Chapter 8
Biological Control as a Tool for Eco-friendly Management of Plant Pathogens

Mamta Sharma, Avijit Tarafdar, Raju Ghosh, and S. Gopalakrishanan

Abstract Crop protection is pivotal to maintain abundant production of high quality. Over the past 100 years, use of chemical fertilizers and pathocides and good agronomical practices enabled growers to maintain improved crop productivity. However, extensive use of chemicals during the last few decades in controlling pests and diseases resulted in negative impacts on the environment, producing inferior quality and harming consumer health. In recent times, diverse approaches are being used to manage and/or mitigate a variety of pathogens for control of plant diseases. Biological control is the alternative approach for disease management that is eco-friendly and reduces the amount of human contact with harmful chemicals and their residues. A variety of biocontrol agents including fungi and bacteria have been identified but require effective adoption and further development of such agents. This requires a better understanding of the intricate interactions among the pathogen, plants and environment towards sustainable agriculture. Beyond the field assessment, the analysis of microbial communities with culture-independent molecular techniques including sequencing technologies and genomics information has begun a new era of plant disease management.

Keywords Biocontrol agent · Plant-pathogen interaction · Eco-friendly plant disease management · Sustainable agriculture · Socio-economic impact

8.1 Introduction

During the last 40 years, the world population has increased by 90%, while food production has increased only by 25% per head. It is estimated that 39% more production is needed worldwide to feed an additional 1.5 billion mouths by 2020

M. Sharma (✉) · A. Tarafdar · R. Ghosh · S. Gopalakrishanan
International Crops Research Institute for the Semi-AridTropics (ICRISAT), Hyderabad, Telangana, India
e-mail: mamta.sharma@cgiar.org

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and the production needed to be doubled by 2050. However, attack by pest and diseases causes a loss to the tune of 40% of the gross crop production. Further, with the rapid change in climatic factors, plant pathogens are becoming more aggressive, breaking the plant resistance, and inhibit the crops to reach its optimum yield. Current practices for integrated disease management are largely based on genetic host resistance and synthetic chemicals. Continuous use of those chemicals in controlling plant diseases has negative effects on the environment, causes pollution in the biosphere and harms the human beings. Further, those chemicals themselves are acting as selective agents, making the pathogens more resistant, and help these pathogens to persist as they are slowly becoming resistant to these agents. Thus, there was a necessity to execute new methods which would supplement conventional strategies for plant disease control and are competent to minimize adverse effects of chemical pathocides on human health and the environs. Control of plant diseases using biological agents like live microbial cells, or byproducts produced by them, is a powerful alternative way, called biological control. Biological control is eco-friendly, and the diversified microbial world provides endless resources for biologically active molecules which can stably inhabit the environment as nondominant species but maintain their effectiveness in suppression of plant pathogens. For instance, in the 1880s, the cottony cushion scale in citrus was the major threat to citrus industry in California. Vedalia beetle (*Rodolia cardinalis* Mulsant), a predatory insect, was introduced in California to cease the effect of the pest (*Icerya purchasi* Maskell). That was the first success story of the biological control. Since this success, scientists have developed diverse techniques to manage a variety of pests and pathogens using diverse biological agents. In recent years, they diverted their attention towards the potential of beneficial microbes. Therefore, dynamic research efforts for developing and exploring innovative tools for the control of diseases have become imperative.

8.2 Why Eco-friendly Management Is Important to Control Plant Pathogen?

Control of the diseases is very important for securing human food sources and agriculture-based industries. There are two main ways to manage diseases and pests, using chemicals (chemical control) and by predators or parasites (natural control/biological control). Controlling of diseases in economically important crops with chemicals has long been practiced in agricultural settings, and use of this method is more acceptable by the farming community, as it is typically less expensive and immediate than natural control methods. But extensive use of those chemicals for an extended period has long lasting negative effects on the environment, including human life and other living organisms existing in the ecological niches. Being detrimental to both beneficial and harmful organisms, they can damage the ecological balance and also contaminate the food chain

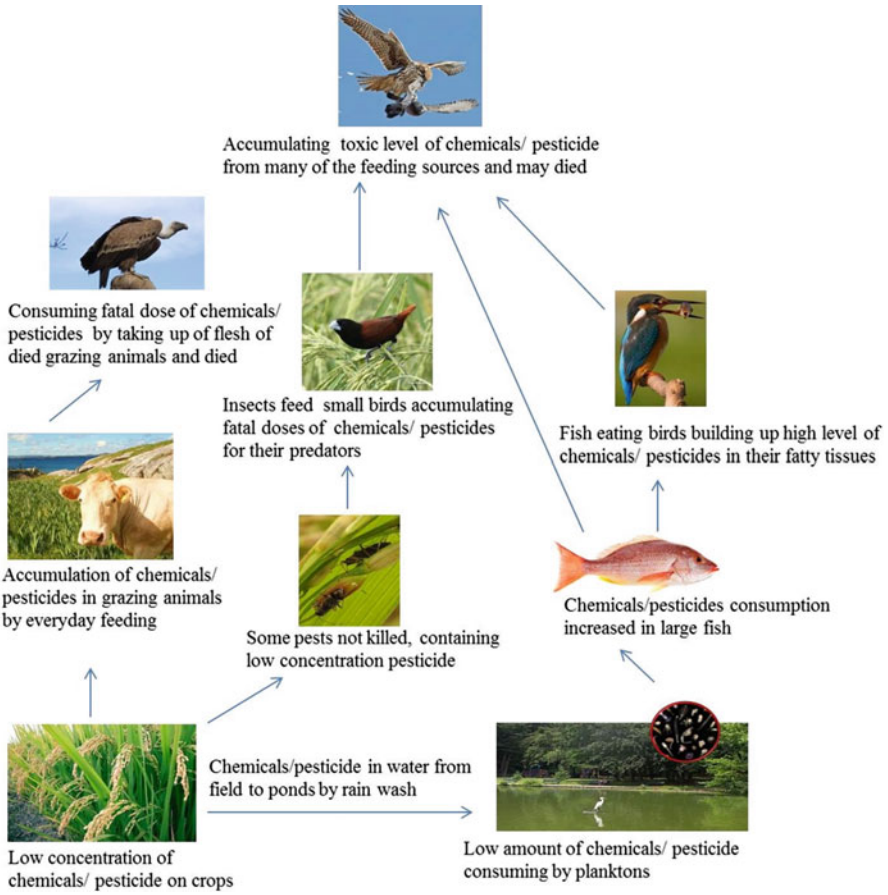

Fig. 8.1 Harmful chemicals enter the food chain and deposition increased tissues of the organisms belonging to the higher tropic level

through bioaccumulation of toxic residues. In this way the chemicals become worse for the organisms belonging to the higher tropic levels (Fig. 8.1).

The term 'biocontrol agent' (biopesticide), as a generic definition, has been applied with a narrow focus on preparations containing living microorganisms, through to a wider definition that includes botanical compounds and semiochemicals (e.g. pheromones) (Kiewnick 2007). The biocontrol agents and the process of biological control have several other benefits.

1. Biocontrol agents are safer both for the environment and the persons who are applying them and avoid environmental pollution (soil, air and water) by leaving no toxic residues.
2. It is comparatively easier to manufacture biocontrol agents, sometimes less expensive than chemical agents.

3. The biggest advantage of using biocontrol agents is that it can eliminate the specific pathogens effectively from the site of infection and can be used in combination with biofertilizers.
4. Biocontrol agents are very effective for a large number of soil-borne pathogen where using of chemical fungicide is not possible.
5. Biocontrol agents do not cause any toxicity to the plants; rather these increase crop yields by enhancing the root and plant growth through the encouragement of beneficial microflora in rhizosphere. It also helps in the mobilization of plant nutrients and makes it available to the plant.
6. Biocontrol agents avoid problems of resistance and also induce systemic resistance among the crop species.
7. Biological control is self-regulating, does not require any intricate management and helps to preserve the ecosystem.

