorporated in the soil compared to the amount of disease in fields not receiving such treatment (Agrios, 2005)

### 2.1.2. Biofumigation or biodisinfection

Better adapted to cooler regions of the world, biological soil disinfection is based on plastic tapping of the soil after incorporation of fresh organic matter (Blok *et al*., 2000). The mechanisms involved in this newly developed technique are not totally understood. Fermentation of organic matter in soil under plastic results in the production of toxic metabolites and anaerobic conditions which both contribute to the inactivation or destruction of pathogenic fungi. Based on the dominant type of mechanisms involved, Lamers *et al*. (2004) proposed the distinction between (i) biofumigation that corresponds to the use of specific plant species containing identified toxic molecules, and (ii) biodisinfection which refers to the use of high quantities of organic matter resulting in anaerobic conditions mainly responsible for the destruction of the pathogens. Many species of the *Brassicaceae* (*Cruciferae*) family contain glucosinolates, a class of organic molecules that can be hydrolyzed by a group of similar-acting enzymes (myrosinases) in toxic compounds such as isothiocyanates. These compounds, analogous to some chemical fumigants act as biocides in controlling various soilborne plant pathogens (Lawrence and Matthiessen, 2004). Traditionally, to avoid problems when brassicas are used as feed for livestock, plant breeders have selected varieties with reduced levels of glucosinolates. On the contrary, cultivars of *Brassicaceae* with a high content in glucosinolates have now been created. Some of them are already available on the market, specifically for biofumigation.

Plants belonging to the *Alliacae* family also contain molecules with either a direct or an indirect effect on pests and pathogens. Degradation of garlic, onion, and leek tissues releases sulphur volatiles such as thiosulfinates and zwiebelanes which are converted into disulfides having biocidal activities against fungi, nematodes and arthropods (Arnault *et al*., 2004). In addition to the effects of these toxic compounds, incorporation of high rates of organic matter in soil followed by plastic tarping result in anaerobic conditions that are also deleterious to many pests and pathogens which need aerobic conditions to survive. Block *et al*. (2000) reported a drastic reduction in the population density of *F. oxysporum f.sp. asparagi* and *R. solani* after addition to soil of either ryegrass or cabbage. These promising methods need to be implemented under various situations to define their conditions of use, both their benefits and their limits. But their use will probably require some changes in the cropping sequence, since the land will not be available for cropping for several weeks during the year. However, in our opinion, these methods will gain popularity since disinfection with methylbromide has now been banned.

### 2.1.3. Biopesticides

Plant diseases cause considerable losses in crop production and storage. Nowadays, growers still rely heavily on chemical pesticides to prevent, or control these diseases. However, the high effectiveness and ease of utilization of these chemicals can result in environmental contamination and the presence of pesticide residues on food, in addition to social and economic problems. Consequently, there is an increasing demand from consumers and officials to reduce the use of chemical pesticides. In this context, biological control through the use of natural antagonistic microorganisms has emerged as a promising alternative. Biopesticide is a mass-produced, biologically based agent manufactured from a living microorganism or a natural product and which is sold for the control of plant pests. The agents used as biopesticides are usually broken down into three categories: (i) microorganisms (bacteria, fungi, oomycetes, and viruses); and (ii) biochemical’s (which include plant products such as essential oils, and various compounds synthesized by other organisms such as chitin and chitosan. Indeed, these biopesticides present many advantages in term of sustainability, mode of action and toxicity compared to chemical pesticides.

#### 2.1.3.1. Interest in the development of biopesticides

As all living organisms, plants must face infections and diseases following the attacks of a mass of plant pathogens and pests from animal, microbial or viral origin. These diseases can be minor causing solely a reduction of plant-growth capacities or can be at the origin of much more severe damage leading to plant death in the worst case. Plant diseases are responsible for the loss of at least 10% of global food production, representing a threat to food security (Strange and Scott, 2005). Agrios (2005) estimated that annual losses caused by disease cost US$ 220 billion. Worldwide, plant diseases were responsible for severe famines in the past (Agrios, 2005). For example, potato blight caused by the plant pathogenic oomycete *Phytophthora infestans* on potato cultures caused more than one million deaths in Ireland during the "the great famine" between 1845 and 1849 (O'Neill, 2009).

To prevent or control these diseases, producers have become increasingly dependent on agrochemicals, especially over the past few decades, as agricultural production has intensified. However, despite the great effectiveness and ease of utilization of these products, their use or misuse has caused many problems including significant pollution of soils and ground water reservoirs, accumulation of undesirable chemical residues in the food chain, emergence of fungicide-resistant strains of pathogens, not to mention health concerns for growers. According to the Stockholm convention on persistent organic pollutants, 10 of the 12 most dangerous and persistent organic chemicals are pesticides (Gilden *et al*., 2010). An example is the synthetic pesticide dichlorodiphenyltrichloroethane, well known as DDT, which was extensively used in agriculture between 1950 and 1980 and was found genotoxic in human and responsible for endocrine disorders (Cohn *et al*., 2007). Consequently, there is nowadays an increasing demand from consumers and authorities for more safe, rational, sustainable and eco-friendly strategies. This has resulted not only in stricter regulations concerning pesticide use, commercialization and production but also in the development of alternative strategies including genetic adaptation of crops, modification of cultural practices and use of biopesticides.

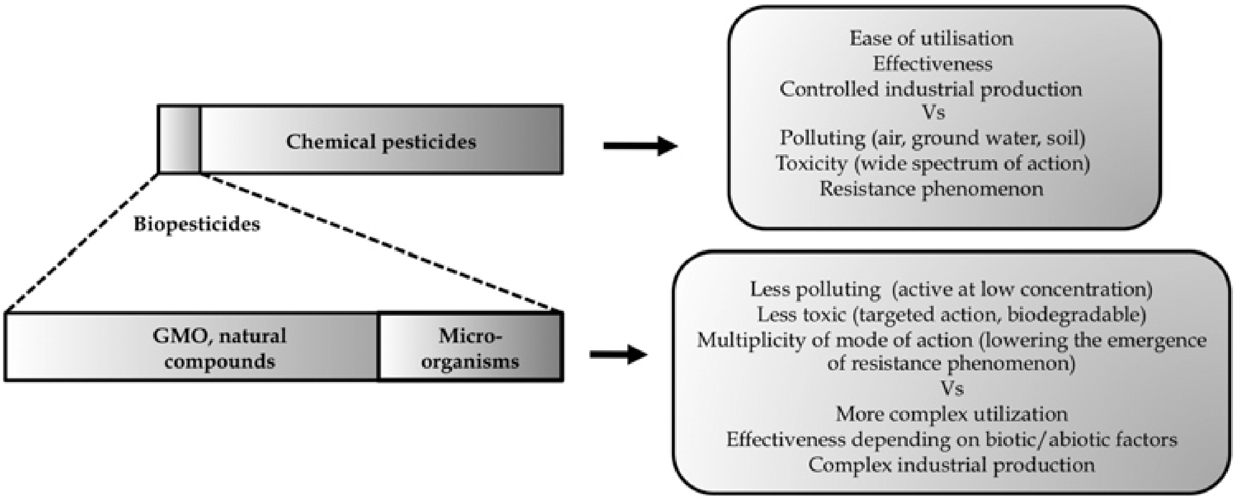

Fig.1. Market share and (dis)advantages of microbial biopesticides versus chemical phytosanitary products.

- Source: (Helene *et al*., 2011)

### 2.1.3.2. Advantages and marketshare of biopesticides

Biopesticides, which are used to suppress pathogen populations, are living organisms or natural products derived from these organisms. They can be divided into four main groups: microorganisms (microbial pesticides), other organisms (nematodes, insects…) used to control pests, natural substances that are derived from living organisms (biochemical pesticides) and plant-incorporated protectants (genetically modified plants) (EPA, 2011). Biopesticides show several advantages when compared to chemical products. They decompose more quickly in the environment and are generally less toxic towards non-target species (Thakore, 2006). Additionally, their modes of actions are usually distinct from those of conventional pesticides. This implies that they can often help suppress resistant pathogens and that they can be applied in alternation with other pesticides to avoid resistance development.

