Chapter 5
Biological Antagonism: A Safe and Sustainable Way to Manage Plant Diseases

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Abstract Biological control is a viable alternatives to the use of synthetic chemicals for plant pathogens management, based on application of microbial antagonists as biological control agents (BCA). Plant health is significantly affected in many ways by a wide variety of pathogens. Cross protection, predation, hyperparasitism, induced resistance, antibiosis and competition are different mechanisms used by BCA. Knowledge is required for successful application of biocontrol in intensive management approaches. BCA can be applied at the site of infection directly or in each crop year, at sites in which they will multiply and spread to other field parts. To keep pathogen populations below economic threshold levels, occasional or one time applications can be adopted. However, due to different environmental conditions, biological control has not always produced encouraging results. To improve the BCA performance in the field, work on formulations is needed. For marketing, strains with better adaptability and field survival should be prospected. Most of biological control work has been centered on management of soil borne or seed borne pathogens. Most of the products containing BCA are applied as seed treatments for protecting major crops such as wheat, rice, sugar beet, corn and cotton. BCA are also used in foliar sprays to manage downy and powdery mildew, leaf spot and blight. Antagonistic microorganisms have also been used against few post-harvest pathogens. In spite of all significant improvements, this area still needs due

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consideration for developing more reliable and stable formulations, especially when for field applications. In this view, more research is required on innovative formulations by exploring novel microorganisms, using nano- and biotechnologies for their improvement, studying the impact of environmental conditions and the mass production of BCA. With a growing of biocontrol demand by growers, the future outlook of biocontrol is bright. By improving biocontrol research it is possible to completely replace chemical pesticides by BCA, improving yields, protection technologies and the environment, leading to a more sustainable agriculture.

Keywords Hyperparasitism · Biological antagonism · Entomopathogenic · Mycoparasitism · Obligate parasite

5.1 Introduction

Biotic agents such as pathogens, harmful pests and weeds are major yield-limiting factors in agriculture. To improve the agricultural products quantitatively and qualitatively, these pest constraints need to be managed. Different agricultural practices are being used to manage pests (Benhamou 2004; Heydari 2007; Cook 1993; Agrios 1988; Islam et al. 2005; Baker 1987; Chisholm et al. 2006; Kloepper et al. 2004; Bargabus et al. 2002, 2004). Most commonly, plant diseases are controlled by different cultural practices and pesticides (Baker 1987; Agrios 1988). However, pesticide pollution is a major, actual concern which led to the development and application of strict regulations towards the use of pesticides. There is also pressure for removal from the market of chemicals which are not eco-friendly. The carcinogenicity and effects on non-target hosts due to their extensive use also raised public pesticide concerns. A further issue about pesticides use regards the insurgence of resistant pathogens, and the difficulty in registration of new chemicals (Cook 1993). All human health and environmental protection concerns forced researchers to rethink and develop alternative strategies for disease management which are eco-friendly (Cook 1993; Baker 1987).

Cook (1993) stated that best alternative to pesticides is biological control. Biological controls means management of one organism's development by the exploitation of other living organisms (Cook 1993; Baker 1987). Advantages related to adaptation of biological control are: environment protection which is eco-friendly, self-sustaining and long lasting; support to existing communities of beneficial species (Cook 1993). Mechanism of action of biological control might include the supplemented release of exotic species or creating favourable conditions for the multiplication of naturally existing microbes or the use of non-pathogenic strains (Schouten et al. 2004; Cook 1993).

According to Agrios (1988), more than 70% of diseases are caused by fungal pathogens. Worldwide, fungal pathogens are the main reason of major annual yield losses in agriculture. Many fungal diseases such as flower and leaf diseases (powdery mildew), vegetables and fruit diseases (*Botrytis* spp.), pathogens of cut and pruning wounds and soil borne diseases, have been successfully managed by BCA (Heydari and Misaghi 1998, 1999, 2003; Cook 1993; Baker 1987; Agrios 1988; Heydari et al. 2004). The interaction among plant, BCA, environment and people still need extensive consideration for better and effective development of biological control strategies. This chapter will (i) illustrate different definitions as well as basic biocontrol mechanisms, (ii) explain the biological control and microbial diversity interaction, (iii) present the recent position of study and implementation of biological controls, and (iv) concisely list forthcoming guidelines that may results in progression of additional and efficient biological control against various fungal diseases (Kessel et al. 2005).

5.2 Biological Control

Biological control (syn. biocontrol) has been used especially in plant pathology and entomology. The use of antagonists or host-specific weed pathogens are also included in biological control. The organisms used to manage other species are termed BCAs. The fermented or extracted natural products (bio-fertilizers or biopesticides) also may be considered as biological control (Cook 1993). The definitions of biological control depend on type, number and source of BCA, targets and human timing and involvement. The biocontrol of plant diseases varies from biocontrol of insect in the following ways (Table 5.1).

5.2.1 Terminology

Predator: organism that normally preys on other or free-living or pest species that are directly devoured. A huge number of preys are killed amongst its entire lifetime.

Table 5.1 Comparison of bio-control for plant diseases and insect pests

Disease	Insects
Control of disease is mainly accomplished by predators and parasites, hyper parasites, competition and antibiosis	Mostly by predators and parasites
Antagonists are not mobile and are broadly passive. Contact of the pathogen is unintentional.	Parasites are dynamic, versatile and look for their prey.
This technique depends largely on local organisms.	Predators/ parasites are normally introduced from different countries
Pathogen free planting materials and seeds are widely used.	Healthy seeds (having no pest) are not utilized.
Mass effect (single types of pathogen many competitors/ antagonists are available.	Single parasite or predator for single prey.

Entomopathogenic: microorganisms that can act as parasites of different insects inducing a disease that seriously deactivates or kills them. **Antagonism**: the activity of any microorganism that overcomes the action of a plant pathogen. ***Parasitoid:*** highly specialized insects that lay their eggs in or on the body of an insect host, which is then utilized as food for the larva development. The host is eventually killed. **Pathogen**: bacteria, fungi, and viruses that disabled or kill their host and are relatively host-specific.