However, despite the fascinating advantages of biocontrol of plant diseases, there might be few adverse effects on humans and the environment. Increasing the population of a certain biological agents artificially could be the reason of paying unexpected concerns. An organism that has been introduced from another area to destroy a pathogen in a new habitat may itself become a pathogen or predator for some beneficial organisms present in natural habitat or crops. Other than that it has the following limitations.

1. Biocontrol agents work slowly and less effectively in comparison to the chemical pesticides, as their efficacy almost completely depends on environmental conditions.
2. Biocontrol agents are mainly used against specific diseases as a preventive measure, not as a curative measure.
3. The antagonists and shelf life of biocontrol agents are short. For example, the shelf life of *Pseudomonas fluorescens* is 3 months and of *Trichoderma viride* is 4 months only. To maintain the effective level of biocontrol agents in cropping area, periodical checking is needed and this requires skilled persons.
4. Skilled persons are also required for multiplying and supplying the biocontrol agents without contamination.
5. At present, biocontrol agents are available only in a few places and in less quantities.

8.3 Groups of Biological Control Agents

After the development of the first commercial biological agent, a range of microorganisms, including virus, bacteria, actinomycetes, fungi, oomycetes, protozoa, etc., were identified for the purpose of plant disease management. Many organisms are found to be very effective against a variety of plant diseases. A few of those organisms are now being used for successful disease management in plants at fields and greenhouse conditions (Table 8.1).

Table 8.1 Groups of successful biocontrol agents that effectively control diseases in economically important crop species

Groups	Biocontrol agent	Target pathogens	Crops	Disease/pest	Experimental condition	References
Virus	*Tomato mosaic virus* (mild strain)	Severe strains of same	Tomato	Mosaic	Field	Fletcher (1978)
	Zucchini yellow mosaic virus (mild strain)	Severe strains of same	Cucarbits	Mosaic	Field	Lecoq and Lemaire (1991)
	Papaya ringspot virus (mild strain)	Severe strains of same	Papaya	Ringspot disease	Field	Tennant et al. (1994)
	Potato virus X (mild strain)	Severe strains of same	Potato	Viral	Field	Webb et al. (1952)
	Mild strain of *Citrus tristeza virus* (mild strain)	Severe strains of same	Citrus	Tristeza	Orchard	Folimonova (2013)
Actinobacteria	***Streptomyces***					
	S. violaceusniger	*Pythium ultimum*	Sugar beet	Damping-off	Field	Trejo-Estrada et al. (1998)
	S. janthimts, *S. cinerochromogenes*	*Pythium coloration*	Carrot	Cavity spot	Field	El-Tarabily et al. (1997)
	Streptomyces species	*Phytophthora medicaginis* and *Phytophthora sojae*	Alfalfa and soybean	Root rot	Controlled condition	Xiao et al. (2002)
	S. lydicus	*P. ultimum*	Cotton and pea	Root and seed rot	Growth chamber	Yuan and Crawford (1995)
	Non-*Streptomyces*					
	Actinoplanes spp.	*P. ultimum*	Beet	Damping-off	Field	Khan et al. (1997)
	A. missouriensis	*Phytophthora megasperma* f. sp. *glycinea*	Soybean	Root rot	Greenhouse	Sutherland and Lockwood (1984)
		Plectosporium tabacinum	Lupin	Root rot	–	El-Tarabily (2003)
	A. philippinensis	*P. coloratum*	Carrots	Cavity spot	–	El-Tarabily et al. (1996a)
		Pythium aphanidermatum	Cucumber	Damping-off	Field	El-Tarabily (2006)

(continued)

Table 8.1 (continued)

Groups	Biocontrol agent	Target pathogens	Crops	Disease/pest	Experimental condition	References
	A. utahensis, *Amorphosporangium auranticolor*, *Micromonospor*a sp.	*P. megasperma* f. sp. *glycinea*	Soybean	Root rot	Greenhouse	Filonow and Lockwood (1985)
	Actinomadura sp.	*Phytophthora cinnamomi*	Snapdragon	Root rot	–	You et al. (1996)
	A. rubra	*P. coloratum*	Carrots	Cavity spot	Field	El-Tarabily et al. (1997)
	Micromonospora sp.	*Gaeumannomyces graminis* var. *tritici*	Wheat	Take all	Field	Coombs et al. (2004)
		Fusarium oxysporum f. sp. *lycopersici*	Tomato	Wilt	–	Smith (1957)
	M. carbonacea	*P. cinnamomi*	Banksia sp.	Root rot	–	El-Tarabily et al. (1996b)
		P. coloratum	Carrots	Cavity spot	Field	El-Tarabily et al. (1997)
		Sclerotinia minor	Lettuce	Basal drop	Greenhouse	El-Tarabily et al. (2000)
	M. chalcea	*P. aphanidermatum*	Cucumber	Damping-off	Field	El-Tarabily (2006)
	M. globosa	*Fusarium udum*	Pigeon pea	Wilt	In vivo	Upadhyay and Rai (1987)
	Microbispora rosea	*P. aphanidermatum*	Cucumber	Damping-off	Field	El-Tarabily (2006)
	Nocardia globerula	*Helminthosporium solani*	Potato	Silver scurf	In vivo	Elson et al. (1997)
	Nocardioides sp.	*Phytophthora fragariae* var. *rubi*	Raspberry	Root rot	In vivo	Valois et al. (1996)
		G. graminis var. *tritici*	Wheat	Take all	Field	Coombs et al. (2004)
	Streptosporangium albidum, *Streptoverticillium netropsis*	*P. coloratum*	Carrots	Cavity spot	Field	El-Tarabily et al. (1997)

	Others					
	Arthrobacter sp.	*Fusarium* sp.	Carnation	Wilt	Greenhouse	Koths and Gunner (1967)
		F. moniliforme var. *subglutinans*	Pine	Pitch canker	Tissue-specific site	Barrows-Broaddus and Kerr (1981)
Bacteria	***Pseudomonads***					
	Pseudomonas chlororaphis	*F. oxysporum* f. sp. *radicis-lycopersici*	Tomato	Foot and root rot	In vitro and in vivo	Bolwerk et al. (2003)
	P. fluorescens	*Phytophthora infestans*	Potato	Late blight	In vitro	Hunziker et al. (2015)
		Sarocladium oryzae	Rice	Sheath rot	Field	Saravanakumar et al. (2009)
		Banana bunchy top virus	Banana	Bunchy top disease	Greenhouse and field	Kavino et al. (2008)
		Dematophora necatrix	Avocado	Root rot	Growth chamber	Cazorla et al. (2006)
		F. culmorum	Rye	Vascular wilt	Greenhouse	Kurek and Jaroszuk-Sciseł (2003)
		Rhizoctonia solani	Rice	Sheath blight	Field	Radjacommare et al. (2004)
		P. ultimum/*Sphaerotheca fuliginea*	Cucumber	Damping-off/powdery mildew	In vivo	Vogt and Buchenauer (1997)
		P. ultimum	Maize	Damping-off	Field	Callan et al. (1991)
		P. ultimum	Cotton	Damping-off	In vivo	Howie and Suslow (1991)

(continued)

Table 8.1 (continued)