Among biopesticides, microorganism-based products represent about 30% of total sales and have a variety of applications. They are used in field crops and greenhouses to reduce diseases on various cereals, legumes, fruits, flowers and ornamental plants caused either by soil-borne, foliar or post-harvest pathogens. These plant protective microorganisms, mainly fungi and bacteria, are often isolated from suppressive environments. In other words, these beneficial microorganisms are generally obtained from aerial or underground parts of plants that are naturally less or not at all affected by a pathogen that devastates a neighboring group of the same plant species (Ryan *et al*., 2009). One of the advantages of microbial biopesticides compared to most other phytosanitary products is the multiplicity of their ways of actions globally based on competition for nutrients and space, direct antagonism of plant pathogen growth and host plant immunization. Compared to genetically modified organisms (GMOs), microbial pesticides benefit from a better consumer acceptance. In Europe, there are also several legal barriers against GMOs. In comparison with natural extracts, microbial pesticides often retain the advantage of having a persistent activity through time. Indeed, microbial agents can establish themselves in the phytosphere and produce continuously bioactive compounds *in situ.* Moreover, as these active molecules are produced in direct contact or very close to the target organisms, only limited quantities are needed for efficacy.

In addition to their potential to directly reduce the incidence of diseases, some microbial products also have other positive effects on crops such as promoting plant growth and nutrition (biofertilizers and phyto-stimulators) and/or facilitating interaction between the host plant and other beneficial organisms (Antoun and Prevost, 2006). A large amount of nutrients present in the soil are in an insoluble form that is unavailable for the crops (Francis *et al*., 2010). Biofertilizers act trough the direct improvement of plant nutrition either by solubilizing these nutrients or by fixing atmospheric $N_2$. In the case of solubilization, several mechanisms may be involved depending on the nature of the nutrient. For example, phosphate can be released from insoluble organic forms by several microbial enzymes like phytases or non-specific phosphatases, while inorganic phosphorus stocks are solubilized through the production of organic acids by the beneficial bacteria. Phytostimulation is the direct promotion of plant growth through the modulation of the plant's hormonal balance. Several microorganisms are capable to produce and excrete a variety of plant hormone-like compounds including auxin, gibberellins, cytokinins etc. Some microbial agents produce enzymes that degrade a precursor of ethylene thus limiting the levels of this hormone in the plant thereby increasing plant growth especially under stress conditions (Francis *et al*., 2010; Lugtenberg and Kamilova, 2009). Both bio-fertilization and phyto-stimulation are important phenomena in the context of the constant need to produce more food on fewer surfaces with the simultaneous wish to reduce reliance on chemical fertilizers. Moreover, a microorganism that possesses a combination of these growth-promoting activities and biocontrol potential offers the advantage to supply the crop in one application with a biopesticide and a bio-fertilizer. In addition, better nutrition of the plant often enhances its overall resistance against pathogens and other stress factors (Bent, 2006).

### 2.1.3.3. Microbial control of plant pathogens

Plant pathogens are controlled naturally to some degree by a range of microorganisms, including fungi, bacteria and viruses. Some of these are being used for biological control using augmentation, classical and conservation strategies. The target plant pathogens include fungi, oomycetes, bacteria, viruses and plant parasitic nematodes. The development of microbial biopesticides of plant pathogens is being driven by the increasing withdrawal of synthetic fungicides following government reviews of their safety, but equally important is the worldwide ban on the use of methyl bromide, which was used widely as a soil sterilant but is being withdrawn because it contributes to depletion of ozone in the atmosphere. The commercialization of microbial biopesticides as control agents of plant pathogens and plant parasitic nematodes is a relatively young endeavor; effective products for disease control have only become commercially available to any extent since the mid-1990s (Whipps and Davies, 2000). In 2000, around 80 products were on sale or close to market (Whipps and Davies, 2000).

The microorganisms exploited for plant disease biocontrol have a wide range of modes of action. There are two broad classes. Microbial antagonists occupy the same ecological niche as the target plant pathogen and interact directly with it. The mechanisms of interaction include parasitism, competition for space, water or food, or 'chemical warfare' using antibiotics or other secondary metabolites that harm the target pathogen. The second class involves an indirect effect in which the control agent induces a resistance response in the plant that gives it protection against virulent plant pathogens. The 'inducer' for this form of control may use a particular strain of the plant pathogen that has low virulence, a different species of microorganism or a natural product, as well as the plant itself. This is very different from the microbial control approach used against insects, which currently relies exclusively on using virulent parasites to directly kill insect pests.

Many microbial antagonists of plant pathogens have more than one way of restricting the development of a target pest. A number of species of the fungal control agent *Trichoderma*, for example, are used against soil-borne plant pathogenic fungi. *Trichoderma* species are able to parasitize plant pathogenic fungi in the soil, they also produce antibiotics and fungal cell-wall-degrading enzymes, they compete with soil-borne pathogens for carbon, nitrogen and other factors, and they can also promote plant growth, possibly by the production of auxin-like compounds (Vinale *et al*., 2008). *Trichoderma* is a common soil fungus and naturally grows in the rhizosphere. Multiple modes of action confer many benefits in terms of disease control, because *Trichoderma* gives good control in a range of conditions. However, it can create problems for the authorities that have to regulate its production and use. Many *Trichoderma* products have been sold on the basis of their plant growth-promoting properties, rather than as plant protection products, and so have escaped scrutiny from regulators in terms of their safety and efficacy.

At the microbial scale, plants present a very diverse set of environments for the microorganisms that are associated with them. The environmental conditions on the leaf surface are very different to those in the root zone, for example. The leaf surface is devoid of many microorganisms as conditions are not conducive to growth and survival. Water and nutrients are in scarce supply, while low humidity and high levels of ultraviolet radiation limit the germination of fungal and bacterial spores. In contrast, the root zone has freely available water and is bathed in large amounts of readily utilizable carbon secreted by root cells. As a result, there are large populations of taxonomically diverse microorganisms inhabiting the root zone and competing for resources. It is critical, therefore, that the ecology of the plant pathogen is understood in detail if biocontrol is to be successful.

#### *2.1.3.3.1. Microbial antagonists*

A number of microbial antagonists are being used as commercial products against plant pathogenic fungi and oomycetes. Microbial control products have been developed for use against soil-borne plant pathogens and pathogens that infect the above-ground parts of plants. The most widely used fungal control agents in the soil are species of *Trichoderma*, such as *Trichoderma harzianum*, which is an antagonist of *Rhizoctonia*, *Pythium*, *Fusarium* and other soil-borne pathogens (Harman, 2005). *Trichoderma* is a parasite of a range of fungi and oomycetes in the soil, but it also inhibits the growth of other organisms by the production of toxic metabolites and cell-wall-degrading enzymes. Specific recognition reactions between parasite and host mediate the release of antimicrobial metabolites by the parasite. Other fungal parasites and antagonists include *Gliocladium virens* and *Coniothyrium minitans*. The latter is applied to the soil to kill *Sclerotinia sclerotiorum*, an important disease of many agricultural and horticultural crops such as oilseed rape, lettuce, carrots, beans and brassicas (Whipps *et al.*, 2008). Bacterial agents can also be used for control of soil-borne diseases. Crown gall, caused by *Agrobacterium tumefaciens*, is a serious disease of a wide range of dicotyledonous plants including pome fruits, vines, ornamentals and vegetables. Bacterial infection causes the formation of tumours in root tissue. Seed and seedlings can be treated with the K84 strain of the non-pathogenic species *Agrobacterium radiobacter*. K84 colonizes root tissues and prevents occupation by *A. tumefaciens*, using an antibiotic (Penalver *et al.*, 1994). Specific strains of *Bacillus subtilis* can also confer protection against some root pathogens, while a number of *Pseudomonas* species, including *Pseudomonas fluorescens* and *Pseudomonas aureofaciens*, reduce damping off and soft rots (Choudhary and Johri, 2009).