5.3 Biological Management of Fungal Plant Pathogens

5.3.1 Beneficial Microbes and Plant Interaction

Plant health has been significantly affected in various ways by the interactions between plants and their pathogens, in many cases throughout their entire life cycle (Fitter and Garbaye 1994; Katska 1994; Agrios 1988; Chisholm et al. 2006; Bull et al. 2002; McSpadden-Gardener and Weller 2001). To study biocontrol mechanisms of action, different interactions must be studied, starting from the direct contact between interacting organisms.

The different types of interactions that can exist between two organisms are: parasitism, mutualism, competition, protocorporation, predation, commensalism, amensalism and neutralism (Hoitink and Boehm 1999; Chisholm et al. 2006; Bull et al. 2002; Fitter and Garbaye 1994; Katska 1994; Bankhead et al. 2004). Both at micro- and macroscopic level, any of these interactions can be observed. For diseases development both plants and pathogens, interact at multiple scales, being the disease the starting point for the development of biological control (Chisholm et al. 2006; Bull et al. 2002; Fitter and Garbaye 1994). Specific or non-specific interactions resulting in a positive, crop beneficial way is considered as a successful biological control from the plants and farmers' view points (Cook 1993; Weller et al. 2002).

Different mechanisms characterizing ecosystem processes that contribute to biological control can be classified and functionally outlined. The beneficial association of two or several species in which every contributing species receives a benefit is called mutualism (Fitter and Garbaye 1994; Kerry 2000; Biermann and Linderman 1983; Chisholm et al. 2006; Garcia-Garrido and Ocampo 1989; Duchesne 1994; Katska 1994). An example is the link of arbuscular mycorrhizal fungi with plants, yielding associations which represent an obligate, biochemical and physical interaction (Fitter and Garbaye 1994; Chisholm et al. 2006; Katska 1994). The association can also be facultative, as in the case of *Rhizobium* bacteria. Rhizobia can survive both in soil and in mutualistic interactions within root nodules of leguminous crops. These associations help plants by improving their nutrition through nitrogen fixation and manipulation/activation of defense mechanisms (Fitter and Garbaye 1994; Chisholm et al. 2006). The prevailing environmental conditions have marked effects on disease suppression and host development, and many BCA are classified as facultative, mutualistic microorganisms (Cook 1993).

The interaction in which one species is neither benefitted nor harmed, while the other one gets a benefit is called commensalism (Fitter and Garbaye 1994). Most of soil inhibiting microbes are commensals, because they are get benefits while the plants rarely show any positive or negative impact (Katska 1994; Chisholm et al. 2006). Commensals raise great challenges to plant pathogens and are responsible for lowering their populations, and ultimately the disease severity (Cook 1993).

Neutralism is a mechanism in which a member of one species has no effect on another one (Berg et al. 2005; Chisholm et al. 2006), while the negative interaction between organisms is defined as antagonism (Cook 1993). the inability of one organism to regulate the population dynamics of another organism (pathogen) is a kind of neutralism, while the antagonistic competition for food, shelter or space may decrease the activity, growth and fecundity of a target species.

The prolonged symbiotic interaction in which a species coexists for a specific time period during which one gets a benefit and the other is harmed, is called parasitism (Lo et al. 1997; Cook 1993; Chisholm et al. 2006). The activity of hyperparasites (parasitizing i.e. a plant parasite) results in biocontrol, as they feed and may regulate densities of plant pathogens (Lo et al. 1997). The stimulation of defense systems in the host after inoculation with a relatively avirulent strain may result in biocontrol and regulation of a virulent strain, an interesting aspect of biological control (Cook 1993). The last (but not least), mechanism of biological control is predation, in which one species consumes and obtains nourishment from another organism, usually by hunting and killing it. This term is applied to animals in higher trophic levels, mesofauna and protists (Cook 1993), but may be also observed at the microcale, including nematodes and bacteria.

As discussed earlier, the type of biological control interaction depends on prevailing environmental conditions. Generally mutualism and antagonism are manipulated for biocontrol of plant pathogenic microorganisms (Chisholm et al. 2006; Bull et al. 2002; Fitter and Garbaye 1994; Katska 1994).

5.3.2 Mechanism Involved in Biological Control

Different experimental approaches were studied to characterize most basic mechanisms of biological control (Howell et al. 1988; Audenaert et al. 2002; Homma et al. 1989; Van Dijk and Nelson 2000; Heydari et al. 1997; De Meyer and Hofte 1997; Ryu et al. 2004; Meziane et al. 2005; Elad and Baker 1985; Islam et al. 2005). In all studies it was revealed that each pathogen was antagonised by other microbes. Selectivity of the antagonist for a given target pathogen and physical contact result in direct antagonism. The most direct antagonism is hyperparasitism, because in this case a suppressive effect is exerted by a single microorganism and no other species is required (Linderman 1994; Harman et al. 2004). In indirect antagonism rather than directly targeting a pathogen, an activation of the host defense pathways

Table 5.2 Antagonistic mechanisms exploited in biocontrol activity

Type	Mechanism	Examples
Direct competition	Predation /Hyperparasitism	*Trichoderma virens*
		Ampelomyces quisqualis
		Pasteuria penetrans
		Lysobacter enzymogenes
		Use of mycoviruses
Mixed-paths	Production of antibiotics	Cyclic lipopeptides
		2,4-diacetylphloroglucinol
		Phenazines
	Lytic enzymes	Proteases, glucanases, chitinases
	Unregulated waste products	Hydrogen cyanide
		CO_2
		Ammonia
	Physical/chemical interference	Soil pores blockage
		Blockage of communication signals
		Remote sensing
Creating harmful environment	Food and space struggle	Consumption of soil resources
		Siderophore foraging
		Destruction of niche
	Activation of resistance of the host	Cell wall degradation
		Destruction of molecular signaling pathway

is produced. An elicited and improved host defense is achieved by non-pathogenic microorganisms (Silva et al. 2004; Leeman et al. 1995; Kloepper et al. 1980; Maurhofer et al. 1994; Lafontaine and Benhamou 1996). There is an increasing attention on studying and establishing the mechanism of biocontrol in a particular host-pathogen interaction. There are a few examples of different mechanisms of biological control that may be operating in the same specific interaction (Table 5.2).