Groups	Biocontrol agent	Target pathogens	Crops	Disease/pest	Experimental condition	References
		Phoma betae, *P. ultimum*	Cotton	Damping-off	Growth chamber	Walther and Gindrat (1988)
		P. ultimum	Sugar beet	Damping-off	–	Howell and Stipanovic (1980)
	P. putida	*Fusarium* sp.	Radish	Wilt	Greenhouse	de Boer et al. (2003)
		Different pathogens in soil micro-flora	Cucumber	Non-specific disease	Greenhouse	Loper and Henkels (1999)
	Bacilli					
	Bacillus pumilus, *B. amyloliquefaciens*, *B. subtilis*	*Cucumber mosaic virus*	Tomato	Mosaic disease	Greenhouse	Zehnder et al. (2000)
	B. amyloliquefaciens, *B. subtilis*, *B. pumilus*	*Tomato mottle virus*	Tomato	Tomato mottle	Field	Murphy et al. (2000)
	B. pumilus	*Erwinia tracheiphila*	Cucumber	Bacterial wilt	Field study	Zehnder et al. (2001)
		Peronospora tabacina	Tobacco	Blue mould	Control condition	Zhang et al. (2002)
	B. subtilis, *B. pumilus*	*Sclerospora graminicola*	Pearl millet	Downy mildew	Greenhouse and field	Niranjan Raj et al. (2003)
	B. cereus	*Corynespora cassiicola*	Tomato	Foliar diseases	Greenhouse	Silva et al. (2004)
		R. solani	Cotton	Root rot	In vivo	Pleban et al. (1997)
	Bacillus sp.	*Phytophthora capsici*	Bell pepper	Blight	Field	Jiang et al. (2006)
		Magnaporthe grisea	Rice	Blast	Greenhouse	Naureen et al. (2009)
		P. capsici	Squash	Blight	Greenhouse	Zhang et al. (2010)

	B. amyloliquefaciens	*F. oxysporum*	Maize	Wilt	In vivo	Koumoutsi et al. (2004)
		Botrytis cinerea	Pepper	Blight	Greenhouse	Park et al. (1999)
	Others					
	Aeromonas caviae	*R. solani* and *F. oxysporum* f. sp. *vasinfectum*	Cotton	Root rot/wilt	Greenhouse	Inbar and Chet (1991)
		Sclerotium rolfsii	Beans	Southern blight		
	Brevibacillus brevis	*B. cinerea*	Cucumber	Grey mould	Field	Konstantinidou-Doltsinis et al. (2002)
	Enterobacter agglomerans	*R. solani*	Cotton	Root rot	Greenhouse	Chernin et al. (1995, 1997)
	Enterobacter cloacae	*Pythium* sp.	Sugar beet	Damping-off	In vitro	Howell et al. (1988)
	Burkholderia sp.	*Fusarium verticillioides*	Maize	Maize rot	Greenhouse	Hernández-Rodríguez et al. (2008)
	Paenibacillus sp.	*F. oxysporum*	Sorghum	Wilt	In vitro	Budi et al. (2000)
	Lysobacter enzymogenes	*F. graminearum*	Wheat	Wilt	–	Li et al. (2008)
	Serratia plymuthica	*B. cinerea*	Many host	–	In vitro	Frankowski et al. (2001)
	Stenotrophomonas maltophilia	*P. ultimum*	Sugar beet	Damping-off	Field	Dunne et al. (2000)
	Agrobacterium radiobacter	*Agrobacterium tumefaciens*	Many hosts	–	Greenhouse	Vicedo et al. (1993)
	Collimonas fungivorans	*F. oxysporum*	Tomato	Wilt	Greenhouse	Kamilova et al. (2007)
	L. enzymogenes	*Pythium aphanidermatum*	Cucumber	Root and crown rot	Greenhouse	Folman et al. (2003)

(continued)

Table 8.1 (continued)

Groups	Biocontrol agent	Target pathogens	Crops	Disease/pest	Experimental condition	References
	Burkholderia cepacia	*Pythium* sp. and *Aphanomyces* sp.	Damping-off, root rot	Peas	Field	Heungens and Parke (2000)
Fungi	***Trichoderma***					
	T. harzianum, *T. harzianum*	*Crinipellis perniciosa*	Witches' broom	Cocoa	–	Marco et al. (2003)
	T. lignorum	*R. solani*	Damping-off	Bean	Greenhouse	Aziz et al. (1997)
	T. viride, *T. harzianum*	*Aspergillus flavus* and *Fusarium moniliforme*	Seed associated	Many hosts	In vitro	Calistru et al. (1997)
	T. harzianum	*Sclerotinia sclerotiorum*	Root rot	Soybean	Greenhouse	Ghisalberti and Sivasithamparam (1991)
	Trichoderma spp.	*Sclerotium rolfsii*	Rot	Common Vegetables	In vitro	Mukherjee and Raghu (1997)
	T. harzianum	*Pyrenophora tritici-repentis*	Tan spot and leaf blotch	Wheat	In vitro	Perello et al. (2003)
	T. virens	*R. solani*, *Pythium ultimum* and *Meloidogyne incognita*	Damping-off	Cucumber	Greenhouse	Yedidia et al. (1999)
	T. viride	*Colletotrichum truncatum*	Brown blotch	Cowpea	Field	Bankole (1996)
	T. viride, *T. pseudokoningii*, *T. koningii*	*Sclerotium cepivorum*	White rot	Onion	In vivo	Clarkson et al. (2002)
	T. harzianum	*R. solani*	Wet root rot	Chickpea	Field	Prasad and Rangeshwaran (2000)
	T. harzianum	*F. udum*	Wilt	Pigeon pea	Field	Prasad et al. (2002)

	T. harzianum	*Penicillium expansum*	Blue and grey mould	Apple	In situ	Batta (2004)
	T. virens, *T. harzianum*	*R. solani*	Stem canker or black scurf	Potato	Field	Brewer and Larkin (2005)
	T. koningii, *T. aureoviride*, *T. longibrachiatum*	*Sclerotinia sclerotiorum*	Head rot	Sunflower	Field	Escande et al. (2002)
	T. asperellum	*F. oxysporum*	Wilt	Tomato	Greenhouse	Cotxarrera et al. (2002)
	T. harzianum	*P. capsici*, *P. erythroseptica*	Crown rot/leaf blight	Chilli	Greenhouse	Khan et al. (2004)
	T. koningii	*Gaeumannomyces graminis* var. *tritici*	Take-all disease	Wheat	Greenhouse	Simon (1989)
	T. virens	*Verticillium dahliae*	Wilt	Cotton	Greenhouse	Hanson (2000)
	Yeast groups					
	Cryptococcus albidus var. *aerius*, *Pichia guilliermondii*, *Debaryomyces hansenii*	*Botryodiplodia theobromae*	Mango	Post-harvest rot	In vitro	Sugiprihatini and Wiyono (2011)
	P. guilliermondii	*Aspergillus flavus*	Soybeans	Post-harvest infection	In vitro	Wisniewski et al. (1991)
	P. membranefaciens, *C. albidus*	*Monilinia fructicola*, *Penicillium expansum*, *Rhizopus stolonifer*	Apple	Post-harvest infection	In vitro	Chan and Tian (2005)
	Rhodotorula glutinis, *C. laurentii*	*P. expansum*, *B. cinerea*	–	–	In vitro	Castoria et al. (1997)
	D. hansenii	*M. fructicola*	Peach	Brown rot	In situ	Stevens et al. (1997)
		Penicillium digitatum	Tangerine	Tomato and sweet potato		
		Rhizopus stolonifer		Soft rot		

(continued)

Table 8.1 (continued)

Groups	Biocontrol agent	Target pathogens	Crops	Disease/pest	Experimental condition	References
	P. guilliermondii	*P. digitatum*	Citrus	Decay of citrus fruit	Large scale in storage house	Droby et al. (1993)
	C. laurentii	*M. fructicola*, *P. expansum*	Peach		In vitro	Yao and Tian (2005)
	Chaetomium globosum	*Phoma betae*, *P. ultimum*, *R. solani*	Sugar beet	Damping-off	Growth chamber	Walther and Gindrat (1988)
	Oomycetes					
	Pythium oligandrum	*Sclerotinia sclerotiorum*	Wheat	White mould/stem rot	Field	Madsen and de Neergaard (1999)
		Pythium spp.	Sugar beet, cress	Damping-off	Greenhouse	McQuilken et al. (1998)
		Verticillium dahliae	Pepper	Wilt	Greenhouse	Rekanovic et al. (2007)
		Pythium dissotocum	Tomato	Root rot	Greenhouse	Vallance et al. (2009)
		F. oxysporum f. sp. *radicis-lycopersici*	Tomato	Wilt	Greenhouse	Benhamou et al. (1997)
		P. parasitica	Tomato	Buckeye rot	Greenhouse	Picard et al. (2000)
		B. cinerea	Tomato	Grey mould	Greenhouse	Lou et al. (2011)
		R. solani	Potato	Black scurf	Field	Ikeda et al. (2012)
		P. ultimum	Cress	Damping-off	Field	Al-hamdani et al. (1983)
		Aphanomyces cochlioides	Sugar beet	Root rot	Greenhouse, field	Takenaka and Ishikawa (2013)