Fungal antagonists used against pathogens that infect leaves and stems include: *Lecanicillium*, which is primarily an insect pathogenic fungus, but some strains have activity also against other fungi; *Ampelomyces quisqualis*, which is used against mildews; and *Nectria inventa* and *Gonatobotrys simplex*, which are parasites of *Alternaria* (Kiss *et al.*, 2004). The fungus *Phlebiopsis gigantea* is used to control *Heterobasidion annosum*, a fungal pathogen that causes rots in freshly cut stumps of pine trees and which can spread subsequently to intact trees by root-to-root contact. *Phlebiopsis* spores are painted on to tree stumps or are incorporated in the lubricating oil used in chainsaws. The fungus occupies the same tissues as *Heterobasidion* and outcompetes it, but causes no damage to the trees (Pratt *et al.*, 1999).

There are also a number of bacterial species that are used as control agents of plant pathogens infecting above-ground parts of plants. Species of *Bacillus*, *Pseudomonas* and *Streptomyces* can prevent colonization of leaf and stem tissue by plant pathogens (Berg, 2009). The activity of these agents is often due to antibiosis brought about through the action of bacterial secondary metabolites. Usually, several kinds of secondary metabolites are produced. Production of metabolites is strain dependent, i.e. different strains of the same species of bacterium can produce different types of metabolites with different effects on target pathogens. Strain selection is therefore a critical part of developing bacterial agents as biopesticides.

Microbial antagonists can be formulated as dusts, granules or liquid suspensions for application to soil, either directly to the roots of plants or in the soil ahead of planting. Antagonists used on leaves, stems or harvested fruit are usually applied as conventional sprays. However, novel application systems are also being developed. Honeybees, used commercially for pollination of blueberries, transport the plant pathogenic fungus *Monilinia vaccinii-corymbosi* between blueberry flowers, leading to berry disease. However, the risk of the disease can be reduced significantly by using the bees as ‘flying doctors’ and treating them with the bacterial biopesticide *B. subtilis*, which is dispensed from a device fitted to the entrance of bee hives and which the bees vector to blueberry flowers (Dedej *et al.*, 2004).

Microbial antagonists are also used as control agents of postharvest diseases, mainly against the causal agents of rots in harvested fruits and vegetables. Yeasts, filamentous fungi and bacteria have all been used (Spadaro and Gullino, 2004). Mode of action is not always clear, although competition with the pathogen for space and nutrients is thought to be important, alongside antibiosis. Sharma *et al*. (2009) list over 40 species of microbial antagonists that have been demonstrated in experiments to give successful control of postharvest disease of fruits and vegetables. Common target pathogens in these experimental programmes included *Botrytis cinerea*, *Penicillium* species (e.g. *Penicillium digitatum*, *Penicillium expansum*, *Penicillium italicum*) and *Mucor piriformis*. At present, however, only nine products are available commercially across the world (Sharma *et al*., 2009). Of these, the most widely used are based on *A. quisqualis* and *B. subtilis*. Application of the control agent may be made pre-harvest, to combat latent infections acquired in the field, although it is not considered a commercially viable strategy. Postharvest application is more practical, and the inoculum is usually applied as sprays or as a dip.

Developers of microbial biopesticides of plant pathogens have tended, quite understandably, to concentrate on species of microbial antagonists that are easy to culture and mass-produce, although it has been pointed out (Alabouvette *et al*., 2006) that if commercialization of these agents is done according to a chemical pesticide model, without proper consideration of the ecological interactions involving the control agent, the target pathogen, the crop plant and the environment, then poor or inconsistent levels of control are certain to occur, which would be damaging to the whole concept of biological control.

### 2.1.3.4. Microbial control of plant parasitic nematodes

Plant parasitic nematodes are susceptible to fungal and bacterial pathogens, a small number of which are available as commercial biopesticides. Nematophagous fungi include species that trap motile nematodes in the rhizosphere using specialized hyphal organs, such as *Arthrobotrys oligospora* and *Arthrobotrys dactyloides*. Endoparasitic fungi, such as *Pochonia chlamydosporia* (= *Verticillium chlamydosporium*) are able to infect female cyst nematodes and their eggs (Kerry, 2000). *Pochonia chlamydosporia* can be mass-produced *in vitro*, and some strains are able to grow saprotrophically within the rhizosphere, making it a potentially valuable augmentation biopesticide. However, the development of microbial control agents of plant parasitic nematodes appears to be relatively slow. Dong and Zhang (2006) list only nine products that have been commercialized based on six different fungal or bacterial species. They attribute this lack of products partly to inconsistent performance in the field, and it is likely that product development is being held back by a lack of knowledge of the complex interactions that occur between nematode, control agent, the plant and the soil, and in particular the rhizosphere.

### 2.1.3.5. Natural Compounds

Biopesticide is a term that includes many aspects of pest control such as microbial (viral, bacterial and fungal) organisms, entomophagous nematodes, plant-derived pesticides (botanicals), secondary metabolites from micro-organisms (antibiotics), insect pheromones applied for mating disruption, monitoring or lure and kill strategies and genes used to transform crops to express resistance to insect, fungal and viral attacks or to render them tolerant of herbicide application (Copping and Menn, 2000). Botanicals include crude extracts and isolated or purified compounds from various plants species and commercial products (Liu *et al*., 2006). Not unlike pyrethrum, rotenone and neem, plant essential oils or the plants from which they are obtained have been used for centuries to protect stored commodities or to repel pests from human habitations and use as fragrances, condiments or spices, as well as medicinal uses (Isman and Machial, 2006). Quantitatively, the most important botanical is pyrethrum, followed by neem, rotenone and essential oils, typical used as insecticides (e.g. pyrethrum, rotenone, rape seed oil, quassia extract, neem oil, nicotine), repellents (e.g. citronella), fungicides (e.g. laminarine, fennel oil, lecithine), herbicides (e.g. pine oil), sprouting inhibitors (e.g. caravay seed oil) and adjuvants such as stickers and spreaders (e.g. pine oil) (Isman, 2006). Plants are capable of synthesizing an overwhelming variety of small organic molecules called secondary metabolites, usually with very complex and unique carbon skeleton structures (Sarker *et al*., 2005). By definition, secondary metabolites are not essential for the growth and development of a plant but rather are required for the interaction of plants with their environment (Kutchan and Dixon, 2005).The biosynthesis of several secondary metabolites is constitutive, whereas in many plants it can be induced and enhanced by biological stress conditions, such as wounding or infection (Wink, 2006). They represent a large reservoir of chemical structures with biological activity. It has been estimated that 14 - 28% of higher plant species are used medicinally and that 74% of pharmacologically active plant derived components were discovered after following up on the ethnomedicinal uses of the plants (Ncube *et al*., 2008). Plants and their secondary metabolites are an important source for biopesticides and the development of new pesticides. The recognition of the important role of these compounds has increased, particularly in terms of resistance to pests and diseases. The intensive use of synthetic pesticides and their environmental and toxicological risks have generated increased global interest to develop alternative sources of chemicals to be used in safe management of plant pests. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutics for plant protection because they are mostly non phytotoxic and easily biodegradable (Isman, 2006).

Currently, different botanicals have been formulated for large scale application as biopesticides in eco-friendly management of plant pests and are being used as alternatives to synthetic pesticides in crop protection. These products have low mammalian toxicity and are cost effective. Such products of higher plant origin may be exploited as eco-chemical and biorational approach in integrated plant protection programs (Dubey *et al*., 2009). In order to increase food safety and develop integrated and sustainable strategies for plant protection, which are safe to the consumer, producer and the environment, the use of natural pesticide need to be promoted.

### 2.1.3.6. Biopesticides and Integrated Pest Management

Biopesticides thus play an important role and are legally accepted for use in integrated pest management and organic agriculture. According to the US Environmental Protection Agency (EPA), Integrated Pest Management (IPM) is an effective and environmentally sensitive approach that relies on a combination of common-sense practices (EPA, 2011). IPM programs use current, comprehensive information on the life cycles of pests and on their interaction with the environment. This information, in combination with available pest control methods, is used to manage pest damage by the most economical means, and with the least possible hazard to people, property, and the environment. IPM may involve a judicious use of pesticides by contrast with organic food production that applies many of the same concepts as IPM but limits the use of pesticides to those that are produced from natural sources, as opposed to chemicals.