5.4 Mycoparasitism

Specific BCA directly attack the pathogen cells or propagules. Hyperparasites may be categorized into four major groups, including Predators, Facultative parasite, Hypoviruses and Obligate bacterial pathogens. Viruses infecting *Cryphonectria parasitica* (the causal agent of the chestnut blight) provide these examples of hypoparasites. These hypoparasites cause the reduction of pathogens virulence and pathogenicity. Hyperparasites have been reported as very successful in managing chestnut blight in different areas (Milgroom and Cortesi 2004).

The interaction of all factors involved in specific biological control, such as hyperparasites, host, pathogen and environment, will determine the success or failure of any specific BCA. Sclerotia-infecting hypoparasites i.e. *Coniothyrium minitans* and those that infect fungal hyphae i.e. *Pythium oligandrum*, have been identified. Multiple hyperparasites can attack a single fungal species. Pathogens causing powdery mildew are been parasitized by a few hyperparasites such as *Gliocladium virens, Ampelomyces quisqualis, Acremonium alternatum, Cladosporium oxysporum* and *Acredontium crateriforme* (Milgroom and Cortesi 2004). In other cases, in which available nutrition is limited, BCA may exhibit a predatory behaviour. *Trichoderma* spp. release a range of cell wall degrading enzymes affecting plant pathogens. If *Trichoderma* develops in a nutrient rich environment it will not directly attack a pathogen such as *Rhizoctonia solani*. In contrast, if available nutrients are limited such as during bark decomposition, or in a condition of limited cellulose availability, *Trichoderma* spp. may release chitinases and will directly parasitize *R. solani* (Benhamou and Chet 1997).

5.5 Antibiosis

Antibiotic are toxins released by different microorganism that can kill other microbes even at low concentrations. The production of compounds with antibiotic activity has been reported for many microorganisms (Shanahan et al. 1992; Homma et al. 1989; Thomashow and Weller 1988; Islam et al. 2005; Thomashow et al. 1990; Howell and Stipanovic 1980; Leclère et al. 2005; Shahraki et al. 2009). The effectiveness of antibiotics in management of plant pathogens and diseases has been studied by different researchers (Howell and Stipanovic 1980; Thomashow and Weller 1988; Shanahan et al. 1992; Homma et al. 1989; Islam et al. 2005; Thomashow et al. 1990, 2002). In all conditions, especially *in-vitro* and *in-situ*, all the studied antibiotic were effective in controlling pathogens and their diseases. It was also demonstrated that any antibiotic which is effective must be produced in required doses to kill pathogens.

The production of antibiotics by biocontrol bacteria has been studied. Comparison of mutant strains showed that those without capacity to produce phloroglucinols and phenazines can colonize the rhizosphere but are incapable of inhibiting the growth of soil borne pathogens. While the corresponding wild strain can colonize and manage these pathogen and the root disease with a significant impact. Also, there are biocontrol strains that produce more than one antibiotic for their biocontrol activity. These types of BCA are supposed to control a wide range of pathogens and allow management of the induced disease (Keel et al. 1989; Thomashow and Weller 1988; Homma et al. 1989; Islam et al. 2005; Howell and Stipanovic 1980; Thomashow et al. 1990; Shanahan et al. 1992).

5.6 Production of Metabolites

BCA produce several metabolites other than antibiotics, that can harm growth and reproduction of target pathogens. Many polymeric compounds, i.e. cellulose, chitin, hemicellulose, proteins and DNA, can be broken by these metabolites such as lytic enzyms (Wilhite et al. 2001; Anderson et al. 2004; Press et al. 2001; Loper and Buyer 1991; Howell et al. 1988; Ordentlich et al. 1988). Many studies showed that these metabolites can directly suppress the development and growth of plant pathogens. The breakdown of complex polymers is important to get carbon which is necessary in the antagonistic activity. For example, chitinase expression by *Serratia marcescens* can suppress growth of *Sclerotium rolfsii* (Ordentlich et al. 1988). In other cases, the production of lytic enzymes by Myxobacteria and Lysobacteria leads to the development of effective biocontrol of many pathogenic fungi (Bull et al. 2002). Howell et al. (1988) showed that the defenses system of the host can be induced by oligosaccharides, fungal cell wall derivatives. The composition and availability of carbon and nitrogen based metabolites in rhizosphere soil are important factors for effectiveness. For example, *Fusarium oxysporum* f.sp. *radices-lycopersici*, the causal organism of root rot, can be managed by application of chitosan (the polymer of β1,4 glucosamine produced from chitin by alkaline deacylation, which is also biodegradable and non-toxic) (Benhamou 2004; Lafontaine and Benhamou 1996).

Another secondary microbe metabolite such as hydrogen cyanide is very effective in controlling plant diseases. The picomolar concentration of HCN is toxic for aerobic microbes and can cause a blockage in the cytochrome-oxidase-pathway (Ramette et al. 2003; Phillips et al. 2004). This is the case of *Pseudomonas fluorescens* which produces both siderophores and HCN. However, it is believed that the biocontrol activity of *P. fluorescens* against *Thielaviopsis basicola* is mainly due to the production of HCN.

Other secondary metabolites are also effective in plant disease control. Howell et al. (1988) reported that suppression of *Pythium ultimum* (the causal agent of cotton damping off) by *Enterobacter cloacae* is due to volatile compounds containing ammonia.