		Cercospora beticola	Sugar beet	Leaf spot	Field	Takenaka and Tamagake (2009)
		Phytoplasma	Tobacco	–	Greenhouse	Lherminier et al. (2003)
	Phytophthora cryptogea	*P. infestans*	Potato	Late blight	In vivo	Stromberg and Brishammar (1991)
	Mycorrhiza					
	Glomus mosseae	*Thielaviopsis basicola*	Tobacco	Root infection	In vivo	Baltruschat and Schoenbeck (1975)
	Vesicular arbuscular mycorrhiza	*R. solani*	Rape	Damping-off	–	Iqbal et al. (1977)
	Glomus fasciculatus	*S. rolfsii*	Peanut	Southern blight	–	Krishna and Bagyaraj (1983)

8.4 Plant Extract

Plants are capable of synthesizing an overwhelming variety of small organic molecules, the secondary metabolites, which help the plants overcome from pathogen infection. Identification of novel effective secondary metabolites as fungicide or insecticide is essential to inhibit increasing resistance rates of the pathogens. The botanical extracts are more effective as insecticidal compounds (Table 8.2). But nowadays plant extracts are being used as effective biocontrol agents for inhibiting fungal diseases of plants. The plant extracts from *Cymbopogon proximus*, *Allium sativum*, *Carum carvi*, *Eugenia caryophyllus* and *Azadirachta indica* were found to have inhibitory effects on some phytopathogens including *Botrytis cinerea*, *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* (Alkhail 2005). The methanolic plant extracts from *Salvadora persica*, *Lantana camara*, *Thymus vulgaris*, *Ziziphus spina-christi* and *Zingiber officinale* have antifungal properties

Table 8.2 Botanical pesticides used to control different pests and pathogens

Botanical compounds	Insect pests	Mode of actions	Plant source
Nicotine	Aphids, thrips, caterpillars	Cholinergic acetylcholine nicotinic receptor Agonist/antagonist	*Nicotiana* spp., *Haloxylon salicornicum*, *Stemona japonicum*
Rotenone	Bugs, aphids, potato beetles, spider mites, carpenter ants	Inhibitor of cellular respiration (mitochondrial complex I electron transport inhibitor, METI)	*Lonchocarpus* spp.
Ryania	Codling moths, potato aphids, onion thrips, corn earworms, silkworms	Affect calcium channels	*Ryania* spp.
Sabadilla	Grasshoppers, codling moths, armyworms, aphids, cabbage loopers, squash bugs	Affect nerve cell membrane action	*Schoenocaulon officinale*
Pyrethrum	Caterpillars, aphids, leafhoppers, spider mites, bugs, cabbage worms, beetles	Sodium and potassium ion exchange disruption	*Chrysanthemum cinerariaefolium*
Essential oils	Caterpillars, cabbage worms, aphids, whiteflies, land snails	Inhibition of acetylcholinesterase (AChE)	*Azadirachta indica*, *Mentha* spp., *Lavendula* spp., *Cedrus* spp., *Pinus* spp., *Citronella* spp., *Eucalyptus* spp.
Neem products/ azadirachtin	Armyworms, cutworms, stem borers, bollworms, leaf miners, caterpillars, aphids, whiteflies, leafhoppers, psyllids, scales, mites and thrips	Hormonal balance disruption	*Azadirachta indica*

against *Fusarium oxysporum*, *Rhizoctonia solani* and *Pythium aphanidermatum* (Hussin et al. 2009). Ethyl acetate extracts of *Lantana camara* showed inhibitory effects against *Colletotrichum gloeosporioides* which causes anthracnose in papaya (*Carica papaya* L.).The mother tincture extract of *Myroxylon balsamum* showed antifungal activity against the filamentous fungi *Fusarium guttiforme* and *Chalara paradoxa*, causing pineapple fusariosis.

8.5 Different Mechanisms of Biological Control

8.5.1 Direct Antagonism

8.5.1.1 Parasitism

Parasitism is an interactive mechanism in which two phylogenetically unrelated organisms live together over a prolonged period of time. In this type of relationship, one organism, usually benefitted, called the 'parasite' and the other called the 'host', is harmed. For instance, *Trichoderma* is a parasite of a range of fungi and oomycetes in the soil, which produce toxic metabolites and cell wall-degrading enzymes and inhibit the growth of others.

8.5.1.2 Hyperparasitism

Hyperparasites are the agents that are parasites of harmful plant pathogens. A classic example is the *Hypovirus*, a hyperparasitic virus on *Cryphonectria parasitica*, a fungus causing chestnut blight. The hypovirulence of *Hypovirus* reduces the disease-producing capacity of *C. parasitica* (Tjamos et al. 2010). Some strains of fungi have hyperparasitic activity against other fungi. The fungus *Ampelomyces quisqualis* grows on mildew pathogen; similarly *Nectria inventa* and *Gonatobotrys simplex* 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 that can spread subsequently to intact trees by root-to-root contact (Pratt et al. 1999). The fungal species, *Acremonium alternatum*, *Acrodontium crateriforme*, *Cladosporium oxysporum* and *Gliocladium virens*, have the capacity to parasitize powdery mildew pathogens and be used as biocontrol agent (Heydari and Pessarakli 2010).

8.5.1.3 Commensalism

Commensalism is a unidirectional association between two unrelated species by living together, in which one population (commensals) benefits from these relationships, while the other (the host) is not harmed. Microbes present in the rhizosphere

control soil-borne pathogens through competition for nutrients and production of antibiotics and help the plants survive pathogen infection (Kumar et al. 2016a, b). On the other hand, the microbes have an important role on the growth of the plant by increasing solubilization of minerals or by synthesizing amino acids, vitamins and growth regulators that stimulate the plant growth.

8.5.2 Mixed-Path Antagonism by Synthesis of Allochemicals

8.5.2.1 Siderophores

Siderophores are ligands with low molecular weight having high affinity to sequester iron from the micro-environment. It has the ability to sequester ferric ion and competitively acquire iron from iron-limiting microenvirons, thereby preventing growth of other microorganisms. Two major classes of siderophores, classified on the basis of their functional group, are catechols and hydroxamate. A mix of carboxylate-hydroxamate group of siderophores is also reported (Hider and Kong 2010) (Table 8.3). Numerous strains of *Streptomyces* spp. have been reported as siderophore producers, namely, *S. pilosus* (Muller et al. 1984; Muller and Raymond 1984), *S. lydicus* (Tokala et al. 2002) and *S. violaceusniger* (Buyer et al. 1989). Biological control of *Erwinia carotovora* by several siderophore-producing and plant growth-promoting *Pseudomonas fluorescens* strains A1, BK1, TL3B1 and B10 was reported for the first time as an important mechanism of biological control (Kloepper et al. 1980). On the other hand, increased efficiency of iron uptake by the commensal microorganisms is thought to dislocate pathogenic microorganisms from the possible infection sites by aggressive colonization in plant rhizosphere.