An example of integration of alternative/biological methods in IPM is given here for the control of lily diseases and pests. This program was developed in a company specialized in the cultivation of lily, located in Holambra, SP, Brazil, with a history of intensive use of fungicides, insecticides and miticides. Phytosanitary problems in lily culture of high value, limit its cultivation. Diseases may originate from several agents such as the fungi/oomycetes *Botrytis elliptica, Phytophthora, Fusarium, Sclerotinia, Penicillium, Rhizoctonia* and *Pythium* or pests such as aphids, fungus gnats, leaf miners, thrips and caterpillars. To solve these problems, over 30 different chemical pesticides had to be used routinely at a cost of US$ 10.00/m$^2$/year in a cultivated area of 13,500 m$^2$. For these products to keep working properly, growers needed to use increasingly higher doses and more toxic products, but losses due to pests and diseases kept increasing. Facing such a situation, the decision was made to change the production system. To achieve integrated control of cultural problems, the use of chemical pesticides was gradually replaced by the integration of biocompatible methods to control pests and diseases like introducing a diversity of microorganisms for biocontrol. Along with this substitution of chemical pesticides, an adaptation of fertilization procedures was needed to improve the survival of the biocontrol agents. The first step was to stop using the most toxic pesticides which took about two years. One additional year was required to successfully replace the use of chemical pesticides of less toxic levels. In general, the current production is based on the treatment of a steam-disinfested substrate with aerobic compost tea and beneficial microorganisms such as *Trichoderma*, *Metarhizium*, *Beauveria* and *Bacillus*. *Clonostachys rosea* and *Trichoderma* sp. are sprayed weekly to control *Botrytis* and other pathogens. When necessary, neem oil, propolis, phosphite and others alternative products are used. Associated with these products and with balanced fertilization, a sanitation program is maintained in all the greenhouses with the elimination of diseased plants or plant's parts. Also, traps and monitors for controlling the relative humidity in greenhouses are used.

Currently, no chemical pesticides are used, except for bulbs, which are treated with imidaclopride before planting to control aphids, in order to comply with phytosanitary standards for exportation. The success is due, not only to the substitution of chemical pesticides by biopesticides and biocompatible products, but also by reconsidering the entire production system. The same strategy is used for the control of disease on *Spathiphyllum*, avoiding any chemical pesticide input and involving *Bacillus subtilis* for the control of *Cylindrocladium spathiphylli* (Wit *et al*., 2009).

## 2.2. Mechanisms of Biological Control Agents

Because biological control can result from many different types of interactions between organisms, researchers have focused on characterizing the mechanisms operating in different experimental situations. In all cases, pathogens are antagonized by the presence and activities of other organisms that they encounter. The different mechanisms of antagonism occur across a spectrum of directionality related to the amount of interspecies contact and specificity of the interactions (Table 1). Direct antagonism results from physical contact and/or a high-degree of selectivity for the pathogen by the mechanism(s) expressed by the BCA(s). In such a scheme, hyper parasitism by obligate parasites of a plant pathogen would be considered the most direct type of antagonism because the activities of no other organism would be required to exert a suppressive effect. In contrast, indirect antagonisms result from activities that do not involve sensing or targeting a pathogen by the BCA(s). Stimulation of plant host defense pathways by non-pathogenic BCAs is 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 biocontrol to particular pathosystems, 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, *Pseudomonads* known to produce the antibiotic 2, 4-diacetylphloroglucinol (DAPG) may also induce host defenses (Iavicoli *et al*. 2003). Additionally, DAPG-producers can aggressively colonize roots, a trait that might further contribute to their ability to suppress pathogen activity in the rhizosphere of wheat through competition for organic nutrients (Raaijmakers and Weller 2001).

Table 1. Types of interspecies antagonisms leading to biological control of plant pathogens.

| **Type** | **Mechanism** | **Examples** |
|---|---|---|
| Direct antagonism | Hyperparasitism/predation | Lytic/some nonlytic mycoviruses<br>*Ampelomyces quisqualis*<br>*Lysobacter enzymogenes*<br>*Pasteuria penetrans*<br>*Trichoderma virens* |
| Mixed-path antagonism | Antibiotics | 2,4-diacetylphloroglucinol<br>Phenazines and Cyclic lipopeptides |
| | Lytic enzymes | Chitinases,Glucanases and Proteases |
| | Unregulated waste products | Ammonia, Carbon dioxide and Hydrogen cyanide |
| | Physical/chemical interference | Blockage of soil pores<br>Germination signals consumption<br>Molecular cross-talk confused |
| Indirect antagonism | Competition | Exudates/leachates consumption<br>Siderophore scavenging<br>Physical niche occupation |
| | Induction of host resistance | Contact with fungal cell walls<br>Detection of pathogen-associated, molecular patterns<br>Phytohormone-mediated induction |

Source: (Pal and McSpadden, 2006)

### 2.2.1. Hyperparasitism and predation

In this mechanism the pathogen is directly attacked by a specific biocontrol agent (BCA) that kills it or its propagules. In general, there are four major classes of hyperparasites: obligate bacterial pathogens, hypoviruses, facultative parasites, and predators. A classical example of *Pasteuria penetrans* is an obligate bacterial pathogen of root-knot nematodes that is used as a BCA. Hypoviruses are hyperparasites; a classical example is the virus that infects *Cryphonectria parasitica*, a fungus causing chestnut blight, which causes hypovirulence, a reduction in disease-producing capacity of the pathogen. The phenomenon has controlled the chestnut blight in many places (Tjamos *et al*., 2010). However, the interaction of virus, fungus, tree, and environment determines the success or failure of hypovirulence. There are several fungal parasites of plant pathogens, including those that attack sclerotia (*i.e. Coniothyrium minitans*) while others attack living hyphae (*i.e. Pythium oligandrum*) and, a single fungal pathogen can be attacked by multiple hyperparasites. For example, *Acremonium alternatum, Acrodontium crateriforme, Ampelomyces quisqualis, Cladosporium oxysporum*, and *Gliocladium virens* are just a few of the fungi that have the capacity to parasitize powdery mildew pathogens (Heydari and Pessarakli, 2010). Other hyperparasites attack plant-pathogenic nematodes during different stages of their life cycles (*i.e. Paecilomyces lilacinus and Dactylella oviparasitica*). In contrast to hyperparasitism, microbial predation is more general and pathogen non-specific and generally provides less predictable levels of disease control. Some BCAs exhibit predatory behavior under nutrient-limited conditions. However, such activity generally is not expressed under typical growing conditions. For example, some species of *Trichoderma* produce a range of enzymes that are directed against cell walls of fungi. However, when fresh bark is used in composts, *Trichoderma* spp. do not directly attack the plant pathogen, *Rhizoctonia solani*. But in decomposing bark, the concentration of readily available cellulose decreases and this activates the chitinase genes of *Trichoderma* spp., which in turn produce chitinase to parasitize *R. solan* (Sharma and Bhat, 2011).

### 2.2.2. Antibiotic-mediated suppression

Antibiotics are microbial toxins that worked at low concentrations, poison or kill other microorganisms. Most microbes produce and secrete one or more compounds with antibiotic activity (Islam *et al*., 2005). In some instances, antibiotics produced by microorganisms have been shown to be particularly effective at suppressing plant pathogens and the diseases they cause. Some examples of antibiotics reported to be involved in plant pathogen suppression are listed in Table 2. In all cases, the antibiotics have been shown to be particularly effective at suppressing growth of the target pathogen *in vitro* and/or *in situ*. To be effective, antibiotics must be produced in sufficient quantities near the pathogen to result in a biocontrol effect. In situ production of antibiotics by several different biocontrol agents has been measured (Thomashow *et al*., 2002). However, the effective quantities are difficult to estimate because of the small quantities produced relative to the other, less toxic, organic compounds present in the phytosphere and several methods have been developed to ascertain when and where biocontrol agents may produce antibiotics detecting expression in the infection court is difficult because of the heterogenous distribution of plant-associated microbes and the potential sites of infection. In a few cases, the relative importance of antibiotic production by biocontrol bacteria has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. For example, mutant strains incapable of producing phenazines (Thomashow and weller, 1988) or phloroglucinols (Keel *et al*., 1989) have been shown to be equally capable of colonizing the rhizosphere but much less capable of suppressing soil borne root diseases than the corresponding wild type and complemented mutant strains. Several biocontrol strains are known to produce multiple antibiotics which can suppress one or more pathogens. For example, *Bacillus cereus* strain UW85 is known to produce both zwittermycin and kanosamine. The ability to produce multiple classes of antibiotics, that differentially inhibit different pathogens, is likely to enhance biological control. 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). Selective examples of BCA's given below are particularly effective. *Pseudomonas fluorescens* F113 produces 2, 4-diacetyl-phloroglucinol against *Pythium* spp. *Agrobacterium radiobacte*r produces agrocin 84, against *Agrobacterium tumefaciens* (Kerr, 1980). *Bacillus subtilis* QST713 produces iturin A against *Botrytis cinerea* and *R. solani* (Paulitz and Belanger, 2001). *B. subtilis* BBG100 produces mycosubtilin against *Pythium aphanidermatum* (Leclere *et al*., 2005). *B. amyloliquefaciens* FZB42 produces bacillomycin and fengycin against *Fusarium oxysporum* (Koumoutsi *et al*., 2004). *Pseudomonas fluorescen*s 2-79 and 30-84 produce phenazines against *Gaeumannomyces graminis* var. *tritici, Trichoderma virens* produces gliotoxin against *Rhizoctonia solani* (Wilhite *et al*., 2001).