5.7 Competition

Competition for food and shelter is a common phenomenon of wildlife. Similarly, microbes also compete for available food resources in the root rhizosphere. The competition of one organism vs another is an important aspect of biocontrol. For a successful biological control activity, a microorganism must compete, after colonization, for obtaining nutrition from senescent tissues, exudates, waste products of insects and leachates (Loper and Buyer 1991; Elad and Baker 1985; Keel et al. 1989). The competition for food of soil (i.e. *Fusarium, Pythium* spp.) and foliar microbes that germinate by producing appressoria and infection, is a more critical factor (Loper and Buyer 1991; Elad and Baker 1985; Keel et al. 1989). In a study on suppression of Fusarium wilt, *Pseudomonas putida* was capable of producing agglutinin. Compared to *P. putida* mutant strain deficient in agglutinin production revealed that *P. putida* producing agglutinin was able to better colonize the rhizosphere, yielding ultimately a better protection as compared to the mutant strain, also highlighting the role of this lectin (Anderson et al. 1988; Tari and Anderson 1988). Similarly, soil microbial communities can protect plants with higher efficacy because of their ability for a quick colonization, thus limiting the resources for other pathogenic microbes.

The availability of micronutrients such as iron, is limited depending on soil pH, oxidization state and aeration. Iron may be present in soil in its ferric form, which is extremely insoluble in water, lowering its concentration. Competition of this essential nutrient is very important for a BCA success. Almost all microorganisms produce siderophores which have ability of bind iron for their survival (Shahraki et al. 2009). There is a direct correlation between biocontrol ability of pseudomonads and siderophore production.

5.8 Resistance: Induced or Systemic

Many stimuli such as light, water or physical stress, nutrients availability and temperature are important as they may induce or affect resistance of host plants (Moyne et al. 2001; Van Wees et al. 1997; Vallad and Goodman 2004; Leeman et al. 1995). Depending on the source, amount and type of stimulus, resistance can be systemic or local (Van Loon et al. 1998; De Meyer and Hofte 1997; Zhang et al. 2002; Audenaert et al. 2002; Kloepper et al. 1980; Leeman et al. 1995; Vallad and Goodman 2004; Van Wees et al. 1997; Van Peer and Schippers 1992). The pathway of systemic or local resistance induced by BCA has been characterized recently. The first pathway relies on the production of salicylic acid after infection by pathogens, which ultimately increases the expression of pathogenesis-related portions (PR proteins) yielding a systemic acquired resistance (Vallad and Goodman 2004). The second pathway is characterized by the production of jasmonic acid or ethylene after infection by a mild or localized pathogen or parasite, which results in induced systemic resistance (Van Wees et al. 1997; Van Loon et al. 1998; Leeman et al. 1995; Audenaert et al. 2002; Kloepper et al. 1980; De Meyer and Hofte 1997; Zhang et al. 2002; Van Peer and Schippers 1992). When multiple stimuli are received and processed, plants may activate the various pathways of resistance. The strength and duration of the host resistance very likely changes over time. If we can control the stimulus then we can control the induction of resistance. The microbial community associated with plants is detected and plants respond to the quantitative or qualitative changes and signals. This interaction also indicates that further induction stimuli exerted by any microbes or chemical will not improve the resistance, health or productivity set in place by the plant (Vallad and Goodman 2004).

5.9 Application Methods

Extensive research, management and knowledge are required for successful application of BCA (Shah-Smith and Burns 1997; Baker 1987; Heydari et al. 2004; Cook 1993). The profitability, appreciation and crop depending requirements need to be considered for biocontrol application. Overall application includes biological products such as microbial fungicides. When these are applied, growth and development of natural soil inhabitants may also be indirectly supported, which in turn further reduces the pathogens activity (Cook 1993: Shah-Smith and Burns 1997; Heydari et al. 2004).

Direct application of BCA to the infection site is another strategy, such as for antagonistic bacteria and fungi for seed treatment and coating. *Trichoderma harzianum* and *P. fluorescens* are applied to stored fruits for protection. Direct application is widely used for application of BCA as shown by success in management of some pathogens (El-Ghaouth et al. 2000; De Capdeville et al. 2002; Janisiewicz and Peterson 2004). Another way of application of fungal biocontrol is one place application. This methods depends on the BCA multiplication and spread. Once are applied at one place with a low population density they will develop and spread to other parts of the plant to protect. In general, hypovirulent applications are considered to be applied once and to develop with time, ultimately spread in whole field without requiring yearly applications. However, to maintain the population of BCAs occasional treatments may be needed (Milgroom and Cortesi 2004).

5.10 Future Prospects

Since 1970s, biological control has become a mature science and got support from both public and private sectors. This is shown by the increasing literature data on biological control published on journals of both plant pathology and entomology, and by the funding from national agencies (i.e. USDA) for biocontrol research. Some research grants include USDA IR-4, Section 406 program, Regional IPM grants and integrated organic programs. Over the last year, researcher learnt much more about biological control. There is still a need to develop new and different strategies that can provide answers on emerging issues and invasive pathogen species. With the advancement of science, researcher are able to characterize both BCA and pathogens increasing the general understanding of many pathosystem. Cellular and molecular studies encouraged researcher to develop new techniques. Some of the areas which need to be addressed for developing fruitful biological control method are indicated as follows.

5.10.1 Antagonistic Microbes Ecology

The establishment of biocontrol agents largely depends on a number of ecological factors, also affecting the activity and performance of the microorganisms applied. At this regard some questions need to be answered and clarified:

1. How are the antagonists and their target pathogens distributed in the environment?
2. How important environmental conditions affect the BCA activity?
3. How do different management practices affect the naturally existing and introduced microorganisms?
4. Which factors regulate the suppressive activity of a biocontrol agent?

5.10.2 Application Method

For enhancing the BCA activity work on application methods is still needed. The investigation must focus on the development of application methods which can increase the BCA effectiveness. The works needed to be done is as follow:

1. Searching for new strains and their variants.
2. Using advanced techniques i.e. genetic engineering of microbes for increasing applications.
3. Developing proper formulations.