Table 8.3 Examples of siderophores produced by various bacteria and fungi

Types of siderophores	Siderophore	Organism
Hydroxamate	Ferrichrome	*Ustilago sphaerogena*
	Fusarinine C	*Fusarium roseum*
	Desferrioxamine B	*Streptomyces pilosus*, *Streptomyces coelicolor*
	Desferrioxamine E	*Streptomyces coelicolor*
	2,3-Dihydroxybenzoylglycine	*Bacillus subtilis*
	Ornibactin	*Burkholderia cepacia*
	Rhodotorulic acid	*Rhodotorula pilimanae*
Catecholate	Enterobactin	*Escherichia coli*, *enteric bacteria*
	Bacillibactin	*Bacillus subtilis*, *Bacillus anthracis*
Mixed ligands	Azotobactin	*Azotobacter vinelandii*
	Pyoverdine	*Pseudomonas aeruginosa*
	Yersiniabactin	*Yersinia pestis*

Sneh et al. (1984) and Elad and Baker (1985) showed a direct correlation between in vitro inhibition capacity of chlamydospore germination of *F. oxysporum* and siderophore synthesis in fluorescent pseudomonads.

8.5.2.2 Antibiosis

The term 'antibiosis' came from the term antibiotics, which refers to organic substances produced by microorganisms that affect the metabolic activity of other microbes and inhibit the growth (Roshan et al. 2013). The result of antibiosis is often death of microbial cells by endolysis and breakdown of the cell cytoplasm. *Agrobacterium radiobacter* K-84, produced commercially as Agricon 84, was first recognized as a valuable control agent of crown gall since 1973. It is very effective against *A. tumefaciens* attacking stone fruit (e.g. plums and peaches), but not effective against *A. tumefaciens* strains that attack grapes, pome fruit (e.g. apples) and some ornamentals. A variety of antibiotics have been identified, including compounds such as 2,4-diacetylphloroglucinol (DAPG), amphisin, oomycin A, hydrogen cyanide, pyoluteorin, phenazine, tensin, pyrrolnitrin, cyclic lipopeptides and tropolone produced by pseudomonads and kanosamine, oligomycin A, xanthobaccin and zwittermicin A produced by *Streptomyces*, *Bacillus* and *Stenotrophomonas* spp. (Kumar et al. 2014) (Table 8.4). For instance, antibiotic 2,4-diacetyl phloroglucinol is reported to be involved in the suppression of *Pythium* spp., iturin suppresses the pathogens *Botrytis cinerea* and *Rhizoctonia solani*, and phenazine carboxylic acid antagonist the pathogen *Rhizoctonia solani* in rice (Padaria et al. 2016) and phenazines control *Gaeumannomyces graminis* var. *tritici* in wheat.

8.5.2.3 Volatile Substances

Apart from the production of antibiotics, some biocontrol agents are also known to produce volatile compounds as tools for pathogen inhibition. Common volatile compounds are hydrocyanic acid (HCN), certain acids, alcohols, ketones, aldehydes and sulphides (Bouizgarne 2013). HCN production is reported to play a role in disease suppression (Wei et al. 1991), for instance, Haas et al. (1991) reported HCN production by strains of *P. fluorescens* that helped in the suppression of black root rot of tobacco. Reports on the production of HCN by beneficial microbes in order to minimize the deleterious effect of pathogenic fungi and bacteria are available (Ahmad et al. 2008; Gopalakrishnan et al. 2011a, b, 2014).

8.5.2.4 Lytic Enzyme Production

Many microorganisms secrete and excrete lytic enzymes that can hydrolyse a wide range of polymeric compounds, including hemicellulose, cellulose, chitin, DNA

Table 8.4 Selected examples of antibiotics produced by biocontrol bacteria

Antibiotic	Source	Target pathogen
2,4-Diacetyl phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium* spp.
2,4-DAPG	*Pseudomonas* sp.	*Xanthomonas oryzae* pv. *oryzae*
2-Hexyl, 5-propyl resorcinol	*P. fluorescens*	*Rosellinia necatrix*
Agrocin 84	*Agrobacterium radiobacter*	*A. tumefaciens*
Amphisin	*P. fluorescens*	*Pythium ultimum* and *Rhizoctonia solani*
Bacillomycin D	*Bacillus subtilis* AU195	*Aspergillus flavus*
Bacillomycin, fengycin	*B. amyloliquefaciens* FZB42	*Fusarium oxysporum*
Cyclic lipopeptides	*Pseudomonas* sp.	*Phytophthora infestans*
Geldanomycin	*Streptomyces hygroscopicus* var. *geldonus*	*R. solani*
Gliotoxin	*Trichoderma virens*	*R. solani*
Herbicolin	*Pantoea agglomerans* C9-1	*Erwinia amylovora*
Iturin A	*B. subtilis* QST713	*Botrytis cinerea*, *P. ultimum*, *R. solani*, *F. oxysporum*, *Sclerotinia sclerotiorum* and *Macrophomina phaseoli*
Iturin A and surfactin	*B. subtilis*	*R. solani*
Kanosamine	*B. cereus*	*Phytophthora medicaginis*
Kasugamycin	*S. kasugaensis*	*Pyricularia oryzae*
Mycosubtilin	*B. subtilis* BBG100	*Pythium aphanidermatum*
Oligomycin A	*S. libani*	*B. cinerea*
Phenazines	*P. fluorescens* 2–79 and 30–84	*Gaeumannomyces graminis* var. *tritici*
	P. chlororaphis	*F. oxysporum*
	P. aureofaciens	*Sclerotinia homeocarpa*
Phenazine-1-carboxamide	*P. chlororaphis*	*F. oxysporum* f. sp. *radicis-lycopersici*
Polyoxin D	*S. cacaoi*	*R. solani*
Pyoluteorin	*P. fluorescens*	*P. ultimum*
Pyoluteorin, pyrrolnitrin	*P. fluorescens* Pf-5	*P. ultimum* and *R. solani*
Pyrrolnitrin, pseudane	*Burkholderia cepacia*	*R. solani* and *Pyricularia oryzae*
	P. fluorescens	*Gaeumannomyces graminis* var. *tritici*
		R. solani
	Enterobacter agglomerans	*A. tumefaciens*, *Clavibacterium michiganense*, *Xanthomonas campestris*, *Pseudomonas syringae* pv. *syringae*

(continued)

Table 8.4 (continued)

Antibiotic	Source	Target pathogen
Polyenes	*S. violaceusniger*	*P. ultimum*
Citrinin	*Penicillium citrinum*	*B. cinerea*
Viscosinamide	*P. fluorescens*	*R. solani*, *P. ultimum*
Xanthobaccin A	*Lysobacter* sp. strain SB-K88	*Aphanomyces cochlioides*
Zwittermicin A	*B. cereus* UW85	*P. medicaginis* and *P. aphanidermatum*
	B. cereus and *B. thuringiensis*	*Phytophthora* spp.
	Bacillus spp.	*S. sclerotiorum*
	B. cereus	*Phytophthora parasitica* var. *nicotianae*

and proteins (Table 8.5). These extracellular hydrolytic enzymes play an important role in the suppression of plant pathogens. Chitinase secreted by *Streptomyces* sp., *Paenibacillus* sp. and *Serratia marcescens* was found to be inhibitory against *Sclerotium rolfsii*, *Botrytis cinerea* and *Fusarium oxysporum* f. sp. *cucumerinum*. Similarly, modifying plant growth substratum with chitosan inhibits the root rot in tomato caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*. β-1,3-Glucanase produced by *Actinoplanes philippinensis* and *Micromonospora chalcea* was found to hydrolyse *Pythium aphanidermatum* in cucumber (El-Tarabily 2006).

8.5.2.5 Unregulated Waste Products

Few soil microbes release a range of unregulated waste products or harmful gases, e.g. ethylene, methane, nitrite, ammonia, hydrogen sulphide, other volatile sulphur compounds, carbon dioxide, etc., and suppress the growth of other pant pathogenic bacteria. This interaction between two species is called ammensalism. *Bacillus megaterium* produces ammonia and has an inhibitory effect on the growth of *Fusarium oxysporum* (Shobha and Kumudini 2012).