Table 2.Mechanisms of specific biocontrol agents for controlling plant pathogens

| **Antibiotic** | **Source** | **Target pathogen** | **Disease** | **Reference** |
|---|---|---|---|---|
| 2, 4-diacetylphloroglucinol | *Pseudomonas fluorescens* F113 | *Pythium spp.* | Damping off | Shanahan *et al.* (1992) |
| Agrocin 84 | *Agrobacterium Radiobacter* | *Agrobacterium tumefaciens* | Crown gall | Kerr (1980) |
| Bacillomycin D | *Bacillus subtilis* AU195 | *Aspergillus flavus* | Aflatoxin contamination | Moyne *et al.* (2001) |
| Bacillomycin, fengycin | *Bacillus amyloliquefaciens* FZB42 | *Fusarium oxysporum* | Wilt | Koumoutsi *et al.* (2004) |
| Xanthobaccin A | *Lysobacter* sp. strain SB-K88 | *Aphanomyces cochlioides* | Damping off | Islam *et al.* (2005) |
| Gliotoxin | *Trichoderma Virens* | *Rhizoctonia solani* | Root rots | Wilhite *et al.* (2001) |
| Herbicolin | *Pantoea agglomerans* C9-1 | *Erwinia amylovora* | Fire blight | Sandra *et al.* (2001) |
| Iturin A | *B.subtilis* QST713 | *Botrytis cinerea* and *R. solani* | Damping off | Kloepper *et al.* (2004) |
| Mycosubtilin | *B. subtilis* BBG100 | *Pythium aphanidermatum* | Damping off | Leclere *et al*.(2005) |
| Phenazines | *P. fluorescens* 2-79 and 30-84 | *Gaeumannomyces Graminis* var. *tritici* | Take-all | Thomashow *et al.* (1990) |
| Pyoluteorin, pyrrolnitrin | *P. fluorescens* Pf-5 | *Pythium ultimum* and *R. solani* | Damping off | Howell and Stipanovic (1980) |
| Pyrrolnitrin, pseudane | *Burkholderia Cepacia* | *R. solani* and *Pyricularia oryzae* | Damping off and rice blast | Homma *et al.* (1989) |
| Zwittermicin A | *Bacillus cereus* UW85 | *Phytophthora medicaginis* and *P.aphanidermatum* | Damping off | Smith *et al.* (1993) |

(Source: Pal and McSpadden, 2006)

### 2.2.3. Cell wall degrading enzymes

Several biological control agents (BCA) produce enzymes able to hydrolyze chitin, proteins, cellulose, and hemicellulose, thus contributing to direct suppression of plant pathogens. There are selective examples of BCA's able to produce enzymes, effective against certain plant pathogens. *Serratia marcescens* chitinases and genes encoding them have been shown to have biocontrol potential in a variety of experiments. A highly chitinolytic strain of *S. marcescens* was found to suppress the growth of *Botrytis spp, Rhizoctonia solani*, and *Fusarium oxysporum* (Ningaraju, 2006). However, such activities are rather indicative of the need to obtain carbon nutrition. *Lysobacter* and *Myxobacteria* are known to produce plentiful amounts of lytic enzymes, and some isolates have been shown to be effective at suppressing fungal plant pathogens (Bull *et al.*, 2002). So, the lines between competition, hyperparasitism, and antibiosis are generally disguised.

### 2.2.4. Competition for space and nutrient

Although difficult to be proven directly, much indirect evidence suggests that competition between pathogens and non-pathogens for nutrient resources is important for restricting disease incidence and severity. Soil-borne pathogens, such as species of *Fusarium* and *Pythium*, infecting through mycelial contact, are more susceptible to competition by other soil and plant-associated microbes than by those germinating directly on plant surfaces which they invade through appressoria and infection pegs. Rhizosphere or phyllosphere biological control agents (BCA) generally protect the plant by rapid colonization, thus consuming completely the limited available substrates so that none is left 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). At the same time, these microbes produce metabolites that suppress pathogens. These microbes colonize the sites where water and carboncontaining nutrients are most readily available and utilize root mucilage. To survive in such an environment, microorganisms secrete iron-binding ligands called siderophores that sequester iron from the microenvironment. Biocontrol based on competition for essential micronutrients, such as iron, has also been examined 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 (Kloepper *et al*., 1980).

### 2.2.5. Induced resistance

Plants respond to a variety of chemical stimuli produced by biological control agents (BCA), such stimuli can either induce host plant defenses through biochemical changes expressing resistance mechanisms against subsequent infection by pathogens. Induction of host defenses can be localized and/or systemic in nature. The determinants and pathways of induced resistance stimulated by BCA's and other non-pathogenic microbes have being occasionally characterized. The first of these pathways, called systemic acquired resistance (SAR), is mediated by salicylic acid (SA), which typically leads to the expression of pathogenesis-related (PR) proteins including a variety of enzymes. A second case, referred to as induced systemic resistance (ISR), is mediated by jasmonic acid (JA) and/or ethylene, which are produced following applications of some non-pathogenic rhizobacteria. Some most striking examples of bacterial determinants and types of disease resistance (ISR) induced by BCA's include a *Bacillus mycoide*s strain able to produce peroxidase, chitinase and β-1,3-glucanase in sugar beet (Bargabus *et al*., 2003). *B. subtilis* GB03 and IN937 producing 2, 3-butanediol in *Arabidopsis* (Ryu *et al*., 2004). *Pseudomonas putida* strains producing a lipopolysaccharide in *Arabidopsis* (Meziane *et al*., 2005). *Serratia marcescens* 90-166 producing siderophore in cucumber (Press *et al*., 2001). 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 T*richoderma sp*. are known to strongly induce host plant defences. 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 *et al*., 1998).

### 2.2.6. Plant Growth Promoting Rhizobacteria (PGPR)

Several PGPR bioinoculants are currently used commercially. They are called different names and operate through a range of mechanisms: (i) bioprotectants, through suppression of plant disease (ii) biofertilizers, through improved nutrient acquisition (iii) biostimulants, through phytohormone production. Bioinoculants include bacteria belonging in the genera *Bacillus, Paenibacillus Streptomyces, Pseudomonas*, *Burkholderia and Agrobacterium*, which are currently used as BCA's also at commercial level. They suppress plant disease through induction of systemic resistance, production of siderophores or antibiotics. Biofertilizers are also available for increasing crop uptake of nitrogen from nitrogen- fixing bacteria (*Azospirillium*), and iron uptake from siderophore-producing bacteria (*Pseudomonas*). Species of *Pseudomonas* and *Bacillus* can produce as yet not well characterized phytohormones or growth regulators that cause extensive root growth, thus increasing the absorptive surface of plant roots. These PGPR are referred to as biostimulants and the phytohormones that they produce include indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production. Current means of delivery of inoculants include peat, granular, liquid and wettable powder formulations. A major determinant of growth promotion is the magnitude of their ability to colonize rhizosphere. Several new studies have contributed to the development of new biofertilizer products that utilize natural antimicrobial compounds produced by diverse antagonists (Marra *et al*., 2006).