5.10.3 New Strains and New Mechanisms

As fungal pathogens always pose a great threat to crops, it must be remarked that each pathogen is different and it ability to cause disease differs consequently. Therefore, it is very important to explore the natural diversity of species to find new strains with different mechanism of biocontrol. The following aspects must hence be also investigated.

1. Characterization of new strains and their use.
2. Genetic study of BCAs to explore new genes or combination of genes that can be manipulated.
3. Instead of using single strain, focus should be given to the development of a combination of strains with diverse mechanisms.

5.10.4 Integrated Pest Management

As integration of biological control strategies with other disease management strategies is very important, some aspects to consider are as follow:

1. Cropping patterns should be chose to get maximum benefit from biological control.
2. IPM and biocontrol strategies must fit each other.
3. Compatibility of BCA and plant cultivars must be considered during breeding programs.

5.11 Research and Development

Biological control can fulfil the gap originated by farmers' reluctance to use chemical pesticides and by the search for new strategies of disease management. Actual lines of action such as crop rotation, breeding programs, use of tillage and/or resistant cultivars etc. are not always sufficient for a successful management. Next step is the application of BCAs as amendments, inoculants or of active ingredients derived directly from natural sources. Biological control has no or just a little effect on environment and other non-pathogenic species (Jacobsen et al. 2004; Guetsky et al. 2001). If growers cannot sustain their production then they can still use less harmful and less specific chemical toxins. However, as biocontrol is very successful under lab condition, when it comes to the commercial application there are still constraints including stability, efficacy, cost and mode of application. As more research and a better understanding of biological control are needed, the research on adoption of BCA as part of IPM is expected to increase in the years to come.

5.12 Biological Control of Nematodes

In the last several nematicides have been withdrawn from the market due to health and environmental hazards associated with production and use. Due to increasing public concern, there has been interest in the development of alternative methods of control, including use of BCA. A number of studies showed that nematophagous fungi and bacteria increase under perennial crops and monocultures. As such they may control some nematode pests, including cyst and root-knot nematodes (Stirling 1985; Stirling 2011).

Nematode-suppressive soils have been reported from around the world and include documented cases of effective biological control (Yang et al. 2012; Giné et al. 2016). Finally, a number of commercial products based on nematophagous fungi and bacteria have been developed.

5.13 Biological Control of Plant Parasitic Nematodes

In soil plant parasitic nematodes are attacked by natural enemies which can be exploited for practical use in field conditions. Many predators such as fungi, nematodes or other predacious organisms such as insects and mites have been identified. Parasitic fungi and bacteria have been investigated as promising BCA. Their development in nematode biocontrol has been reviewed (Stirling 2011; Timper 2014; Devi and George 2018).

5.14 Fungi as Biocontrol Agents of Nematodes

Among various microorganisms which parasitize or prey on plant parasitic nematodes, fungi have vital position and possess great biocontrol potential. In various soil types plant parasitic nematodes are destroyed by fungi on continuous basis. The biocontrol potential of fungi against females of cyst nematodes was observed firstly by Kuhn in 1877. There are more than 70 genera and 160 fungal species which are associated with nematodes.

5.14.1 Predacious Fungi

There are more than 50 species of this group of fungi which kill nematodes. The nematode trapping efficiency decreases with the life span of the fungi. Their efficacy can be increased by soil amendments with organic matter. According to predacious activities, they can be classified as endoparasitic or trapping fungi.

5.14.1.1 Endoparasitic Fungi

These are mostly specific to single species or group of nematodes. Being obligate parasites, therefore, they are difficult to culture in absence of the host. *Hirsutella, Meria, Nematophthora* and *Nematoctonus* are ideal BCAs against nematodes. These fungi in general attack the nematode host by adhesive spores, from which a germ tube develops which later penetrates the nematode cuticle. The fungal hyphae divide and multiply throughout the nematode body and absorb its contents. The hyphae then emerge from the dead carcass. *Catenaria vermicola* often attacks *Heterodera schachtii*, while *Nematophthora* and Pchonia chlamydosporia have been reported as parasites of *H. avenae* (Kerry 2000). These fungi have a key role in natural regulation of the population dynamics of plant nematodes, in some soils.

5.14.1.2 Trapping Fungi

The nematode trapping fungi develop adhesive networks, sticky knobs or constricting rings formed by the mycelium. All are specialized hyphal structures capable to capture nematodes. The fungi then digest the nematode internal tissues. The nematode trappers may be grouped as follows.

(I) *Sticky branches*: the mycelium bears small lateral branches which join to form loops (anastomose). The plant nematodes are trapped in this loop, as those produced, for example, by *Dactylella lobata.*
(II) *Sticky networks*: the hyphae curl around and anastomose forming similar branches. These loops form three dimensional structures. Nematodes are trapped in the network due to the hyphae sticky surface as those, for example, of *Arthrobotrys oligospora*.
(III) *Sticky knobs*: small spherical or sub-spherical lobes are present on short lateral hyphae, the terminal lobe being sticky to trap nematodes. *Dactylella ellipsospora* illustrates this mechanism of trapping.
(IV) *Constricting rings*: the short branch of a fungal hypha anastomoses with its base to form a ring. Whenever a nematode enters the ring, a swelling of the inner ring wall occurs, dramatically reducing the ring cavity and eventually strangling and/or immobilizing the nematode. Subsequently, hyphae penetrate and kill the prey, as for example in *Dactylaria bembicodes*.
(V) *Non-constricting ring:* This trap is similar to the previous one but the ring develops as an infective structure and kills the nematode. *Dactylaria candida* forms such kind of ring.

Trapping fungi are easy to produce *in vitro* and have wide host ranges. Although nematode trapping fungi did not attain much popularity A commercial product named Royale 300® formulated from one isolate of *Arthrobotrys* sp. has been commercialized for some time for nematode management of *Ditylenchus myceliophagous* in mushroom production. Another product based on *Arthrobotrys* spp. (Royale 350®) has been commercialized for control of root knot nematodes (Cayrol 1983).