8.5.2.6 Detoxification and Degradation of Virulence Factor

Biological control by detoxification involves production of a protein that binds with the pathogen toxin and detoxifies pathogen virulence factors, either reversibly or irreversibly, ultimately decreasing the virulence potential of pathogen toxin. For example, the biocontrol agents *Alcaligenes denitrificans* and *Pantoea dispersa* are able to detoxify albicidin toxin produced by *Xanthomonas albilineans*. Similarly, strains like *B. cepacia* and *Ralstonia solanacearum* can hydrolyse fusaric acid, a phytotoxin produced by various *Fusarium* spp. The protein has the ability to bind reversibly with the toxins of both *Klebsiella oxytoca* and *Alcaligenes denitrificans*, as well as irreversibly with the toxin albicidin in *Pantoea dispersa*.

Table 8.5 Examples of lytic enzymes produced by biocontrol bacteria

Enzyme	Producing bacteria	Target phytopathogen	Host plant
Chitinases	*Aeromonas caviae*	*Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *vasinfectum*	Cotton
		Sclerotium rolfsii	Beans
	Arthrobacter sp.	*Fusarium* sp.	Carnation
		F. moniliforme var. *subglutinans*	Southern pines
	Streptomyces sp.	*Macrophomina phaseolina*	Sorghum
	Enterobacter agglomerans, *Bacillus cereus*	*R. solani*	Cotton
	B. circulans and *Serratia marcescens*	*Phaeoisariopsis personata*	Peanut
	E. agglomerans, *B. cereus*	*R. solani*	Cotton
	Paenibacillus illinoisensis	*R. solani*	Cucumber
	Pseudomonas sp.	*F. oxysp*orum f. sp. *cucumerinum*	Cucumber
	Serratia plymuthica	*Botrytis cinerea* and *Sclerotinia sclerotiorum*	Cucumber
	Serratia marcescens	*S. rolfsii*	Beans
		R. solani	Cotton
	Streptomyces lydicus	*Pythium* and *Aphanomyces*	-
	Serratia plymuthica	*Botrytis cinerea*	Many host
Glucanases	*Streptomyces* sp.	*Phytophthora fragariae*	Raspberry
	Pseudomonas cepacia	*R. solani*, *S. rolfsii*, *Pythium ultimum*	
	Actinoplanes philippinensis and *Micromonospora chalcea*	*Pythium aphanidermatum*	Cucumber
	Lysobacter enzymogenes	*Pythium*	Sugar beet
	Paenibacillus, *B. cepacia*	*F. oxysporum*, *R. solani*, *S. rolfsii* and *Pythium ultimum*	-
Chitinases and glucanases	*Serratia marcescens*, *Streptomyces viridodiasticus*, *Micromonospora carbonacea*	*Sclerotinia minor*	Lettuce
	L. enzymogenes	*F. graminearum*	Wheat
	Streptomyces sp. and *Paenibacillus* sp.	*F. oxysporum* f. sp. *cucumerinum*	Cucumber
Chitinases, proteases and cellulases	*B. subtilis*, *Erwinia herbicola*, *Serratia plymuthica* and *Actinomycete*	*Eutypa lata*	Grapevine
Proteases	*Stenotrophomonas maltophilia*	*P. ultimum*	Sugar beet
Laminarinase	*Pseudomonas stutzeri*	*F. solani*	-

8.5.3 Indirect Antagonism

8.5.3.1 Competitive Root Colonization

From the microbial perspective, living plant surfaces and soils are often nutrient-restricted environments. Nutrient limitation is an important mode of action of some biological control agents. Carbon plays an important role for competition of root colonization for nutrients such as *Trichoderm*a spp. (Sivan and Chet 1989). Carbon competition between pathogenic and non-pathogenic strains of *F. oxysporum* is one of the main mechanisms in the suppression of *Fusarium* wilt (Alabouvette et al. 2009). The disease suppression of bacterium *Erwinia amylovora* causes fireblight by the closely related saprophytic species *E. herbicola* due to competition of the nutrient on the leaf surface. Competition between rhizosphere bacteria and *Pythium ultimum*, a common cause of seedling damping-off for the same carbon source, has resulted in an effective biological control of the latter organism in several crops. Germination of the conidia of *Botrytis cinerea* is inhibited by *Pseudomonas* species due to competition for amino acids. This mechanism may not be useful in suppressing biotrophs such as powdery mildews and rusts, because they do not require exogenous nutrients for host infection.

8.5.3.2 Plant Growth Promotion Through SAR and ISR

Chemical stimuli are produced by some biocontrol agents, i.e. non-pathogenic plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF), or by soil- and plant-associated microbes. Such stimuli can either induce a sustained change in the plants which increase the capacity of tolerance to infection by pathogens or induce the local and/or systemic host defences of the whole plant against broad-spectrum pathogens. This phenomenon is known as induced resistance. Two types of induced resistance are distinguished in plants, systemic acquired resistance (SAR) and induced systemic resistance (ISR). The first of the two pathways is mediated by salicylic acid (SA) which is frequently produced after pathogen infection and induces the expression of pathogenesis-related (PR) proteins that include a variety of enzymes. The second method is mainly jasmonic acid (JA) and/or ethylene mediated following the applications of some nonpathogenic rhizobacteria (Fig. 8.2). The SAR-induced resistant was observed when *Trichoderma harzianum* was inoculated in roots and leaves of grapes, and it provides control of diseases caused by *Botrytis cinerea* from the site of application of *T. harzianum* (Deshmukh et al. 2006). It was found that the biocontrol agent *P. fluorescens* strain CHAO induces accumulation of salicylic acid and by inducing SAR-associated proteins confers systemic resistance to a vira1 pathogen in tobacco. Colonization of *Glomus intraradices* on the roots of *Oryza sativa* conferred resistance through induction of defence-related genes (Campos-Soriano et al. 2012). *Penicillium simplicissimum* enhanced the resistance of barley to *Colletotrichum orbiculare* by inducing salicylic acid accumulation, formation of active oxygen species, lignin deposition