### 2.2.7. Hypovirulence

A highly specialized mode of action concerns the use of hypovirulent isolates of fungal pathogens. Hypovirulent fungal isolates contain mycoviruses that intrinsically cause the fungus to be less fit. When hypovirulent isolates are introduced into plant tissues infected with a virulent pathogen isolate, the viruses can be transmitted via hyphal anastamoses, spreading the viral infection, and decreasing disease. The classic example of this process is that of hypovirulent isolates of *Cryphonectria parasitica*, containing unencapsidated double-stranded RNA viruses of the virus family Hypoviridae which have been used to control Chestnut blight (Heiniger and Rigling, 1994). Hypovirus infection is persistent and non-lytic, and is associated with inability to effectively penetrate the host plant, reduced sexual sporulation, female infertility, and reduced pigmentation. Genetically modified strains of *C. parasitica* transformed with a severe hypovirus are being explored for control of Chestnut blight in the United States (Dawe and Nuss, 2001).

### 2.2.8. Production, formulation and application of biocontrol agents (BCA)

Production, formulation and application of BCAs have been investigated extensively with the aim of producing successful and cost-effective products (Hall and Menn, 1999). A major aim is to produce the greatest quantity of viable propagules with the best quality for formulation as cheaply as possible, preferably using inexpensive growing media such as industrial wastes. Production of bacteria and fungi can be done using large-scale liquid fermentation which often involves manipulating the culture medium to induce production of the desired propagules for formulation. Factors which are often manipulated include temperature, pH and osmotic potential, as well as nutritional factors such as carbon source and C:N ratio (Jackson, 1997). Recently, solid-state fermentation has been used for the production of fungal biomass. For example, conidia produced by solid-state fermentation are incorporated into the wettable granule formulation of the commercial *C. minitans* product, Contans WG (De Vrije *et al*., 2001).

Unless inocula of BCAs are used immediately following production, cells or biomass are usually dried and formulated as products capable of storage, distribution and application (Fravel, 2005). Drying can be done by a range of different methods, including air- and freeze-drying, drying on silica gel and spray- and fluid bed-drying. These methods reduce the metabolic rate of the inoculum by removing the available water, which tends to preserve the inoculum with high viability depending on the BCA. Once the inoculum is dried, it is usually mixed with various components such as carriers, bulking agents, diluents and food bases. BCAs have been formulated as dusts, gels, emulsions, prills, pellets and granules for seed treatments, dips, wettable powders and sprays for application to aerial plant parts, and drenches for incorporation into soil and growing media (Fravel *et al*., 1998). Most work on formulation closely involves agrochemical, biotechnology or seed-treatment companies and, unfortunately, tends not to be published. The final formulated product should be convenient to use, safe to handle and have an adequate shelf life with stability for at least 1 year. Other desirable characteristics of a formulation include compatibility with application machinery, and ease of integration into integrated pest and disease control systems.

Both quality assurance and technical support are important to ensure that the formulated product contains the appropriate active BCA without contamination, and is applied correctly to ensure efficacy. Quality assurance and extensive technical support have been instrumental in the success of Serenade, a product containing the bacterium *Bacillus subtilis*, and used to reduce post-harvest diseases of citrus and pome fruit (Janisiewicz and Korsten, 2002). The success of products based on *Bacillus* is largely related to their ability to form spores and their ease of formulation and storage (Schisler *et al*., 2004).

Large-scale field application of BCAs poses practical problems in terms of producing sufficient amounts required to reach the target plant pathogen, and achieve efficacy, as well as concerns over production costs. The target and timing of application depends on the BCA, the pathogen and also the crop.

There has been extensive research directed at improving the application and performance of BCAs, and reducing the amounts required for control. One way of reducing the amount of BCA required to control both seed and soil-borne diseases is to apply the agent to seed rather than in-furrow, or as a soil or growing medium incorporation (McQuilken *et al*., 1998). Application of BCAs to seed has the potential to deliver the agent ‘in the right amount, at the right place and at the right time’. However, the process of applying BCAs to seeds presents a special set of technical considerations. For example, sufficient numbers of the BCA must survive the process, and be able to grow and colonize the environment of the germinating seed fast enough to provide control. The

BCA must also be able to survive a period of low water activity as the seed has to be stored at low moisture levels. Colonization of seeds by BCAs during germination can be improved by incorporating the agent during seed priming, a process used for the physiological enhancement of germination (McQuilken *et al*., 1998). This has been done successfully for *Trichoderma* spp. applied through solid matrix priming (Harman, 1991), and recently in the United Kingdom through drum priming for several bacterial species, including *Bacillus subtilis* and *Pseudomonas fluorescens* (Wright *et al*., 2003). The use of drum priming is a major advance in the application of BCAs to seeds and has significant commercial potential. There has been considerable interest in the use of insects to apply and disseminate BCAs to aerial microbiomes. For example, honey and bumble bees have been used successfully to spread both bacterial and fungal BCAs to specific sites such as soft and pome fruit flowers to control diseases including grey mould and fireblight, caused by *Botrytis cinerea* and *Erwinia amylovora*, respectively (Thomson *et al*., 1992). As part of a 3-year field study, beehives were equipped with dispensers containing the commercial *B. subtilis* product Serenade (Dedej *et al*., 2004). The honey bees were effective in spreading the BCA to blueberry flowers to suppress mummy berry disease caused by *Monilinia vaccinii-corymbosi*.

### 2.2.8.1. Methods of application of antagonists

**Overall application**: Successful application of biological control strategies requires more knowledge-intensive management (Heydari *et al*., 2004). Understanding when and where biological control of plant pathogens can be profitable, requires an appreciation of its place within integrated pest management systems (Shah-Smith and Burns, 1997). In general, the foundation of a sound pest and disease management program in an annual cropping system begins with cultural practices that alter the farm landscape to promote crop health (Heydari *et al*., 2004). These include crop rotations that limit the availability of host material used by plant pathogens (Cook, 1993). Proper use of tillage can disrupt pathogen life cycles and prepare seed beds of optimal moisture and bulk density. Careful management of soil fertility and moisture can also limit plant diseases by minimizing plant stress (Cook, 1993). In nurseries and greenhouses environmental control can be more tightly regulated in terms of temperature, light, moisture and soil composition, but the design of such systems cannot wholly eliminate disease problems (Paulitz and Belanger, 2001). The second layer of defense against pests consists of the quality of crop germplasm. Breeding for pathogen resistance including fungal pathogens contributes substantially to crop success in most regions (Cook, 1993). Newer technologies that directly incorporate genes into crop genomes, commonly referred to as genetic modification or genetic engineering, are bringing new traits into crop. Other technologies, such as seed washing, testing for pathogens and treatments are also used to keep germplasm pathogen-free. In perennial cropping systems, such as orchards and forests, germplasm quality may be more important than cultural practices, because rotation and tillage cannot be used as regularly (Cook, 1993). Upon these two layers, growers can further reduce pathogen pressure by considering both biological and chemical inputs. Biologically based inputs such as microbial fungicides can be used to interfere with pathogen activities. Registered biofungicides 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).

**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 *et al*., 2004), antagonists applied to fruit for protection in storage, e.g., *Pseudomonas fluorescens* (Janisiewicz and Peterson, 2004) and application to soil at the site of seed placement (Heydari and Misaghi, 2003). These types of applications are the most commonly used procedures which have resulted in the successful control of several fungal plant pathogens.

**One place application:** in this procedure, biocontrol microorganisms are applied at one place (each crop year), but at lower populations which then multiply and spread to other plant parts and give protection (augmentative application) against fungal pathogens. An Example of this method is Plant Growth Promoting Rhizobacteria (PGPR) and atoxigenic *Aspergillus flavus* on wheat seed scattered on the soil to spread to cotton flowers where they displace aflatoxin producing strains of *A. flavus* and fungal antagonists added to soil (Islam *et al*., 2005).

**Occasional application:** One time or occasional application maintains pathogen populations below threshold levels. In theory, parasites of the pathogen, or hypovirulent (disease carrying) strains of the pathogen, might be used and not require yearly repetition (e.g., hypovirulent strains of the chestnut blight pathogen) in which host plant is inoculated with attenuated strains of pathogenic that protects the host plant against the virulent strains of pathogen (Milgroom and Cortesi, 2004).