5.14.2 Parasitic Fungi

A number of fungi from this group has elicited more interest in management of plant parasitic nematodes, as compared to other fungal groups discussed previously. They can survive even in absence of their hosts and can be cultured axenically. These parasitic fungi can be isolated from eggs, juveniles or adult nematodes. Many of them have preferential hosts, and have a certain degree of nematode density regulation. They are also called as opportunistic fungi because they parasitize some nematode stages whenever in contact. Sedentary stages of cyst and root knot nematodes are susceptible to these fungi, present in soil or as endophytes in roots. Whenever egg masses or nematode cysts come in contact with such fungi, these develop on them and eventually parasitize eggs. *Cylindrocarpon, Exophila, Fusarium, Gliocladium, Paecilomyces, Phoma* and *Pochonia chlamydosporia* are most common examples of such fungi. Their damaging action occurs through the enzymatic disruption of nematode structural elements such as the egg shells or cuticle. Physiological effects on nematodes, or when endophytic, on roots, may also occur including, but not limited to, the biosynthesis of toxic metabolites.

5.15 Bacteria as Biocontrol Agents of Nematodes

Nematode parasitic bacteria may be grouped into the following categories:

- Obligate parasites
- Rhizosphere bacteria
- Antagonistic bacteria

5.15.1 Obligate Parasite

Pasteuria spp. are Gram+ obligate bacterial parasites, forming durable endospores. They are parasitic to a number of nematodes, including plant parasitic and free living species. Species of *Pasteuria* include *P. penetrans*, *P. thornei, P. usgae* and *P. nishizawe* parasitizing different nematode hosts. *Pasteuria* spp. are worldwide in distribution and have been reported from 323 nematode species belonging to several different genera of free-living, predatory and plant-parasitic nematodes (Stirling 2011). *Pasteuria penetrans* is one of the most important natural antagonist of root-knot nematodes (*Meloidogyne* spp.) and is highly specific, even at the host population level. The host specificity biocontrol potential of these bacteria has been revealed on many crops. *Pasteuria nishizawe* attacks the soybean cyst nematode *Heterodera glycines*, whereas *P. usgae* is associated to sting nematodes (*Belonolaimus* spp.). These bacteria have a significant role in some suppressive soils. An immediate control root-knot nematode can be achieved by applying up to 10^5 endospores /g of soil, while at 1000–5000 endospores/ g of soil it may take 3 years for establishment in soil (Chen and Dickson 1998; Stirling 2011; Kokalis-Burelle 2015). Field studies, however, showed that *P. penetrans* and other similar species may persist in soil for a long time (Ciancio and Quénéhervé 2000).

The endospores of *Pasteuria* spp. are non-motile and remain in soil, and get attached to the cuticle of passing nematode. Several hundreds of such spores may attach to the cuticle of a single a nematode. However the host may become infected by only one such propagule. The endospore germinates and the germ tube penetrates the cuticle, producing micro colonies in the nematode body. Parasitized nematodes become sterile because as the reproductive system does not develop. Moreover, spore-encumbered juveniles may also fail to reach the root These bacteria are also compatible with certain nematicides i.e. no impact was found for some nematicides on endospore survival and infectivity.

Prior to the development of industrial artificial methods for mass culturing, *P. penetrans* was produced for experimental purposes using the host *Meloidogyne* spp., on a suitable host plant. The nematode-infected roots containing females filled with endospores are powdered, sieved through fine mesh and used as a powder (Stirling 2011). Formulates based on *P. penetrans* endospores for seed coating are now commercially available.

5.15.2 Rhizosphere Bacteria

Another strategy used for nematode biocontrol is based on the introduction of bacteria colonizing the host plant rhizosphere, called rhizobacteria. They grow in the rhizosphere providing a certain defense from pathogens attacks and are considered ideal as biocontrol agent. Some rhizobacteria also have positive effects on plant growth. They are known as plant growth promoting rhizobacteria (PGPR) or plant health promoting rhizobacteria (PHPR). Applications to sugar beet and potato seed significantly lowered early root infestation by the sugar beet cyst nematode *H. schachtii* and the potato cyst nematode *Globodera pallida*.

Many bacteria antagonistic to nematodes are from genus *Pseudomonas*. Others belong to *Agrobacterium, Anthrobacter*, *Bacillus* (i.e. *B. subtilis*, *B. cereus, B. sphaericus*), and *Serratia*. The role of *Ps. fluorescence* as biocontrol agent has been investigated as it appeared effective against both root-knot and cyst nematodes. Bare root dip treatments of tomato seedlings in a suspension of *P. fluorescence* proved to be effective against root-knot nematodes. *Agrobacterium radiobacter* and *B. sphaericus* produce toxic metabolites which affect penetration of *G. pallida*, consequently increasing crop production. *Azotobacter*, including aerobic, Gram- and nitrogen fixing species, is also gaining importance in management of plant parasitic nematodes.

5.15.3 Bacterial Antagonists

Many soil bacteria produce butyric acid, cyanide, exotoxins and hydrogen sulphides. These compounds are antagonistic to nematodes. Compounds such as ammonia and hydrogen sulphide have poisonous effects on root- knot nematodes of rice. *Bacillus thuringiensis* var. *thuringiensis*, *Chromobacterium* spp., *Clostridium butyricum* and *Desulfovibrio desulfuricans* are important antagonistic bacteria against plant parasitic nematodes. *Bacillus thuringiensis* possesses a biocontrol potential. It is an aerobic, Gram+ and produces endospores. There are more than 200 isolates of *B. thuringiensis.* Although it is well known for its pathogenicity to insects, some strains have been reported to be effective against the eggs and juveniles of root-knot nematodes, or against other nematodes such as seed gall, leaf and bud, and lesion nematodes, free living and animal parasitic species (Zuckerman et al. 1993; Sharma 1994; Leyns et al. 1995; Wei et al. 2003).