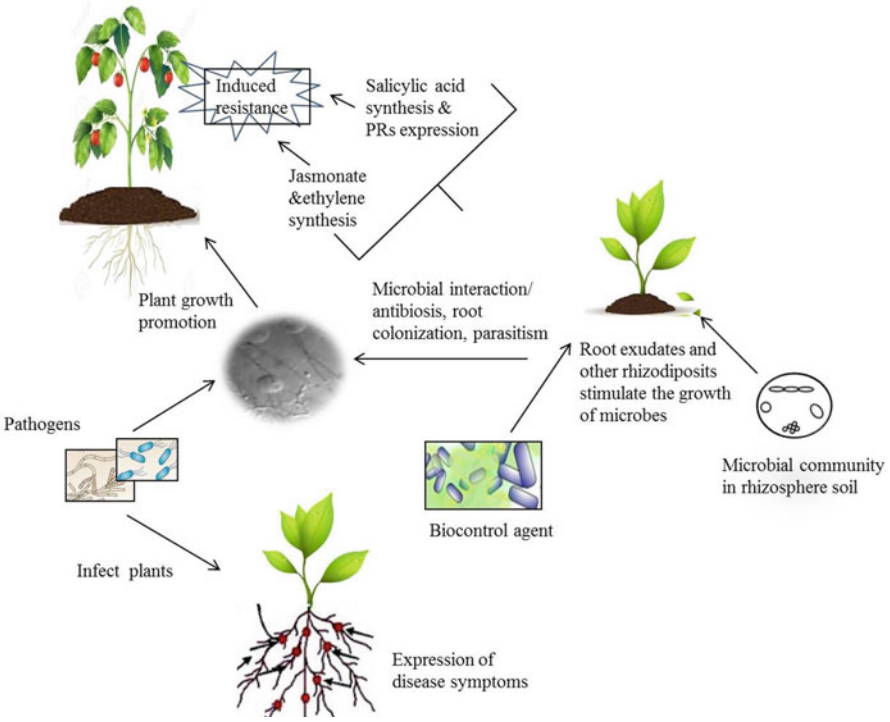

Fig. 8.2 Interaction within the plants, pathogenic microorganisms and biocontrol

and activation of defence genes. In addition, *Fusarium equiseti* and *Phoma* sp. elicited *Arabidopsis thaliana* systemic resistance *against Pseudomonas syringae* pv. tomato and *Pythium oligandrum* against *Ralstonia solanacearum*. However, different ISR elicitors like secondary metabolites and proteins involved in mycoparasitism and antibiosis have also been identified. Secondary metabolites like trichokinin, alamethicin, harzianopyridone, harzianolide and 6-pentyl–αpyrone have antagonist effects at high doses but in low doses act as ISR inducers. Expression of endochitinase *Ech42* of *Trichoderma atroviride* was found to act as an ISR inducer in barley, resulting in an increased resistance to *Fusarium* sp. infection. Similarly, chitinase *Chit42* of *T. harzianum* expression increased resistance in potato and tobacco against the foliar pathogens, *B. cinerea*, *Alternaria solani* and *A. alternata*, and soil-borne pathogen, *Rhizoctonia solani*.

8.6 Genomic Approaches of Biocontrol Agent

Recent advances in molecular technologies have brought a revolution in microbial worlds and unzipped the immense diversity in microbial population helping scientific community to find out novel biocontrol agents (Kumar et al. 2014; Sharma

et al. 2016). Utilizing bioinformatics tools and inexpensive sequencing techniques has led to the assembly of genomic data for microbial biocontrol agents and exploring the untapped and novel microbial isolates for important secondary metabolites and enzymes.

Seventy-eight percent of the genes functionally associated with antagonism were found to be distributed in *Trichoderma* species, described as the best fungal biocontrol agent till date. This is followed by *Coniothyrium*, *Pythium* and *Clonostachys* with 6, 5 and 4%, respectively. The way of antagonism is different in different microbes and sometimes depends on the pathogens. The genes associated with antagonism are diverse and involved in antibiosis, signalling, parasitism or transport. Of the identified genes, 44% are related to mycoparasitism, and 26% were for the antibiosis, whereas ISR-, signalling- and competition-related genes represent only 12, 11 and 5%, respectively. The role of different glucanases and chitinases during mycoparasitism is demonstrated with the functional characterization by gene-by-gene study in *Trichoderma* spp. (Daguerre et al. 2014). However, molecular mechanisms involved in the antagonism are not well known for all the cases. Now metatranscriptomic analyses appear as a more powerful tool as they provide generous information on different aspects of the antagonism allowing for comparison from the early stages to the later ones. The use of metatranscriptomic analyses prior to functional characterization seems to be the most sensible strategy. However, functional characterization is needed for verifying and ensuring the molecular mechanisms of antagonism.

The use of advanced molecular technique and genomic approaches in the identification of novel biocontrol agent is in its initial stages, but in the near future, latent biochemical products may arise as the key of antagonism of major phytopathogens as well as PGP in crops. For example, a total number of six genera of actinobacteria, viz. *Corynebacterium*, *Mycobacterium*, *Arthrobacter*, *Frankia*, *Rhodococcus* and *Streptomyces*, have been sequenced and analysed for potential secondary metabolite and gene diversity (James and William 2013).

8.7 Commercially Available Eco-friendly Biological Agents

Formulation of biopesticide based on a variety of microorganisms, e.g. nematodes, protozoa, fungi, bacteria, viruses, etc., is known as microbial pesticides or biocontrol agents. Predominantly five microbes, *P. fluorescens*, *B. subtilis*, *Gliocladium* spp., *Verticillium lecanii* and *Trichoderma* spp., are used for the purpose of commercial microbial pesticides. Several biopesticides are commercially available (Table 8.6) globally. However, in India only 35 microbes have been included in the Insecticides Act (1968) till now for commercial production of biocontrol agent, since the first biopesticide was notified in the *Gazette of India* dated 26 March 1999. In India, Singh (2006) identified novel *Trichoderma* strain with enhanced nematicidal, fungicidal and growth promotion property and used for developing biocontrol agent. The technology was transferred to Department of Agriculture, Government of UP, for its commercial production. Later on, this technology has also been

Table 8.6 List of biocontrol products commercially available for the control of plant pathogens

Product	Biocontrol agent	Target disease/organism	Crop	Manufacturer
Actinovate	*Streptomyces lydicus*	Soil-borne disease	Greenhouse and nursery crops, turf	Natural Industries, Inc., USA
Avogreen	*Bacillus subtilis*	*Pseudocercospora purpurea*	Avocado	Stimuplant, South Africa
Alfa guard	*Aspergillus flavus* AF36	*Aspergillus flavus*	Cotton	Circle One Global, USA
AQ10 Biofungicide	*Ampelomyces quisqualis* isolate M10	Powdery mildew	Apples, cucurbits, grapes, ornamentals, strawberries and tomato	Ecogen, Inc., USA
Aspire	*Candida oleophila* I182	*Botrytis* spp., *Penicillium* spp.	Citrus, pome fruit	Ecogen, Inc.
Ballad Plus	*Bacillus pumilus*	Rust, powdery mildew, cercospora, brown spot	Soybean	AgraQuest, USA
Biobest	*Bacillus subtilis*	Sheath blight, blast, brown spot	Rice	Appliedchem, Thailand
BioJect SpotLess	*Pseudomonas aureofaciens* strain TX-1	*Pythium*, *Rhizoctonia solani*	Vegetables and ornamentals in greenhouses	Eco Soil Systems, Inc.
Biosave 10LP, 110	*Pseudomonas syringae*	*Botrytis cinerea*, *Penicillium* spp., *Mucor piriformis*, *Geotrichum candidum*	Pome fruit, citrus, cherries and potato	Village Farms LLC
BlightBan A506	*Pseudomonas fluorescens* A506	*Erwinia amylovora* and russet-inducing bacteria	Almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry, tomato	NuFarm Inc.
Cedomon	*Pseudomonas chlororaphis*	Leaf stripe, net blotch, *Fusarium* sp., spot blotch, leaf spot and others	Barley and oats; potential for other cereals	BioAgri AB
Companion	*Bacillus subtilis* GB03, other *B. subtilis*, *B. licheniformis*, *B. megaterium*	*Rhizoctonia*, *Pythium*, *Fusarium*, *Sclerotinia* and *Phytophthora*	Greenhouse, nursery and ornamental crops	Growth Products, USA
Contans WG, Intercept WG	*Coniothyrium minitans*	*Sclerotinia sclerotiorum* and *S. minor*	All types of crops	Prophyta Biologischer Pflanzenschutz GmbH, Germany