### 2.2.9. Commercialization of biocontrol

Commercial use and application of biological disease control have been slow mainly due to their variable performances under different environmental conditions in the field (Fravel, 2005). Many biocontrol agents perform well in the laboratory and green house conditions but fail to do so in the field. This problem can only be solved by better understanding of the environmental parameters that affect biocontrol agents (Wang *et al*., 2003). In addition to this problem, there has also been relatively little investment in the development and production of commercial formulation of biocontrol-active microorganisms probably due to the cost of developing, testing, registering and marketing of these products (Ardakani *et al*., 2009).

Biological control agents are generally formulated as wetable powders, dusts, granules and aqueous or oil-based liquid products using different mineral and organic carriers (Ardakani *et al*., 2009). Currently in the market, a number of biologically based products are being sold for the control of fungal plant diseases (Ardakani *et al*., 2009). A growing number of companies are also developing new products that are in the process of registration. Many of these companies are small, privately owned firms with a limited product-line. Others are publicly traded and have substantial capitalization values. In addition, larger companies with more diverse product lines that include a variety of agrochemicals and biotechnological products have played a significant role in the development and marketing of products for the control of plant pathogens (Ardakani *et al*., 2009).

Biocontrol products are either marketed as stand-alone products or formulated as mixtures with other microbials. Some products with biocontrol properties may not be registered, but are sold instead as plant strengtheners or growth promoters without any specific claims regarding disease control (Ardakani *et al*., 2009). To help improve the global market perception of biopesticides as effective products, the biopesticide Industry Alliance is establishing a certification process to ensure industry standards for efficacy, quality and consistency. To improve commercial use and application of biological disease control it is extremely important to emphasize and concentrate on several factors including training of growers, formulation of biocontrol microorganisms and studying the role of environmental factors.

### 2.2.10. Factors affecting variable efficacy and constraints on commercial developments

Inconsistency in efficacy of potential biological control agents (BCA) when evaluated in large-scale glasshouse or field trials is one of the major constraints in biological disease control. This can arise from various causes, especially extrinsic factors of the environment, reflecting the biological nature of the BCA. The BCA must first survive potential stresses of formulation and application procedures, and then remain active at the target site during the period when effective control is required. In addition, it must survive fluctuations in the natural environment, especially temperature, as well as the action of indigenous and competitive microbiota. Consequently, poor disease control at the scale-up stages of evaluation is always likely to be high (Whipps and Lumsden, 2001). In an attempt to resolve this problem and increase the number of BCAs reaching the market, it is recommended that all selection, screening and development processes adopt an ecological approach which takes into account the extrinsic factors of the environment of use (Whipps and Lumsden, 2001). It is unfortunate that most BCAs are only active under particular environmental conditions. Consequently, biological disease control in environmentally controlled structures, such as glasshouses and polytunnels, tends to be more successful and cost-effective compared to large-scale field application.

Economical, mass production of stable inoculum and appropriate formulation is imperative for the successful development of BCAs. Potential BCAs must also be easy to use and cost-effective, or they will never reach the market or be used by growers. Currently, many fungicides are relatively cheap and more effective than BCAs, and are unlikely to be substituted for by BCAs unless they are withdrawn from the market. Very few growers or extension workers know how to store and use BCAs, which often results in inadequate disease control and subsequent poor sales. Clearly, there is need to train growers on how to use BCAs effectively and integrate their use into crop protection programmes.

Another constraint to the development of bacteria and fungi as commercial BCAs has been poor long-term storage stability. Good long-term stability, preferably for 18-24 months at room temperature (21°C), is required to improve market competitiveness. Despite the hurdles in obtaining stability, considerable progress has been made, with stability of most current commercial products often being achieved by mixing propagules with various additives during formulation (Jones and Burges, 1998). Improved stability can also be achieved by treatment before formulation, for example, by appropriate growth conditions during production and by processing after production, such as drying. Furthermore, regulation of water availability in the formulation is important for stability.

Application technology can have a significant impact on the efficacy of BCAs. Unfortunately, this has often been neglected in the past, especially for the application of BCAs to aerial microbiomes, resulting in poor efficacy. Targeted delivery, deposition and coverage of the infection court are essential for good disease control. In laboratory experiments, Scherm *et al.* (2004) reported significant activity of the commercial product Serenade (*B. subtilis* QST713) against blueberry flower infection by *Monilinia vaccinii-corymbosis*. However, disease suppression was unsatisfactory when the *B. subtilis*-based product was applied in the field with a standard sprayer. This was likely due to low and variable coverage of the stigmatic infection court, which presents a difficult spray target. In a recent laboratory study, air-assisted electrostatic spraying significantly increased deposition of *B. subtilis* QST713 and coverage on the stigmatic surfaces of detached blueberry flower clusters compared to conventional hydraulic spraying (Scherm *et al*., 2007). The increased deposition and coverage together with the excellent bacterial survival in the formulated product bodes well for electrostatic application of the product for disease control in the field.

One of the major economic hurdles in the commercialization of BCAs is in risk assessment of toxicity and environmental impact of the organism, and its formulation (Scherwinski *et al*., 2007). Extensive trials are essential to generate data for registration purposes to show that potential commercial BCAs are safe both to humans and to other non-target organisms. Quality and efficacy data as well as additional technical protocols are also required by the registration authorities. All this can be extremely time-consuming and very expensive to generate as well as the cost for the assessment process itself. High registration costs have clearly been responsible for delaying or preventing the commercial development of BCAs in the past, especially by small–medium-size enterprises (SMEs) which are the main producers of BCAs. This has led to a large number of products appearing on the market which actually work by controlling plant pathogens but are claimed to be soil conditioners, plant-growth promoters or biofertilisers that do not require registration. However, without toxicological and efficacy data, safe use cannot be assured and consistent disease control and crop growth are not always observed (Cook *et al*., 1996).

Large-scale use of commercial products is still limited because of variability and inconsistency in terms of disease control. Coupled with a very competitive market with chemical pesticides, manufacturers of BCAs are finding it increasingly difficult to make sufficient profit from sale of commercial products to maintain the costs of registration. Unfortunately, this has resulted in the withdrawal of a number of products from the market. For example, Trichodex (*Trichoderma harzianum* T-39) introduced in 1993 for control of *B. cinerea* on grapes and greenhouse crops in Europe and Israel was withdrawn from the market in 2005 due to insufficient sales and increased registration costs.

### 2.3. Controlling plant disease using biological and environmentally friendly approaches

Since the 1960s, aggregate world food production has increased by 145%, with production increasing by 280% in Asia, nearly 200% in Latin America and 140% in Africa. Food production started from a higher base in industrialized countries, although it still grew by 68% in Western Europe (Pretty, 2008). During this period, world population doubled to more than six billion, although *per capita* agricultural production has exceeded population growth, with the result that for each person there is an additional 25% more food today compared to the 1960s (Pretty, 2008). Despite these increases in productivity, there are still some 800 million people hungry, with inadequate access to food (Pretty, 2008). In the United States in 2004, 38.2 million people, including 13.9 million children, lived in food-insecure households (Nord *et al.*, 2005), while in India in 2004, many people went hungry despite bumper harvests (Thurow and Solomon, 2004). The problem is of income distribution rather than food shortages the hungry are too poor to buy the food (Hazell and Wood, 2008).