5.16 Nematodes as BCA of Nematodes

Predatory nematodes may contribute to biocontrol of plant parasitic nematodes. In 1917 Cobb reported about the effectiveness of *Mononchus* sp. against plant parasitic nematodes. Predatory nematodes may offer some advantage over fungi and bacteria as BCA because of their active movement and host searching ability. These nematodes are provided with specialized teeth to catch and swallow the prey. Addition of organic amendments helps to increase their multiplication, given the increase in free living species. Predatory nematodes belong to four orders i.e. Aphelenchida, Diplogasterida, Dorylaimida and Mononchida, differing for their feeding parts, searching behavior and feeding mechanisms (Aatif et al. 2015).

5.16.1 Aphelenchida

The members of this order have piercing and sucking sort of stylet. Their prey nematodes are *Acrobeloides* spp., *Bursilla labiate* and other *Aphelenchoides* spp., such as *A. richardsoni*.

5.16.2 Dorylaimida

They occur in many soil types and habitats and are characterized by piercing and sucking stylets. They actively search their preys. *Eudorylaimus obtusicaudatus* was reported as feeding on eggs inside *H. schachtii* cysts. The host range includes nematodes such as *Aphelenchus avenae*, *Panagrellus redivivus*, *Anguina tritici* and *Tylenchulus semipenetrans* (Bilgrami 2008).

5.16.3 Diplogasterida

Members of this group have cutting-sucking mouths, and may feed also on bacteria. *Diplogaster* or *Koerneria* spp. may be multiplied on both prey nematode and different bacterial species, either *in vitro* and *in vivo*, because of their facultative feeding habit. *Mononchoides fortidens* was cultured on *Rhabditis* spp. on agar with skim milk powder (Devi and George 2018).

5.16.4 Mononchida

These predators have cutting and sucking mouths, and feed on nematodes such as *Meloidogyne* spp., *Pratylenchus* spp., *Paratylenchus* spp., *Meloidodera* spp. and *Tylenchorhynchus* spp. which may even be swallowed entire. A single *Mononchus papillatus* was found to destroy more than one thousand juveniles of *H. radicicola* during its life (Bilgrami 2008; Devi and George 2018).

5.16.5 Symbionts of Entomopathogenic Nematodes

Entomopathogenic nematodes (EPN) are also used in biocontrol programs of plant nematodes. EPN of genera *Heterorhabditis* and *Steinernema* are BCA of many insects, and are mutually associated with endosymbiotic bacteria *Photorhabdus* and *Xenorhabdus*, respectively. A potential antagonistic effect of this symbiosis has been reported for plant parasitic nematodes, that were suppressed by the production of secondary metabolites with a nematicidal effect Such as ammonia, indole and stilbene derivatives. These products are toxic to J_2 stages and eggs of root-knot nematode and adults and J_4 of pine wood nematodes (Hu et al. 1999). Some other features such as competition among nematode groups for root exudates and space in the rhizophere support the EPN suppressive effect on plant parasitic nematodes. Some nematodes are also available commercially as NemAttack™, NemaSeek™ etc.

5.17 Environmental Concerns

The establishment and activity of nematode natural enemies depend on various factors such as species, density and rate of development of the natural enemy, soil conditions, and host plant. Temperature and relative humidity are important factors affecting the biocontrol effectiveness. The understanding of these interactions is essential for effective biocontrol program. Temperature directly affects sporulation and growth of most BCA as well as of targeted species. Soil humidity also influences the survival and growth of bacteria as well as nematodes. In most cases, however, it does not limit growth of fungi. Soil structure and texture also affect the activity of nematode, as well as the growth and spread of microorganisms.

The incorporation of BCA in soil is difficult, and broadcasting methods are not worth due to costs. The use of agents in cereal crops depends on the organisms present in the root zone, and BCA are applied either on seed or in rows. Residual soil microflora also has a static effect, opposing introduced species and competing for energy sources. Consequently, this process may affect the BCA performance. As plant parasitic nematodes are mostly mobile, at least during their larval stages, to infect these pests the BCA evolved adhesive spores or traps. Sedentary nematodes may be parasitized by fungal hyphae in the root zone without forming special infective structures. Cyst nematode females may be destroyed by fungi when they are exposed on the root surface, or the fungi can reduce their fertility rate or parasitize the eggs. In the case of root knot nematodes the eggs are exposed in the root zone, whereas the females remain inside roots. Therefore, the egg parasitic fungi must rapidly kill their eggs to avoid competition by other soil inhabitants. The degradation of soil amendments by non-parasitic microbes releases nematicidal compounds. For example chitin degrading bacteria releases NH_3, which is lethal to many nematodes.

5.18 Future Prospects

The BCA of plant parasitic nematodes have an important role in regulating their population densities. *Pasteuria penetrans, Ps. fluorescens* and nematicidal strains of *B. thuringiensis* have potential to act as effective BCA. The obligate parasite *P. penetrans* possess essential capacities for biocontrol, except its high host specificity. Its obligate behavior confers a certain degree of independence from other soil bacteria, as concerns competition for food sources. Also a number of opportunistic fungi such as *Trichoderma* and *Pochonia* are suitable as BCA of plant parasitic nematodes. They are ubiquitous with a rapid dispersal, and are abundant in the rhizosphere. They can be easily produced in axenic culture for introduction into soil.

The development of integrated pest management program encouraged the integration of multiple control practices, including use of BCA. Researchers must focus on the following features for the management of plant parasitic nematodes, with the help of potential BCA, specific for any concerned ecosystem.

(i) Identification and selection of effective strains of natural enemies.
(ii) Development of a standardized and effective rearing, culturing, storage, handling, release and evaluation procedures.
(iii) Understanding the biology, ecology, physiology, genetic behavior of BCA.
(iv) Identifying most efficient host genotypes-symbionts.
(v) Developing mass culture techniques for field applications.
(vi) Full demonstration and assessment of BCA benefits under field conditions.
(vii) Education of public for BCA effective utilization of.