Cyd-Xe	Codling moth granulosis virus	Codling moth	Apple, pear, walnut and plum	Thermo Trilogy Corp., Columbia
Deny	*Burkholderia cepacia type*	*Rhizoctonia*, *Pythium*, *Fusarium* and disease caused by lesion, spiral, lance and sting nematodes	Alfalfa, barley, beans, clover, cotton, peas, grain sorghum, vegetable crops and wheat	Stine Microbial Products
EcoGuard	*B. licheniformis*	Dollar spot, anthracnose	Turf	Novozymes, Denmark
Frostban	*P. fluorescence* strain A506	Fire blight, bunch rot	Fruit crop, tomato, potato	Plant Health Technologies
Galltrol	*Agrobacterium radiobacter* strain 84	*Agrobacterium tumefaciens*	Fruit, nut and ornamental nursery stock	AgBioChem Inc., USA
GB34	*Bacillus subtilis* strain GB34	*Rhizoctonia*, *Fusarium*	Soybean	Gustafon, USA
HiStick N/T	*Bacillus subtilis* MBI600	*Fusarium*, *Rhizoctonia*, *Aspergillus*	Soybean, alfalfa, dry/snap beans, peanuts	Becker Underwood Inc., UK
Intercept	*Burkholderia cepacia*	*Rhizoctonia solani*, *Fusarium* spp., *Pythium* sp.	Maize, vegetables, cotton	Soil Technologies Corp.
Kodiak	*Bacillus subtilis* GB03	*Rhizoctonia solani*, *Pythium*, *Fusarium* spp., *Alternaria* spp. and *Aspergillus* spp. that attack roots	Wheat, barley, peas, cotton, legumes, soybean and vegetable crops	Bayer CropScience, USA
Larminar	*B. subtilis*	*Alternaria* spp., *Botryodiplodia* sp., *Colletotrichum* sp., *Corticium* sp., *Fusarium* spp., *Phytophthora* spp.	Vegetables, fruit trees, ornamentals, rice and field crops	Appliedchem, Thailand
Messenger	*Erwinia amylovora* HrpN harpin protein	Many	Field, ornamental, and vegetable crops	EDEN Bioscience Corporation,
Mycostop	*Streptomyces griseoviridis* strain K61	*Fusarium* spp., *Alternaria brassicola*, *Phomopsis* spp., *Botrytis* spp., *Pythium* spp. and *Phytophthora* spp. that cause seed, root and stem rot, and wilt disease	Field, ornamental, vegetable crops and tree seedlings	Kemira Agro Oy, Finland
Nogall	*Agrobacterium radiobacter* K1026	*Agrobacterium tumefaciens*	Fruit, nut, and ornamental nursery stock	Biocare Technology, Australia

(continued)

Table 8.6 (continued)

Product	Biocontrol agent	Target disease/organism	Crop	Manufacturer
Primastopsoil guard	*Gliocladium catenulatum*	Soil-borne pathogens that cause seed, root and stem rot and wilt disease	Ornamental, vegetable, spices and tree crops	Kemira Agro Oy, Finland
Rhapsody	*B. subtilis*	*Rhizoctonia*, *Fusarium*, *Pythium*, *Phytophthora*	Turf, ornamental, vegetables and fruits in greenhouse	AgraQuest, USA
RootShield, PlantShield	*Trichoderma harzianum* strain KRLAG2 T22	*Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp.	Trees, shrubs, transplants, all ornamentals, cabbage, tomato, cucumber	Bioworks Inc., USA
Serenade	*Bacillus subtilis* QST716	Powdery mildew, downy mildew, *Cercospora* leaf spot, early blight, late blight, brown rot, fire blight, *Botrytis* and *Sclerotinia* disease and others	Cucurbits, grapes, hops, vegetables, peanuts, pome fruits, stone fruits and others	AgraQuest Inc., USA
SoilGard	*Gliocladium virens*	Damping-off and root rot pathogens especially *Rhizoctonia solani* and *Pythium* spp.	Ornamental and food crop plants grown in greenhouses, nurseries	Certis Inc., USA
Sonata	*B. pumilus*	Rusts, powdery and downy mildews	Vegetable and fruit crops	AgraQuest, USA
Subtilex	*B. subtilis*	*Rhizoctonia*, *Fusarium*, *Aspergillus*	Field, ornamental and vegetable crops	Becker Underwood, USA
Taegro	*B. amyloliquefaciens*	*Rhizoctonia*, *Fusarium*	Tree seedlings, ornamentals and shrubs	Novozymes, Denmark
Trichodex	*Trichoderma harzianum* T-39	*Botrytis cinerea*	Most of the food crops	Bio works, USA
YiedShield	*Bacillus pumilus* GB34	Soil-borne fungal pathogens causing root diseases	Soybean	Gustafson Inc., USA

transferred to Gujarat State Fertilizer and Chemicals Limited (GSFC), Gujarat Green Revolution Company Limited (GGRC) and Balaji Crop Care Pvt. Ltd., Hyderabad, for commercial production. The products 'Sardar Eco Green Biofungicide' and 'TRICHA' based on a potential strain of *Trichoderma harzianum* NBRI-1055 are in market for controlling phytopathogenic fungi. A talc-based formulation of *Trichoderma viride* strain 2953 has recently been transferred to Balaji Crop Care Pvt. Ltd., Hyderabad, for large-scale production.

8.8 Socio-economic Impact, Ethical Issues Winding with the Biocontrol

Global assessment of biocontrol agents' commercial availability in markets shows that the percentage of users and land have steadily increased since the late 1990s and the projected growth is continuing at a rate of 15.6% per year (Glare et al. 2012). Lehr (2010) reported that the global sales of biocontrol agents were estimated at US$ 396.48 million in 2003 and have continued to increase with projections to reach up to US$ 1.068 billion by 2010. With the successful implementation of biological agents in field for integrated plant disease management, demand for commercial biocontrol agent is increasing within the growers. There are approximately 225 microbial biocontrol agents which were manufactured in 30 member countries and registered by the Organization for Economic Development and Cooperation (Kabaluk and Gazdik 2007) for commercialization. The rest of the global market share is distributed among the countries within the Oceania at 20%, Latin and South American countries at 10% and less than 5% each accredited to Asia and India (Thakore 2006). The chances of future market expansion within the latter countries are likely to be variable. Organic and conventional producers are anticipating the use of alternative biocontrol products that pose a lower-risk exposure to human health than synthetic chemicals. Worldwide evolutionary exploration with the microbial products and the illustrating actions of the government personnel within the country, the growers and the industry have led to changes in strategy, management and research initiatives. On the other hand, legislation concurrently is supporting to make new policy that encouraged the registration of lower-risk pest control products.

Quality of the inoculants available in the market, however, needs to be carefully monitored as the formulation available in the market should contain sufficient population of the biocontrol microbes to produce an economic gain. Many countries such as the Netherlands, Thailand, Russia, France, Australia, Canada and the UK have regulations for inoculant quality which lead to improvements in the quality of commercial inoculants (Bashan et al. 2014). Canada and France have set norms that formulated products should have 10^6 viable cells per seed with no detectable contaminants (Catroux et al. 2001). However, that is not the case in developing countries as most of the inoculants produced are of poor or sub-optimal quality.

Brockwell and Bottomley (1995) observed that most of the inoculants produced in the world is of relatively poor quality and 90% of all inoculants has no practical effect on the productivity of crops for which it is used. Further, the presence and nature of contaminants encountered in inoculants may represent a risk for humans, plants and for the environment, which remains to be assessed. Hence, quality of inoculants available in the market needs to be closely monitored, and make sure that farmers use the quality inoculants so that they will have trust on biocontrol.

8.9 Future Prospects for Biocontrol

In the past five decades, an increasing number of chemical fertilizer and biocidal molecules were the main cause for a substantial increase in crop production and quality. Because of environmental issues and health concerns, continuous and extensive use of those molecules has raised serious debate, and often various biological control methods based on natural pest and pathogen-suppressing organisms are being recommended as a substitute. Globally the registrations of microbial biocontrol agents are increasing significantly. The changes in legislation in the country level, development of new policies and management structures to address the reduction of chemical uses are the expanding scope of biocontrol agents. On the other hand, the researchers worldwide have been supported to discover new biocontrol agents to reinforce for entering in the industry. Being practical, at present biocontrol agents are not comparable to chemical pesticides in meeting efficacy which is needed for market expectations, but they still have a promising future if knowledge and methods of various fields of biotechnology are utilized. The availability of recent molecular technologies has significantly facilitated for surveying and identification of candidate agents, and helped to interpret the modes of action after field applications. These new technologies like proteomics and functional genomics will give new possibilities for insights in ecological constraints and will help to see hitherto unseen possibilities to determine the physiological status and expression of crucial genes present within the biocontrol agents during mass production, formulation, storage and application.

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