Increases in food production in the past 50 years have resulted from increasing the intensity of production on agricultural land, with increased use of machinery, fertilizers and pesticides. Indeed, the use of pesticides in agriculture has increased hugely, now amounting to some 2.56 billion kg/yr (Pretty, 2008). However, the inefficient use of these inputs has resulted in considerable damage to the environment, with increased agricultural area contributing greatly to the loss of habitats and biodiversity (Scherr and McNeely, 2008). Interest in the sustainability of agricultural systems arose out of concern for the damaging effects of agricultural practices on the environment that began to surface in the 1950s–1960s (Pretty, 2008). Current concerns about sustainability revolve around the need to develop agricultural technologies and practices that: (a) have no adverse effects on the environment, (b) are effective and can be easily accessed by farmers, and (c) lead to increased food productivity, while yielding positive effects on environmental goods and services (Pretty, 2008). The key principles for sustainability are to integrate biological and ecological processes (e.g. nutrient cycling, soil regeneration, predation and parasitism) into food production processes, minimize use of non-renewable inputs that harm the environment, make use of the knowledge and skills of farmers in order to substitute human capital for costly external inputs, and to encourage people to work together to solve common agricultural problems (Pretty, 2008). A number of different terms have been used to imply greater sustainability in some agricultural systems than others, including eco-agriculture (Scherr and McNeely, 2008), ecological agriculture (Magdoff, 2007), and low input agriculture (Pretty, 2008). Many of these approaches involve minimizing or even eliminating the use of pesticides in favour of biologically based approaches to crop protection. If food production is to increase to feed the ever-rising world population, either the intensity of agricultural production needs to increase or more land is converted to agriculture. At the same time, the environmental consequences of food production need to be tackled, while scientists grapple with the persistent problems of fungicide insensitivity and breakdown of host resistance. This is a tall order and in terms of crop protection, will require a multi-faceted approach to controlling diseases, pests and weeds.

#### 2.3.1. Biological based disease control methods of crop protection practice

As mentioned above, effective disease control requires a multi-faceted approach, using a number of different methods. Control of certain crop diseases will require biologically based methods to be integrated into disease control programmes, along with other approaches. For other diseases, for example, those for which no adequate control exists, biologically based methods might offer the only hope of reducing disease to acceptable levels. Sustainable approaches to agriculture, including many biologically based methods of disease control, might be particularly appropriate for fragile and low-yielding farming systems located, for example, in dry lands, uplands, near-deserts and hillsides (Hazell and Wood, 2008). In many developing countries, integrated management practices are used to control important pathogens and pests (Phiri *et al.*, 2007). For example, bean common mosaic virus (BCMV) and bean common mosaic necrotic virus (BCMNV) are controlled using virus-free seed, intercropping with non-host crops, and use of resistant varieties, while loose or head smut, caused by *Sphacelotheca reiliana,* is managed through rotation, deep ploughing and destruction of plant debris, and use of resistant varieties (Phiri *et al.*, 2007). Interestingly, in China, which grows in excess of 28 million hectares of wheat, ‘ecological’ control of the stripe rust fungus, *Puccinia striiformis,* has been considered as a major strategy for sustainable disease control (Chen *et al.*, 2007). This approach involves: (a) improving cultivar resistance, (b) changing cultural practices, (c) eradicating volunteer wheat seedlings, (d) regulating wheat planting date and (e) returning land to forestry and pastures (Chen *et al.*, 2007). Irrespective of the system into which biologically based disease control methods are slotted, their use in crop protection programmes will first require a number of issues to be resolved and barriers to be overcome.

#### 2.3.2. Biologically based disease control

##### 2.3.2.1. Efficacy of disease control

In most developed countries, high crop yields are maintained through the use of improved varieties, together with fertilizers and pesticides. Indeed, farmers and growers in these countries are accustomed to achieving high levels of disease control with fungicides, although the development of fungicide resistance can erode fungicide efficacy. In contrast, levels of disease control obtained with many biologically based control methods are lower than those achieved using fungicides. In addition, many biologically based methods tend to provide inconsistent disease control. For example, although induced resistance can provide high levels of disease control on some crops, with many crops, disease control is less impressive. Expression of induced resistance in crop plants can also be variable, depending on a number of factors, including genotype and environment. There are also problems of variability and inconsistency of disease control with some biological control agents (BCAs) (Whipps, 2007). Perceived problems with inadequate and inconsistent disease control will not persuade farmers and growers to adopt biologically based approaches. Minimizing the effects of these problems requires further research.

##### 2.3.2.2. Regulatory issues

Despite the considerable effort by researchers to develop novel biologically based solutions for disease control (e.g. BCAs, plant-derived substances, induced resistance agents), few products have reached the marketplace. The high cost of registration, coupled with limited market size for some products, has been identified as a major barrier (Kleeberg, 2007). However, this problem has been recognized by regulatory authorities and in the United Kingdom, for example, the Pesticides Safety Directorate (PSD) launched a direct scheme for biopesticides in 2004, allowing the requirements for registration to be modified to the product type and importantly, offering a significant reduction in the application fee (Whittaker, 2007). This pilot scheme has since evolved into a permanent Biopesticides Scheme run by the PSD. However, this experience contrasts with elsewhere in Europe, where the biopesticide industry has failed to engage effectively with the regulatory authorities (Whittaker, 2007). Unless this situation changes, significant problems, getting biopesticides into commercial practice, will continue.

### 2.3.2.3. Farmer adoption of biologically based practices

There is evidence that improved agricultural technologies, such as disease-resistant varieties, precision farming and improved water management practices, can increase crop yields while reducing chemical use (Pingali *et al.*, 1997). However, farmers have been slow to adopt these new practices. There are a number of reasons for this reluctance to switch to improved practices. For example, it might be due, in part, to the continuing subsidies on water and agrochemicals provided by many governments, that is by making these inputs less expensive, subsidies encourage farmers and growers to be more wasteful in their use (Hazell and Wood, 2008). In addition, many of these improved practices are more labour and knowledge intensive than the existing practices, which can make it difficult and costly for farmers and growers to adopt them (Pingali *et al.*, 1997). Changing farmer attitudes to the adoption of new agricultural practices is not easy, but ultimately, unless farmers and growers are prepared to use such technologies, most of them will never find their way into crop protection practice.

## 3. CONCLUDING REMARK AND FUTURE OUTLOOK

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. A successful biocontrol requires considerable understanding of cropping system; disease epidemiology; the biology, ecology, and population dynamics of biocontrol organisms; and the interactions among these variables. 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 biocontrol agents.

Many factors have to be considered in deciding whether a biological system is feasible for the control of a particular pathogen. Of prime importance is the availability of a suitable antagonist capable of maintaining itself on the host plant. The environment under which the crop is grown will play a significant part in determining whether effective population levels of an antagonist can be established in competition with the existing microflora. Environment may also govern the choice of antagonist; for example, yeasts can survive on leaves more readily than non-spore-forming bacteria under adverse humidity conditions. It is essential that the primary mechanism by which antagonism is brought about should be known. 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.

With people turning more health conscious Biological control seem to the best alternative to disease suppression. Bio-agents bring the disease suppression with no environmental hazards. Research has proved that the bio agents trigger the growth of plants. Bio agents themselves being non-pathogenic to plants need to be formulated in a way that favours the activity and survival of microbe it contains. Moreover, the organism that suppresses the pathogen is referred to as the biological control agent (BCA). More broadly, the term biological control also has been applied to the use of the natural products extracted or fermented from various sources. These formulations may be very simple mixtures of natural ingredients with specific activities or complex mixtures with multiple effects on the host as well as the target pest or pathogen. And, while such inputs may mimic the activities of living organisms, non-living inputs should more properly be referred to as biopesticides or biofertilizers, depending on the primary benefit provided to the host plant.

Over the past few years, the novel applications of molecular techniques have broadened our insight into the basis of biological control of plant diseases. New molecular approaches have been available for assessment of interaction between the antagonist and pathogen, ecological traits of antagonists in rhizosphere and improving the efficacy of bacterial, fungal and viral biocontrol agent. Consequently, there has been a significant increase in the number of biological disease control agents registered or on the market worldwide in the last few years.

Currently, biological control will thus be an alternative strategy for the control of plant diseases given the history of fungicides in the near future. However, other methods in IPM for crop disease control are still necessary in various environmental conditions, because an agro-ecosystem is a variable and functioning system that includes several factors that influence disease and crop development. Consequently, for economic threshold, other control strategies of IPM besides biological control should be also considered and applied to effectively reduce the disease development and the yield loss of crops in the different crop systems.

### Future outlook

Some of the research criteria that will advance our understanding of biological control and the conditions under which it can be most fruitfully applied. Ecological factors play very important roles in the performance and activity of biocontrol-active microorganisms. Application strategies still there are some areas which should be investigated and developed for the enhancement of the effectiveness of biocontrol microorganisms. Introducing new strains and mechanisms of fungal/bacterial plant pathogens are very diverse and their pathogenicity is different on host plants, it is therefore very important to look for new and novel biocontrol microorganisms with different mechanisms.

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