5.19 Major Developments in Biomanagement of Plant Viruses

Plant viruses are devastating pathogens of the plants. They are unique and distinguished pathogens because of their intrinsic properties. Unlike other plant pathogens, once infected by a virus, the plant will support the virus multiplication throughout its whole life-cycle. The viral infection may have a systemic nature. So far, control and eradication of plant viruses through chemicals is not successful due to the virus biology. Although, biological control of plant diseases caused by fungi, bacteria and nematodes is gaining importance, plant viruses still need new technologies to get any breakthrough.

There is a lot of literature available for the suppression and management of bacteria, fungi and nematodes, but few data are available regarding biocontrol of virus diseases.

Plant viruses, unlike other pathogens, are difficult to control but only can be managed by different methods under an integrated disease management strategy. Selective and non-selective approaches to their control are referred as i) cultural practices and legislations, ii) host resistance and biological control, respectively. There is also a vital interaction between the insect vectors, their BCA use and the application of chemicals or physical (i.e. insect traps, nets etc.) methods.

The progress of viral infection depends on the initial source of inoculum. When biocontrol and host resistance are applied, they will only work for the specific virus targeted (Bos 1992; Buddenhagen 1977; Thresh 1980, 1981, 1982; Jones 2006).

For virus management, the virus-vector relationship need to be understood. Among BCA, PGPR, including a mixture of microorganisms which are beneficial for the plant health (Pal and McSpadden Gardener 2006), play an important role in strengthening the host by improving its nutritional status. Success biocontrol stories against viruses are under the way and need to be investigated for effective management of virus epidemics.

Tomato leaf curl virus (ToLCV) is an important and destructive virus in India and major tomato growing areas in Asia. It is one leading limiting factor in tomato production and is responsible of substantial losses. The virus is transmitted through whitefly (*Bemisia tabaci*) (Muniyappa and Veeresh 1984). The major symptoms are upward curling, yellowing of leaves and abortion of flowers (Gafni 2003). ToLCV belongs to the geminivirus group, family *Geminiviridae* (subgroup – III) (Saikia and Muniyappa 1989; Harrison et al. 1991). Management was attempted through different approaches (Mishra et al. 2014), including the use of agrochemicals vs the insect vectors, that may also imbalance the natural microbial community of beneficial species. However, the virus biocontrol is difficult as there is a complex interaction with the vectors. Therefore, although chemicals may control the vectors in the short term, they may induce the evolution of new resistant insects, in absence of beneficial organisms in the field. To avoid the use of chemicals, resistant plant varieties represent a more favourable option that can be referred to as biological control. However, lack of dominant resistant genes and emerging of new virus races are important constraints in using new varieties (Mishra et al. 2014).

PGPR commonly used for growth promotion may sustain effective biocontrol for virus management. Elicitors were considered to sustain the plant physiology, as shown by comparison between healthy and virus infected samples. The elicitors usually trigger defense mechanisms against fungi, bacteria and viruses. Among them, chitosan was the most important helping in regulating resistant genes. Chitin and chitosan performed well in controlling some plant viruses (Abdelbasset et al. 2010).

Streptomyces, *Pseudomonas*, *Gliocladium*, *Bacillus*, and *Trichoderma* spp. with different PGPR showed a potential against viruses in tomato and other crops (Kandan et al. 2007; Srinivasan et al. 2005; Kavino et al. 2008; Kirankumar 2008). ToLCV was managed through different rhizobacterial isolates with or without PGPR and chitosan treatments (Mishra et al. 2014). Three different PGPRs viz., *Pseudomonas* 206(4), *Pseudomonas* B-15 and *Pseudomonas* JK-16 were used against ToLCV. Chitosan in combination with *Pseudomonas* spp. not only suppressed the virus but also greatly improved plant growth, biomass, chlorophyll content, and yield. High phenolic compounds were observed in chitosan and rhizobacterial treatments against ToLCV. Additionally, peroxidase (PO) and phenylalanine ammonia lyase (PAL) activities were also increased. Polyphenol oxidase (PPO) and chitinase activities were also high in plants treated with chitosan and PGPRs (Mishra et al. 2014).

Kandan et al. (2007) observed an increase in phenolic compounds that contributed in protecting cowpea against **Tomato spotted wilt virus** when treated with *P. fluorescens*. PAL activity plays an important role in defense reactions such as the phenyl propanoid metabolism (Harish 2005). Harish (2005), managed **Banana bunchy top virus** (BBTV) through a biocontrol approach based on the increase in peroxidase activity. Production of hydrogen peroxides and lignification is linked with PO and PPO activities which in turn directly inhibit the pathogen or indirectly restrict its development (Silva et al. 2004). Chitosan was used in potato plants and reported a response involving callose, ribonuclease and β-1,3 glucanase against **Potato virus X** (PVX).

Several *Streptomyces* spp. were used for management of **Cucumber mosaic virus** (CMV) in cucumber plants, in relation to a hypersensitive response. Isolates of *Streptomyces* could inhibit the production of local lesions in treated cucumber plants, as compared to control. Induced systemic resistance was detected through different biological assays (El-Dougdoug et al. 2012).

Mixture of bacterial isolates has been used for management of **Cotton leaf curl virus** disease (CLCuD) (Ramzan et al. 2016). *Bacillus* spp. and *Pseudomonas* spp. antimicrobial activities were reported for the phosphate solubilization and production of indole-acetic acid.

Some rhizosphere species may result beneficial for the host plant growth. Sometimes they are used to reduce the impact of a disease (Murphy et al. 2000; Kandan et al. 2005). These microorganisms may provide protection to the plants from viruses through different mechanisms, such as by improving growth or indirectly through antibiosis, production of lytic enzymes, and induced systemic resistance (Haas and Keel 2003; Jeger et al. 2009). Management of CLCuD through BCAs is not a common practice as only one attempt has been reported (Ramzan et al. 2016).

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